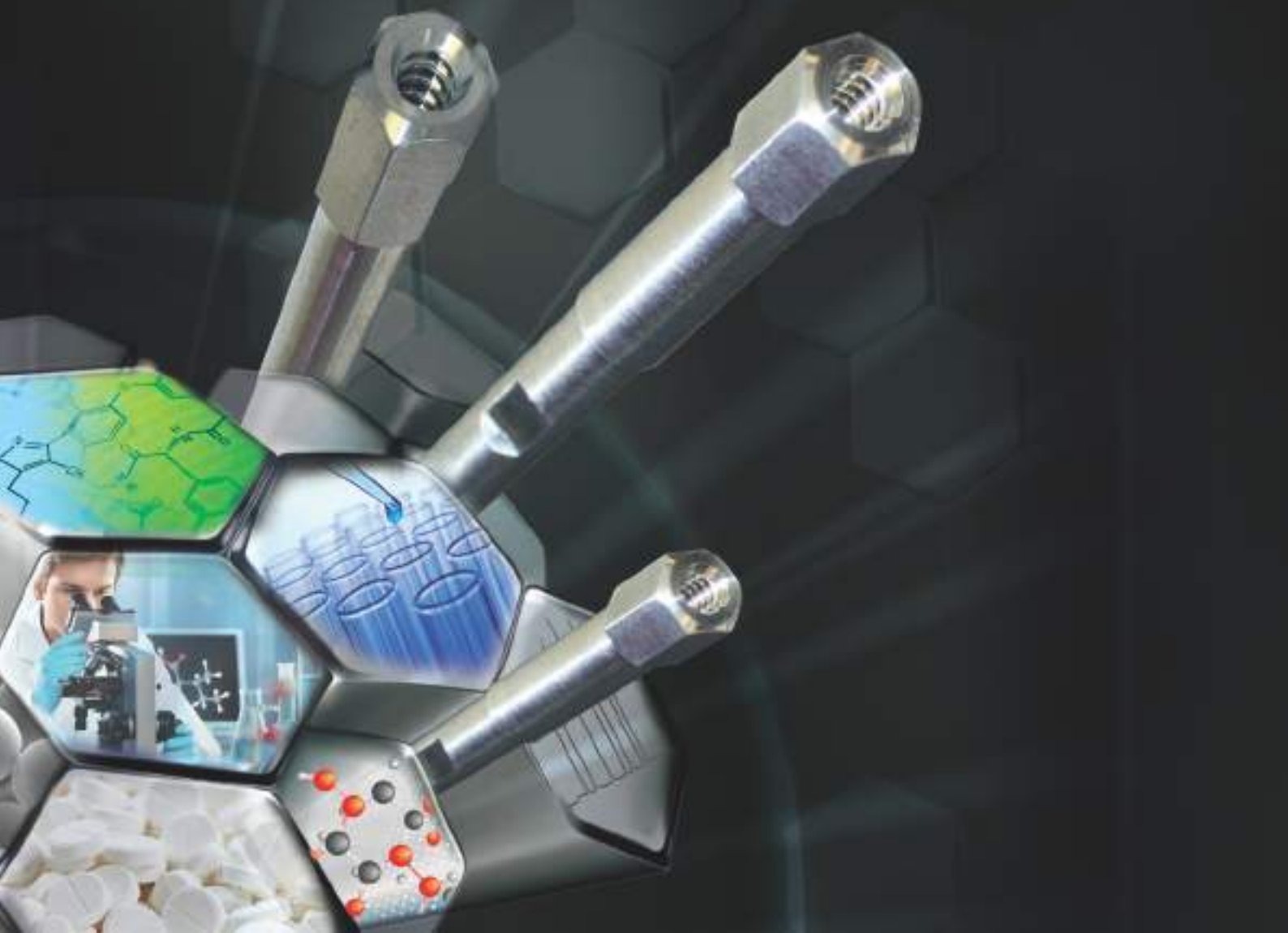
# HICHROM

Chromatography Columns and Supplies

**INTERNATIONAL CATALOGUE 9**



# INTRODUCTION

Based in the UK and founded in 1978, Hichrom is dedicated to supplying the highest quality HPLC and UHPLC columns and other chromatography products to the scientific community. As a leading global LC column manufacturer, we have gained an unrivalled reputation for excellent quality, competitively priced products and fast delivery – all backed up with expert technical support and after-sales service. Our worldwide network of distributors currently supplies Hichrom columns to laboratories in over 100 countries.

In addition to our own manufactured products, Hichrom is a distributor for many of the major HPLC column suppliers, including Advanced Chromatography Technologies, Advanced Materials Technology, Akzo Nobel, CERI, Chiral Technologies, Dikma, Eprogen, ES Industries, GL Sciences, Grace, Macherey-Nagel, Merck Millipore, MicroSolv, Mitsubishi, Nacalai Tesque, Nomura, PolyLC, Princeton, Regis, RStech, Shinwa, Shodex, SIELC, Thermo Scientific, Tosoh Bioscience, Waters, YMC and ZirChrom.

We also supply an extensive range of SFC, GC, SPE, TLC, Flash, CE, LC-MS and GC-MS products. Our technical staff will be happy to respond to any customer enquiries - please see pages 6 to 9 for further details of Hichrom technical services.

HICHROM Manufactured Columns
ISO 9001 and ISO 14001

**Hichrom Manufactured Columns -** We guarantee that any HPLC or UHPLC column produced by Hichrom will be manufactured to the highest possible quality standards, using only the original manufacturers' silica material. Please contact us for details of Hichrom manufactured columns not found in this catalogue. Details of the original silica manufacturers can be found in the appropriate material section. For further details of Hichrom's column packing and custom packing services, please see page 8.

**Quality and Environmentally Assured -** Hichrom aims to supply the highest quality products to the chromatography industry. We are an ISO 9001:2008 accredited company and have been accredited since 1994. We also aim to minimise the impact of our business on the environment. We are an ISO 14001:2004 accredited company and have been accredited since 1996.

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**Environmental Statement -** This catalogue is printed on paper sourced from environmentally aware forest product companies, and the pulp used in the manufacture of the paper is sourced from responsible forest resources. The paper used in this catalogue is a certified FSC® grade. The pre-press process is 100% digital, which means that no film or any of the associated chemicals are used. No optical brighteners are used on the paper. This catalogue is printed on chlorine-free paper. Please recycle this catalogue where possible.

**Despatch -** The majority of Hichrom manufactured columns are available on a 24 hour despatch basis. Other items may vary by supplier and product type, but we will always despatch goods in an efficient and timely manner. Goods can be despatched by express carrier (subject to geographical location). An additional delivery charge may apply

**Prices and Terms -** Every effort is made to keep prices stable, although, owing to our policy of continuous product development and the wide variety of products contained in this catalogue, we reserve the right to change prices and specifications without notice. Please contact Hichrom or our distributors for a current price on any item. Further details of Hichrom's Terms and Conditions of Sale can be found on page 228 of this catalogue.

**Warranty -** All Hichrom manufactured products are under warranty against defects in material or workmanship. We will promptly replace any such goods unless the defects are attributed to customer misuse.

**Damaged and Returned Goods -** Notification of goods damaged in transit or order discrepancies must be received in a timely manner. See Hichrom's Terms and Conditions of Sale on page 228. We will endeavour to assist with customer returns if possible but all returns are at Hichrom's sole discretion. A handling charge may be applicable. It is always important to contact us before sending any goods back. We will provide you with relevant returns information/numbers and, where appropriate, any decontamination certificates.

## Contact Information

Please contact us if you have any questions about our products or services.


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## LC COLUMNS (continued)



## GAS CHROMATOGRAPHY



## SAMPLE PREPARATION

## LC CONSUMABLES and ACCESSORIES



## APPENDICES

## ACE® UltraCore™

- Ultra-inert solid-core UHPLC/HPLC columns
- 2.5 and 5µm particle sizes
- SuperC18™ and SuperPhenylHexyl™ phases
- Extended pH stability (1.5 – 11.0)


ACE® UltraCore™ is a new range of ultra-inert UHPLC/HPLC solid-core (superficially porous) 2.5 and 5µm particle columns from Advanced Chromatography Technologies Limited. The monodisperse particle distribution of these phases combines high efficiency and performance with a low column back pressure. ACE UltraCore is available with SuperC18™ and SuperPhenylHexyl™ bonding. Both phases feature proprietary Encapsulated Bonding Technology (EBT™), which results in excellent peak shape and phase stability across an extended pH range of 1.5 to 11.0. Encapsulated Bonding Technology dramatically increases the ligand coverage of the silica surface and effectively eliminates the effect of unbonded silanol groups. This higher ligand coverage results in improved inertness, chromatographic performance and stability. These phases enable selectivity to be exploited at low, intermediate and high pH for rapid, systematic method development, with all the efficiency, robustness and lower pressure attributes of solid-core columns.

Please contact Hichrom for ordering details of ACE UltraCore columns.

## RAPID Sealing Products for 96 Well Plates

- Ideal for LC-MS applications
- Minimise sample contamination from adhesives
- Minimise needle blockage

Micro well plates are widely used in the pharmaceutical and biotech industries for storage, assays and analysis. Ideally, plates should be covered to prevent contamination, cross contamination between wells and solvent evaporation.

Bio Chromato, Inc of Japan manufactures the RAPID range of seals for 96 well plates. RAPID EPS and RAPID Slit Seals minimise problems of needle blockage and contamination from adhesives. Both seals are ideal for LC-MS applications and will maintain a seal even down to -80°C.

**RAPID EPS** (**E**asy **P**iercing **S**eal) seals are designed for sealing the surface of multiple well plates including 96, 384 and 1536 well plates, as they are available in sheet or roll format. The seal material is a polyolefin and uses synthetic rubber adhesive instead of silicon adhesive. No contamination of adhesive into samples occurs and no residual adhesive remains on the plates after removing the seals. A key feature of these seals is their high resistance to organic solvents such as methanol, acetonitrile and DMSO. RAPID EPS seals have an embossed surface for easy piercing.

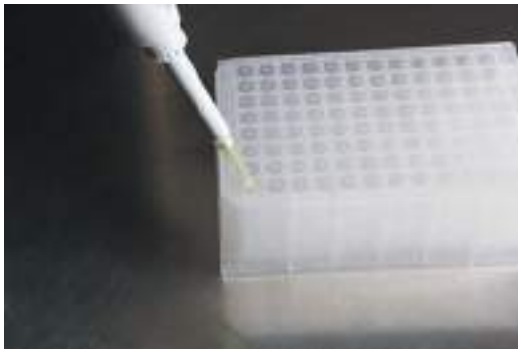
Rapid EPS

The unique **RAPID Slit Seals** are designed for sealing the surface of 96 well plates. The seal material consists of a triple layer structure of PET film/silicon rubber/PET film. It is adhesive free on the well position to prevent needle blockage. The seals have a self closing function after piercing to minimise solvent evaporation, enabling multiple penetrations to be performed without damaging the sealing of the plate.

### Product Specifications

| | RAPID EPS | RAPID Slit Seal |
|---|---|---|
| Material | Polyolefin | Polyethylene, silicon adhesive |
| Applicable well plates | 96, 384, 1536 well plate | 96 well plate |
| Operating temperature | -80°C to 80°C | -80°C to 37°C |
| Organic solvent compatibility | Yes | No |
| Sterile condition | Not sterile | Not sterile |
| Genetic cleanliness | Not DNA free | Not DNA free |

Please contact Hichrom for ordering details of RAPID sealing products.

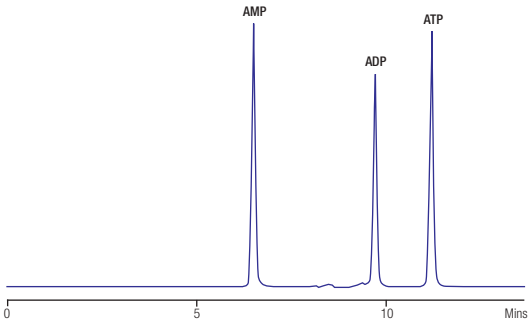

Rapid Slit Seal

# NEW PRODUCTS (continued)



## MicroSolv Cogent UDA™

Cogent UDA™ is a new unique selectivity phase based on MicroSolv's TYPE-C Silica and silica hydride surface chemistry. This silica hydride surface is bonded with an eleven carbon chain terminating in a carboxylic acid (undecanoic acid), giving the phase weak cation-exchange properties in addition to aqueous normal-phase (ANP). All the features and benefits of TYPE-C Silica, such as fast equilibration, precision, multi-mode and robust column-lifetime are maintained. The Cogent UDA columns have applications in metabolomics and have been used for the analysis of nucleotides. The figure below shows the separation of energy adenine nucleotides on a Cogent UDA column. These three nucleotides are separated in order of increasing polarity, as expected when ANP chromatography is used.




Column: Cogent UDA (4µm, 50 x 2.1mm)
Eluent: A: 16mM ammonium acetate in $H_2O$
B: $CH_3CN$ – 16mM ammonium acetate in $H_2O$

| Gradient: Time (mins) | %B |
|---|---|
| 0 | 95 |
| 0.5 | 95 |
| 10 | 70 |
| 15 | 30 |
| 20 | 30 |
| 20.1 | 95 |

Flow rate: 0.4ml/min
Temperature: 25°C
Detection: UV, 254nm

Please contact Hichrom for ordering details of Cogent UDA columns.

## RStech Corporation Products

RStech Corporation of Korea manufactures the OPTIMAPAK® and HECTOR ranges of HPLC columns and the INOPAK range of SPE columns. OPTIMAPAK columns are available in C18, C8, $NH_2$ and Silica chemistries. The newer HECTOR HPLC column ranges include several series of bonded phases, offering the customer a wide choice of selectivities.

HECTOR columns are designed for HPLC, LC-MS, SFC and SMB. All phases have low levels of metal impurities and are endcapped for reproducibility and durability. Columns are available packed with 3, 5 and 10µm particles and with microbore to preparative dimensions. HECTOR columns are suitable for a variety of applications in the pharmaceutical, chemical, environmental and food separation areas.

The **HECTOR** product line-up includes five different ranges:

**HECTOR-M** phases (100Å) are monomerically bonded and available with C18, C8, C4, $NH_2$, Diol, CN, Phenyl and Sil chemistries.
**HECTOR-A** C18 and C8 phases (100Å) contain a hydrophilic functional group, enabling them to be compatible with highly aqueous eluents and provide additional retention for polar compounds.
**HECTOR-T** has trifunctional C18 bonding (100Å), which confers a higher acid and base durability than monofunctional C18 phases.
**HECTOR-W** is a 300Å wide-pore silica available with C18, C8, C4 and $NH_2$ bonding, for the separation of peptides, proteins and oligonucleotides.
**HECTOR-ACD** ion-exchange phases (WCX and SCX) are specifically designed for the separation of acidic compounds.

INOPAK SPE columns have a wide range of applications, each showing high selectivity and enhanced recovery. They offer reproducible results and the potential for automation. INOPAK sorbents include C18, C8, $NH_2$, Silica and Florisil packed in 1, 3 and 6ml syringes.


Please contact Hichrom for ordering details of any RStech product.

# NEW PRODUCTS (continued)



## Coresep™ Mixed-mode Core-Shell Columns

- Silica based core-shell phases with mixed-mode bonding
- 2.7µm particles with 90Å pore size
- LC-MS compatible eluents
- 3 bonded phases

SIELC Technologies has developed Coresep™, a new generation of unique stationary phases which combine mixed-mode bonding and core-shell technologies. Three different bonded phases have been developed, based on the mixed-mode bonding technology of the established Primesep and Obelisc ranges. Mixed-mode columns offer higher capacity and retention than traditional reversed-phase columns. Since the surface area of core-shell particles is reduced, mixed-mode phases address potential loadability issues whilst maintaining the selectivity advantages of multiple interactions. Optimised ligand density and ionic/hydrophobic ratios further increase the capacity of mixed-mode core-shell columns.


**Coresep 100** is a reversed-phase core-shell phase with embedded acidic ion-pairing groups. It can show an improved retention of basic compounds by a cation-exchange mechanism. In addition, it can separate acids by ion-exclusion. Figure 1 shows the separation of a mixture of 7 hydrophilic and ionic compounds on Coresep 100. All compounds have low hydrophobicity, and adequate retention on a reversed-phase column is difficult to achieve. Using the reversed-phase and ion-exchange mechanisms of Coresep 100, retention and complete separation of all components was achieved within 5 minutes.

**Coresep S** is a normal-phase core-shell material with embedded acidic ion-pairing groups. It is particularly suitable for separation of common sugars and other neutral or very polar molecules. Weak bases can be separated by an ion-exchange mechanism, whilst acids are separated by ion-exclusion.

**Coresep SB** is a reversed-phase core-shell material with embedded strong basic ion-pairing groups. It shows improved retention of acidic compounds by an anion-exchange mechanism and separates bases by ion-exclusion.

Please contact Hichrom for ordering details of Coresep columns.

Column: Coresep 100 (2.7µm, 50 x 3.2mm)
Eluent: A: 30mM amm formate, pH 3.5 – $CH_3CN$ (90:10)
B: 70mM amm formate, pH 3.5 – $CH_3CN$ (35:65)
Gradient 100% A to 100% B over 5 mins
Flow rate: 1.2ml/min
Detection: UV, 270nm

1. Adenosine
2. 3,4-Difluoroaniline
3. 4-Amino-2-chloropyridine
4. 4-Amino-3-chloropyridine
5. 5-Aminoindole
6. 2-Amino-5-methylthiadiazole
7. 4-Ethylaniline

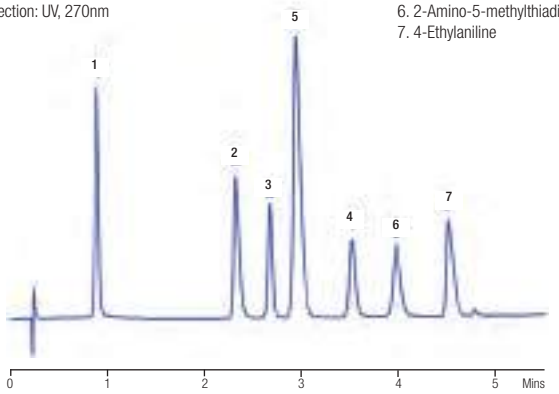



Figure 1. Separation of mixture on Coresep 100

## MCI GEL™ Mixed-mode Column

MCI GEL™ CHK40/C04 is a new mixed-mode column manufactured by Mitsubishi Chemical Corporation. This is a low capacity cation-exchange resin based on 4µm particle size sulphonated poly(ethylstyrenedivinylbenzene) copolymer, with separations based on both hydrophobic and hydrophilic interactions. Mitsubishi Chemical Corporation have found that the column gives high performance for polar compounds, including amino acids, nucleic acids and small peptides.

Please contact Hichrom for ordering details of MCI GEL mixed-mode columns.

# NEW PRODUCTS (continued)

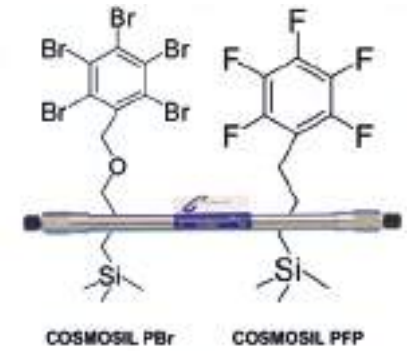


## COSMOSIL® PFP and PBr HPLC Columns

Nacalai Tesque have launched COSMOSIL® 5PFP, a pentafluorophenyl-bonded HPLC column with alternative selectivity to C18 columns. In addition to a hydrophobic separation mechanism, the phase offers dipole-dipole and π-π interactions. COSMOSIL 5PFP shows good retention for compounds with strong dipole moments, such as cationic or halogenated compounds, and exhibits high steric selectivity for structural isomer separations. Columns with analytical to preparative dimensions are available.

The new COSMOSIL PBr column is a pentabromobenzyl bonded phase designed for the reversed-phase separation of polar compounds that may not require HILIC separation conditions. Separations are achieved using a combination of hydrophobic and dispersion forces.

Please contact Hichrom for ordering details of COSMOSIL PFP and PBr columns.



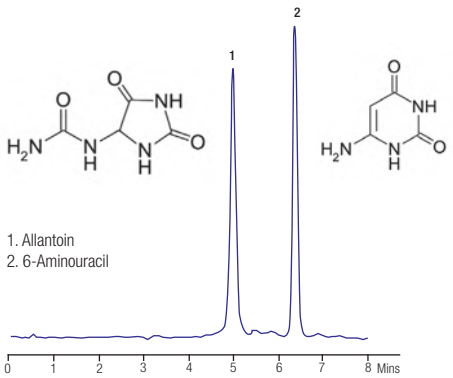

## MicroSolv Cogent Diol™

Cogent Diol™ is the latest addition to the MicroSolv TYPE-C Silica™ range of stationary phases. This 4µm diol bonded phase is suitable for the reversed-phase or aqueous normal-phase (ANP) analysis of polar compounds and is LC-MS compatible. Columns show fast equilibration between gradient runs and can be successfully used in studies of pathways in human pathology. The figure below shows the LC-MS separation of uric acid metabolites in human urine. Allantoin and 6-aminouracil are used as biomarkers for different disease states. Their peaks are well separated and the use of formic acid in the eluent ensures good MS sensitivity.

Column: Cogent Diol (4µm, 150 x 3.0mm)
Eluent: A: 0.1% Formic acid in $H_2O$
B: 0.1% Formic acid in $CH_3CN$

| Gradient: Time (mins) | %B |
|---|---|
| 0 | 95 |
| 1 | 95 |
| 6-7 | 30 |
| 8 | 95 |

Flow rate: 0.4ml/min
Detection: ESI (+)




Please contact Hichrom for ordering details of MicroSolv Cogent Diol columns.

# MISSION STATEMENT

Hichrom aims to supply the highest quality HPLC columns and consumable products to the chromatography industry. We measure the quality of our service not only in the product itself but in the nature of our technical consultancy and after-sales service. In doing so we aspire to achieve complete customer satisfaction and make an important contribution to the future of the life sciences and related chemical industries.

## Quality and Traceability – ISO 9001:2008

We believe that quality assurance is fundamental to our operations at every level, and in 1994 we gained official recognition with the award of ISO 9001 accreditation. Every Hichrom manufactured column is supplied with documentation which enables a full audit trail to be performed from the point of manufacture to the time of use.

## Environmental Awareness – ISO 14001:2004

Hichrom also places a great emphasis on environmental awareness, and in 1996 became the first chromatography company to receive approval of an ISO 14001 Environmental Management System. In doing so it adheres to the Control of Substances Hazardous to Health regulations (1998), its amendments and other related legislation.

## Column Sales Policy

We offer very high performance, competitively priced, pre-packed liquid chromatography columns of guaranteed efficiency. Every column is individually tested prior to despatch and only those which meet Hichrom's strict specifications are accepted. To maintain this high quality we utilise only selected batches of raw materials. We guarantee to replace any column which does not give full customer satisfaction.

## Column Reproducibility

By purchasing raw materials in as large a batch size as possible and checking selectivity where appropriate, Hichrom endeavours to minimise batch-to-batch variation. We are also happy to supply columns from customer specified batches of packing material.

## Column Despatch

The majority of columns are available on a 24-hour despatch basis. Columns of non-standard dimensions, columns packed with alternative material or those for use with specific instrumentation can be supplied on request. Telephone, fax or email orders are welcome.

## Column Durability

Each type of column we manufacture has been tested for durability over a 48-hour period. After initial evaluation, a typical eluent is passed through the column for a 48-hour period and its performance re-measured. A column is only considered satisfactory if it maintains its original performance.

# TECHNICAL SUPPORT

## Column Supply and Sourcing Solutions

Hichrom Limited offers a complete column sourcing solution for any laboratory. We supply many of the world's leading column brands and work closely with the key manufacturers. Additionally, we have excellent relationships with a wide number of the less well known column producers and are experts at sourcing some of the more 'obscure' column brands from all over the globe. Furthermore, if your specific column requirement is no longer available, for whatever reason, we will be happy to recommend a suitable alternative to meet your needs.

## Column Lifetime Advice

Column lifetime is an important factor for any analyst and it pays to make sure that you are getting the longest possible lifetime for your column and application. No column lasts forever but the additional cost and inconvenience of replacing a column too frequently is something to be avoided. The quality and aqueous stability of HPLC silicas have improved over time. However, the inherent chemical instability of bonded phases at low pH and the dissolution of silicas at high pH, remains an underlying problem for all chromatographers. Hichrom offers advice and support on all column lifetime issues, including the use of modified silica technologies, column protection, column storage and column usage conditions.

## Troubleshooting Support

Hichrom's expert Technical Support team are available to provide useful advice and support on any HPLC/UHPLC troubleshooting issue. Whether you have questions or problems with method reproducibility, impurity peaks, sample cleanliness, method transfer, scale-up or any other area of chromatography, our experts are available by phone or email with a quick and helpful response.

## Column Selection Guidance

There are numerous HPLC silica based column ranges and phases available for today's chromatographer to select from. The options are further increased when alternative base materials (polymer, titania, zirconia, graphite etc.) and technologies (UHPLC, superficially porous, hybrid, monolithic, Type-C, mixed-mode etc.) are considered. Hichrom scientists are familiar with the huge range of column brands and technologies available and are able to provide informed advice on the best column choices for your methods. We maintain a comprehensive library of general and manufacturers' literature, both current and historic. Our practical experience in manufacturing and evaluating our own columns, together with those of many other manufacturers, ensures that we always have extensive and up-to-date experience of column selection. Simply contact our technical group for valuable, time-saving, free of charge advice.

## SFC, GC, SPE, TLC, Flash, CE, LC-MS, GC-MS and New Developments

Hichrom offers free technical support and advice in all other areas of chromatography including SFC, GC, SPE and other sample preparation techniques (eg. SLE, MMSE, pipette tips), as well as TLC, flash chromatography, capillary electrophoresis and hyphenated techniques such as GC-MS, LC-MS and LC-MS/MS. We are also well placed to keep you up to date with the latest developments in the industry, whether that's a new technique or a new product.


**For professional technical advice and assistance:**

Tel: +44 (0) 118 930 3660 Fax: +44 (0) 118 932 3484
Email: technical@hichrom.co.uk www.hichrom.co.uk

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Theale, Reading, Berkshire, RG7 4PE, United Kingdom

# APPLICATION & METHOD DEVELOPMENT SUPPORT

## Application Support

Hichrom has an extensive in-house reference database of HPLC and UHPLC applications, covering a large number of analytes, separated on a wide range of column materials. This database includes applications related to chiral analyses. We also have access to a vast number of additional applications and method recommendations through our extensive supplier and partner network. In addition to HPLC and UHPLC applications, we also offer application support on SFC (both chiral and achiral), as well as GC, CE and SPE methods.

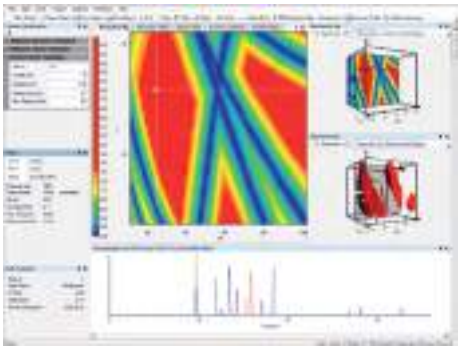
Applications cover a broad range of interest areas including biochemical, food, ion analysis, organics, environmental, pharmaceutical and polymers to name a few. Searches can be performed on individual compounds, a mixture of components or a specific column. With this resource we are able to offer useful advice and information to aid you in the selection of a suitable column, or for identifying a suitable alternative column, for any given assay.

## Method Development and Optimisation Strategy

With our extensive experience of columns, column technologies and HPLC methods, and utilising resources such as our applications database, Hichrom technical staff are able to discuss your method requirements with you, to offer useful advice and direction for your specific assay. This includes suggesting appropriate method development strategies, suitable LC conditions (including buffer, pH and temperature), column types and specifications, starting points, robustness concerns and resolution criteria. If you are looking to speed up your method, or convert from HPLC to UHPLC or superficially porous technology, we can provide useful information on how best to do this and the pitfalls to avoid. If you have an existing method but need to further optimise it, our experts will be able to offer you the support and assistance you need.

## DryLab Computer Aided Method Development

Hichrom offers the DryLab computer aided method development software, which enables you to develop better, more robust HPLC/UHPLC methods, faster and more efficiently. It also allows you to optimise or modify existing HPLC methods for successful transfer to alternative UHPLC/HPLC equipment. DryLab is so powerful, that from just 12 practical runs in the laboratory, you can model up to 1,000,000 possible chromatographic solutions, saving you a huge amount of time and money. A new robustness module additionally allows you to simply and quickly explore the boundaries of robustness, cumulatively or independently, for all specified parameters. Please see page 206 for additional details or contact us to discuss DryLab method development software further.




## Column Screening Services

As the availability of HPLC silicas and phases increases, selection of the optimum column for a particular assay is becoming an increasingly difficult and time consuming task. Hichrom maintains an extensive HPLC column library and has valuable expertise in the area of column screening and column comparisons. As such we are well placed to advise you on the selection of the optimum phase for your assay.

Working with our partners we also offer a number of column screening options (both chiral and achiral), where your sample can be run on a number of different column options in order to establish the ideal column for the specific application. Confidentiality is guaranteed and confidentiality agreements are offered to support this.

**For application and method development support:**

Tel: +44 (0) 118 930 3660 Fax: +44 (0) 118 932 3484
Email: technical@hichrom.co.uk www.hichrom.co.uk

Hichrom Limited, 1 The Markham Centre, Station Road,
Theale, Reading, Berkshire, RG7 4PE, United Kingdom

# COLUMN PACKING AND CUSTOM PACKED COLUMNS

## Extensive Range of Dimensions and Silicas

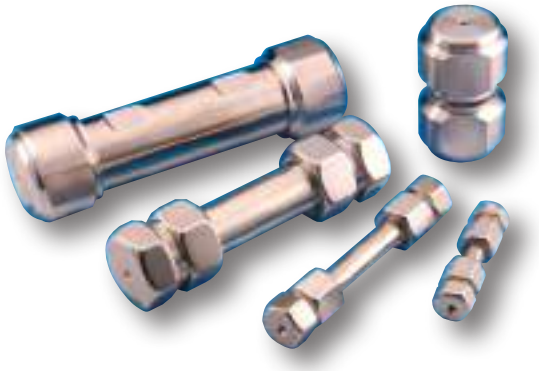
Based in the UK, Hichrom is a leading global manufacturer of HPLC and UHPLC columns. We offer unrivalled experience in the manufacture of superior quality HPLC columns, in an extensive range of dimensions from capillary to preparative. We are also able to pack columns from any commercially available material. If you cannot find the column dimensions/silica you require in this catalogue, please contact our technical team who will be happy to check availability and discuss the option of a custom manufactured column. We are highly experienced in the manufacture of unique dimension/silica columns for specific applications and are able to offer many products that are not listed in this catalogue.


## Customer Supplied Packings

In addition to the wide range of materials listed in this catalogue, Hichrom can also manufacture columns from any commercially available material and pack columns from customer supplied materials. Please contact us to discuss this service. We will be pleased to sign a confidentially agreement if required.

## Batch Reservation Service

Hichrom recognises that column-to-column reproducibility is of the utmost importance and all Hichrom columns are manufactured to very stringent quality specifications. Additionally, column manufacture is optimised on the largest batch size of silica available. However, for particularly searching applications, Hichrom also offers a no charge batch reservation service which completely eliminates any batch related reproducibility concerns. Based on your projected column usage (large or small), Hichrom will reserve the quantity of silica you require and pack columns from this material as and when you require them.

**For details of our column packing service:**

Tel: +44 (0) 118 930 3660 Fax: +44 (0) 118 932 3484
Email: technical@hichrom.co.uk www.hichrom.co.uk

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John Dolan

Mel Euerby

## Keep Up-to-Date with the Latest Developments

Our experts are at the forefront of developments in the chromatography industry and will always ensure that the very latest information is available to you. We specialise in providing scientists with the chromatography skills and knowledge they need to make efficiency gains and productivity improvements in the laboratory. Whether you are a relative newcomer to HPLC or a seasoned veteran, you can benefit from one of our courses.

## Full Day and Short 1-2 Hour Chromatography Courses

Hichrom offers a wide range of chromatography courses to suit everyone's needs and budgets. Full one and two day courses provide the ideal environment for those who need to develop a thorough understanding of a topic in as short a time as possible. Shorter 1-2 hour seminars are ideal learning experiences for busy chromatographers who simply want to brush up on their skills or to develop experience in a new field or topic.

## Cost Effective

We are confident that the techniques you will hear about on our courses will provide you with many ideas for improving efficiency in the laboratory – in fact we guarantee it! If after six months you have not been able to recover the registration fees by applying what you have learnt from the course, we will happily refund your money.


## IBMS CPD Accreditation, RSC and ChromSoc Members

All our listed courses are IBMS CPD accredited and certificates will be issued to delegates on request. Additionally IBMS, RSC and ChromSoc members all receive preferential rates on our courses.

## Request a copy of our latest HPLC Workshops Training Book

For full details of all our courses simply request a copy of our latest HPLC Workshops Training Book at seminars@hichrom.co.uk.

### Full day courses include:

An Introduction to HPLC
Stepwise Introduction to 'Practical' HPLC
HPLC and UHPLC Troubleshooting
Making HPLC Methods Work
Transferring HPLC Methods to UHPLC
HPLC Method Development
Advanced HPLC Method Development
Validating HPLC Methods
LC-MS for Chromatographers
Big Molecules – Big Challenges
Advances in Chiral Analysis
An Introduction to Gas Chromatography
GC Troubleshooting
GC Method Development

### Short courses include:

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# COLUMN EVALUATION



Hichrom columns are rigorously tested prior to sale. Every column is supplied with a unique chromatogram corresponding to the column purchased (Figure 1). Efficiency measurements and two peak asymmetry calculations are recorded for each component of the quality control test mixture.

Details of the Hichrom quality control test mixtures are available on request. Information regarding the care and use of Hichrom columns is displayed on the reverse of every chromatogram.

It is recommended that the performance of a column is tested on arrival and periodically during use. A column performance history enables the chromatographer to readily decide the most suitable time for column replacement or regeneration. Performance parameters are as defined below.

The peak asymmetry factor determined by ratioing plate efficiencies at 10% and 50% peak height is more sensitive to 'tailing' and 'fronting' effects than the conventional method using peak half-width ratios and therefore a better test of column performance.

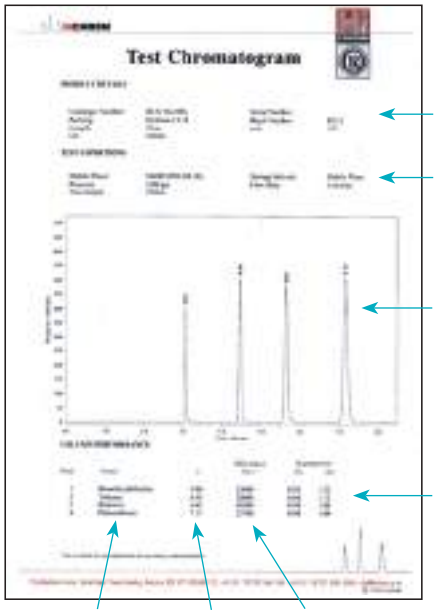
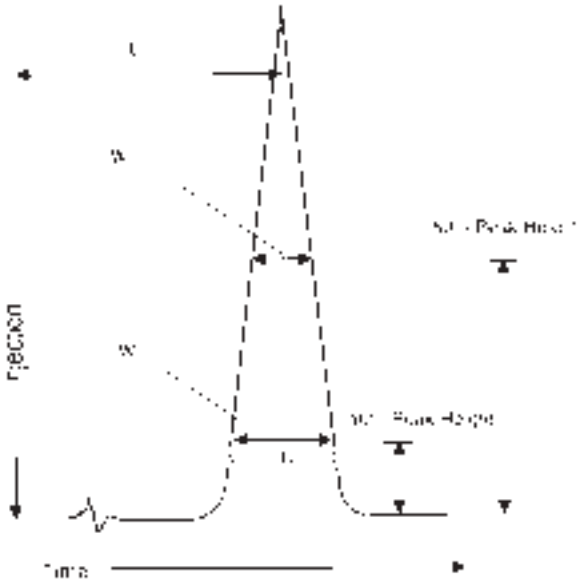



Figure 1. Test chromatogram

## Asymmetry and Efficiency Measurements



# ANALYTICAL AND MEDIUM BORE COLUMNS



Traditionally, columns of either 4.6 or 4.0mm i.d. and 150 or 250mm length have been used as the industry's standard for analytical applications. Such columns are still the most popular, although there is an on-going transition to shorter and narrower i.d. columns. The increased application of hyphenated techniques, such as LC-MS and LC-MS/MS, capable of providing analyte characterisation data, has necessitated the use of lower solvent volumes and hence smaller columns. Increased assay sensitivity and decreased sample requirements have also led to the use of smaller i.d. columns, including 3.2mm i.d. medium bore columns.

## Silica

Silica is the most popular base material for HPLC phases. Despite its necessary porosity, it has a high physical strength and a surface to which a broad range of ligands can be attached using well established silanisation technology. The majority of separations are performed using these bonded materials under reversed-phase conditions.

Spherical silica particles are easier to pack than irregular particles and enable high performance, stable and low back pressure columns to be reproducibly obtained.

## Performance

The efficiency of a column depends on the choice of particle size and column length. The mode and quality of manufacture will also affect its performance. Particle size refers to the average diameter of the spherical silica particle. Commonly used silicas have a distribution of diameters; hence a material of nominal particle size 5µm can typically contain silica particles between 4.0 and 6.5µm diameter (Figure 1).

Smaller particles give higher efficiencies for constant column length, but also give higher column back pressure, resulting in an effective maximum column length when used with conventional HPLC systems.

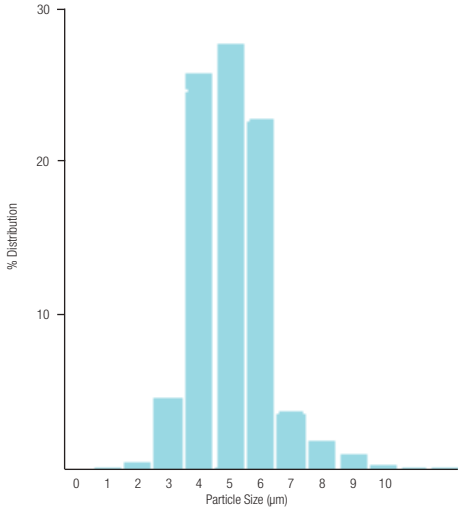



Figure 1. Typical silica particle size distribution

## Silica Phases

| Silica Particle Size[1] (µm) | Maximum Recommended Column Length (mm) | Plate Efficiency (plates/metre) | Use of Columns |
|---|---|---|---|
| 3 | 150 | 120-200,000 | Fast analysis without loss of efficiency or sacrificing column life |
| 5 | 250 | 80-120,000 | Routine analysis requiring a high separation performance under moderate operating pressure |
| 10 | >250 | 40-60,000 | Moderate efficiency at low operating pressures. Routine QC labs or as scout column for scaling-up methods |
| >10 | >250 | <40,000 | Primarily for preparative applications |

[1] For ≤2µm UHPLC columns, please see page 15

## Medium Bore Columns

For analysts who do not have access to more modern, low dispersion volume HPLC equipment, intermediate i.d. medium bore columns (3.2mm i.d.) represent a compromise between using standard 4.6mm i.d. columns and microbore columns of 2.1mm i.d. or less.

Medium bore columns offer a 50% saving in solvent consumption for the same linear dynamic flow without necessarily requiring a change to lower dispersion volume injectors and flow cells. This reduces costs and environmental issues associated with solvent disposal. In practice chromatograms obtained from 3.2 and 4.6mm i.d. columns with the same linear dynamic flow are very similar (Figure 2). For satisfactory use of 2.1mm or lower i.d. columns, systems need to be adjusted for system dead volume (see page 12).

A flow rate of 0.76ml/min through a 4.0mm i.d. and 0.48ml/min through a 3.2mm i.d. column gives the same relative flow rate as 1ml/min through a 4.6mm i.d. column.

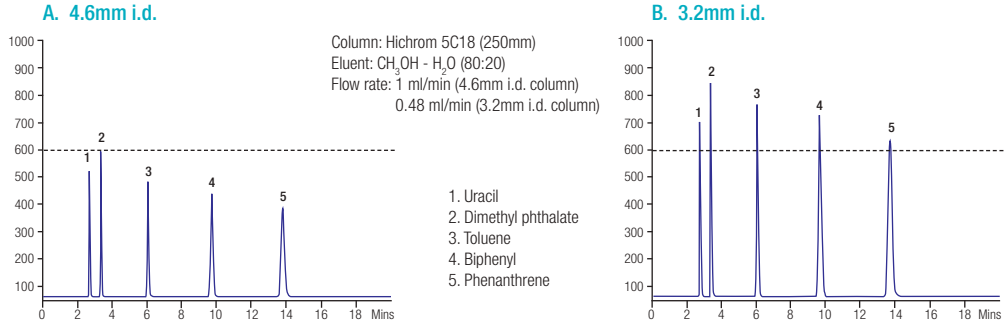



Figure 2. Comparison between 4.6mm and 3.2mm i.d. columns

# MICROBORE (2.1 AND 1.0mm i.d.) COLUMNS

- Solvent consumption reduced by 80-95%
- Sensitivity increased x5 to x21
- Significant cost-saving
- LC-MS applications

Microbore columns (1.0 and 2.1mm i.d.) offer significant theoretical advantages over conventional 4.6mm i.d. analytical columns. Hichrom currently offer a comprehensive range of 1.0 and 2.1mm i.d. microbore columns, which offer minimal loss in performance compared to the corresponding analytical columns and can be used with optimised conventional equipment.

## Comparison of Microbore and Standard Column Parameters

| Column[1] i.d. (mm) | Internal Volume (µl) | Relative Flow Rate (ml/min) | Solvent Reduction (%) | Theoretical Sensitivity Increase | Recommended Flow Cell (µl) | Injection Volume (µl) |
|---|---|---|---|---|---|---|
| 4.6 | 1500 | 1.0 | - | - | 8.0 | 20 |
| 4.0 | 1133 | 0.76 | 24 | 1.3 | 8.0 | 15 |
| 3.2 | 725 | 0.48 | 52 | 2 | 2.0-8.0 | 10 |
| 2.1 | 300 | 0.20 | 80 | 5 | 1.0-2.0 | 5 |
| 1.0 | 71 | 0.047 | 95 | 21 | ≤1.0 | 1 |

[1] 150mm length

## Column Evaluation

For optimum performance of microbore columns, HPLC system dead volume must be minimised. If the calculated theoretical plate value is less than 90% of that measured by the manufacturer, there is probably an excessive amount of extra column volume in the system for the column being used. Early eluting peaks are less diluted and have smaller volumes than later eluting peaks and will consequently be more susceptible to the detrimental effects of excessive extra column volume.

Several measures can be undertaken to minimise the effect of extra column volume.

- Use connecting tubing that has an internal diameter of 0.010˝ (0.254mm) or less. Tubing with an i.d. of 0.007˝ (0.177mm) or lower is preferable for use with microbore columns (see Table 1).
- Keep the connecting tubing as short as possible between the injector and the column, and the column and the detector. For microbore columns, it is desirable to keep these distances less than 5cm.
- Use only 'low dead volume' fittings and unions. A fingertight column coupler (throughbore 0.007˝) HI-081 is recommended to connect guard and microbore columns (see page 20).
- Use a low volume (microflow) detector flow cell (≤2.0µl).

### Table 1. Connection Tubing Internal Volumes

| Tubing i.d. (inches) | Volume per 5m length (µl) |
|---|---|
| 0.006 (0.152mm) | 0.9 |
| 0.010 (0.254mm) | 2.5 |
| 0.020 (0.508mm) | 10.1 |
| 0.030 (0.762mm) | 22.8 |

Figure 1 illustrates what can happen when a microbore column is used with a typical HPLC system. The microbore column (B) is unable to achieve the baseline separation provided by the 4.6mm i.d. column (A), unless the HPLC system is 'optimised' (C). In the latter case, the optimised system has less than 10µl of extra column volume, so excellent resolution and peak shape can be obtained.

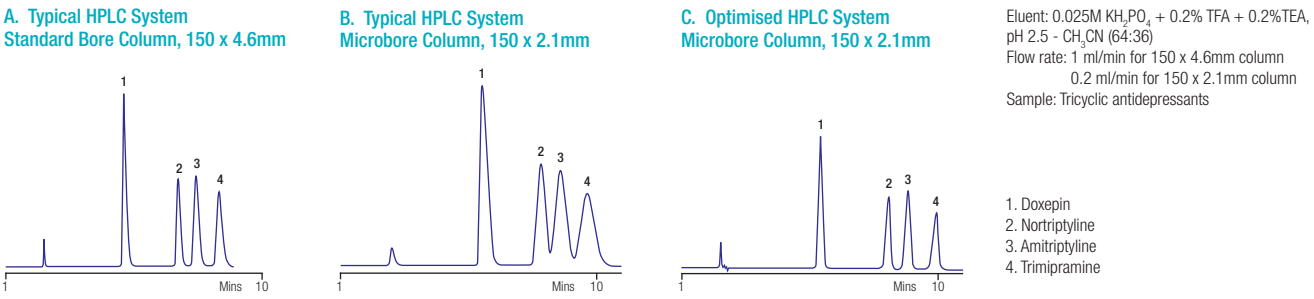



Figure 1. HPLC systems must be optimised to obtain the best performance from microbore columns

# CAPILLARY AND NANO (<1.0mm i.d.) COLUMNS



- High sensitivity
- Low sample mass and volume applications
- LC-MS and LC-MS/MS applications
- Very low solvent consumption

Capillary and nano HPLC are gaining acceptance in applications where limited sample amounts lead to problems in detection sensitivity. High sensitivity and high resolution separations can now be achieved for small sample volumes. This is relevant in the areas of pharmacokinetics, trace analysis and particularly the rapidly expanding field of bioanalytical and proteomic analysis. Figure 1 shows the analysis of a protein mixture digest on a nano column.

Capillary and nano columns are ideal for use with detectors requiring the lowest flow rates, such as the electrospray LC-MS detector. Indeed, the on-line coupling with a mass spectrometer has been a major driving force behind the development of capillary chromatography.

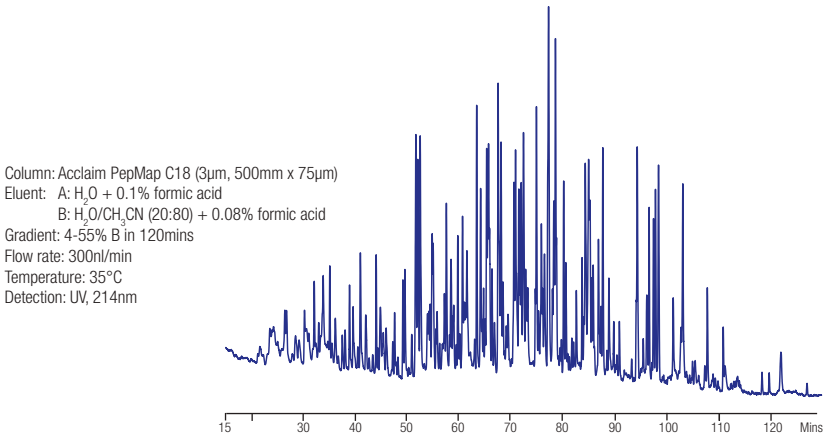



Figure 1. Protein mixture digest

## Sensitivity

The introduction of capillary and nano columns has made possible high sensitivity and high resolution separations for small sample volumes. Table 1 shows the theoretical sensitivity increase of various i.d. capillary columns compared with a 1mm i.d. microbore column. The use of a 0.075mm (75µm) i.d. column, in theory, can decrease detection limits by a factor of >3500 relative to a 4.6mm i.d. column when the same sample size is used, due to lower chromatographic dilution of the sample.

Table 1.

| Column i.d. (mm) | Typical Flow Rate (µl/min) | Theoretical Sensitivity Increase |
|---|---|---|
| 1.0 | 40 - 240 | - |
| 0.5 | 10 - 60 | 4 |
| 0.3 | 3 - 18 | 11 |
| 0.15 | 0.05 - 5 | 44 |
| 0.075 | 0.02 - 1.5 | 178 |

## Instrument Modifications

In order to fully exploit the benefits of using capillary dimensions, the HPLC system must be capable of handling sample volumes in the sub-microlitre range. The use of columns of <1mm i.d. requires either a specially designed micro-LC instrument or extensive modifications of a standard HPLC instrument. The major principles in the conversion are flow rate reduction and elimination of dead volume.

Flow rate reduction can be achieved using a high performance, low µl/minute pump or by incorporating a flow splitting tee between a standard HPLC pump and the injector. The majority of the flow can be spilt to waste or recycled.

To ensure that band spreading is kept to a minimum, low dispersion column hardware must be used throughout the system. Connecting capillaries must be dead volume free and as short as possible. A micro-scale injector allowing injection of sub-microlitre sample volumes should be used. Micro flow cells with appropriate internal volume (<1µl) and path length should be used for UV detection.

## Column Availability

Capillary and nano columns, ranging in i.d. from 0.05mm to 0.8mm, are available in several manufacturers' ranges – please contact Hichrom for further information.

## Trap Columns

Short capillary or nano dimension columns are typically used for on-line preconcentration or desalting of protein and peptide samples prior to HPLC separation with MS detection. This enables fast clean-up of large volume injections. Typical trap column dimensions are 10 or 20mm length and 75 to 500µm i.d. Trap columns can be applied for 1D or 2D HPLC approaches. Please enquire for availability of trap columns.

# LC-MS COLUMNS



- Column diameter ≤2.1mm
- Rapid analysis
- Characterisation technique
- High sensitivity
- Quantitative analysis
- Peak deconvolution ability

## Introduction

LC-MS is a leading technique which combines the separation capabilities of HPLC with the mass analysis capabilities of mass spectrometry. LC-MS is used for many applications, providing high sensitivity and high selectivity for small sample quantities. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combines high detection sensitivity with high analyte specificity.

## Column Dimensions

LC-MS interface techniques such as Atmospheric Pressure Chemical Ionisation (APCI), Electrospray (ESI), Particle Beam or Thermospray can typically handle maximum flow rates of 200µl/min. As microbore columns (e.g. 2.1mm i.d.) utilise such flow rates, they are commonly used in LC-MS applications. A 50 x 2.1mm i.d. column is used for fast speed analysis applications, whilst a 250 x 2.1mm i.d. column is preferred for more complex separations. When larger bore columns (3.0-4.6mm i.d.) are used, a flow splitter is used to reduce the eluent flow into the mass spectrometer. Capillary columns (see page 13) are frequently used for proteomics applications, where sample quantities are limited.

## Column Availability and Eluent Conditions

Eluents used for LC-MS must be volatile and preferably non-ion pairing. Suitable buffers include formate and acetate, while phosphate buffers must be avoided. Although there are limitations to eluent conditions, selectivity can be adjusted by changing bonded phase chemistry.

Many high purity silica materials, with low column bleed and high reproducibility, are now available for LC-MS. Generally, bonding chemistries which offer low bleed (e.g. C18, C8) are preferred over traditional PFP and polar embedded phases. However, the advent of newer bonding technologies has led to columns with alternative selectivity to C18, but which retain exceptionally low bleed characteristics.

Many methods which have a low requirement for buffer salts or modifiers are directly adaptable for LC-MS. For LC-MS and LC-MS/MS analyses, fully porous, superficially porous and monolithic reversed-phase columns are all used, as well as HILIC phases. Many suitable columns are discussed in later sections of this catalogue. Please contact us for further information and advice.

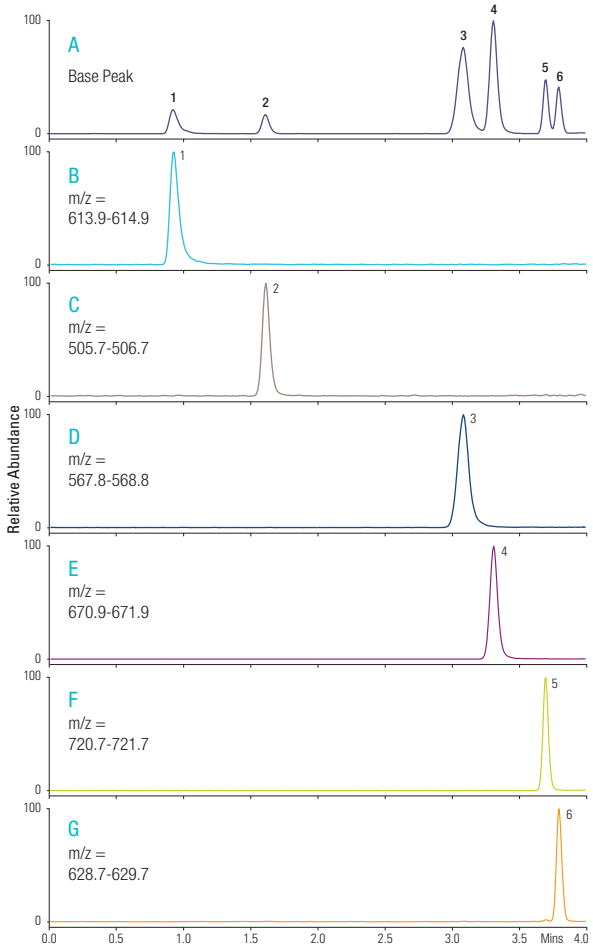
## Applications

LC-MS and LC-MS/MS have numerous applications in a wide range of areas. These include:

- Drug metabolism structure elucidation and quantitative studies
- Clinical assays e.g. steroids, biogenic amines, vitamin D
- Protein and peptide identification and sequencing in proteomics studies
- Agrochemical identification and quantitative studies
- Food and beverage products e.g. additives and contaminants
- Forensic science e.g. drugs of abuse

Preparative LC-MS can also be used for fast and mass directed compound purification.

Figure 1 shows the use of single-ion monitoring in the analysis of a mixture of protease inhibitors. The base peak chromatogram is shown in (A), with the single-ion monitoring traces of each individual component shown in chromatograms (B) to (G).




Column: Hypersil GOLD aQ (1.9µm, 50 x 2.1mm)
Eluent: A: $H_2O$ + 0.1% formic acid
B: $CH_3CN$ + 0.1% formic acid
Gradient: 0 – 2.2mins at 35% B then 100% at 4mins
Flow rate: 0.5ml/min
Temperature: 30°C
Detection: +ESI-MS

1. Indinavir
2. Nelfinavir
3. Saquinavir
4. Amprenavir
5. Ritonavir
6. Lopinavir

Figure 1. Single-ion monitoring of protease inhibitors

# UHPLC COLUMNS (≤ 2µm Particle Size)



- Decrease in analysis time
- Shorter columns with high separation efficiency
- Significant improvement in resolution
- Increased detection sensitivity

## Introduction

There is an increasing demand for high throughput analysis and columns capable of ultra fast run times. A requirement for increased column efficiency has led to the development of smaller particle size materials. The introduction of ultra high pressure pumps and low dispersion hardware has enabled the use of smaller particle sizes to become more practical. However, many smaller particle columns have now been specifically designed so that they may still be used on conventional HPLC equipment, in addition to high pressure UHPLC systems.

## Features of UHPLC Silica Particles

### Resolution Equation

$$R_s = \frac{1}{4}\frac{(\alpha - 1)}{\alpha}\sqrt{N}\,\frac{k}{1+k}$$

As the resolution equation shows, resolution (Rs) is proportional to the square root of separation efficiency (N). Efficiency is inversely proportional to particle size (dp). This means that when particle size is reduced, column length can also be reduced, whilst still maintaining constant efficiency. Using a shorter column with smaller particles, at the same flow rate as the larger particle column, means that analysis time can be considerably reduced. The optimal flow rates for particles of ≤2µm are higher than for 3 and 5µm particles (see Figure 1).

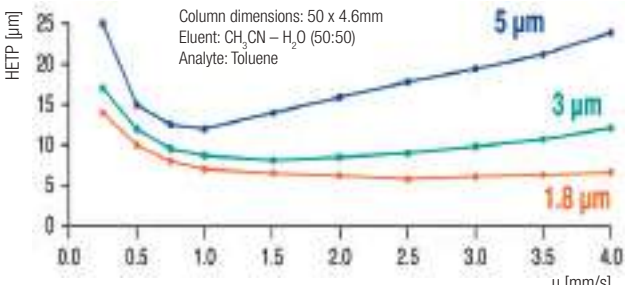



Figure 1. Van Deemter plot

**Table 1. Particle size effects**

| Particle Size (µm) | Resolution (Rs) | Efficiency (N) (plates/m) | Pressure (psi) |
|---|---|---|---|
| Proportional to | $1/dp^{1/2}$ | 1/dp | $1/dp^2$ |
| 2 | 2.97 | 200,000 | 5000 |
| 3 | 2.57 | 150,000 | 2250 |
| 3.5 | 2.39 | 130,000 | 2000 |
| 5 | 2.00 | 90,000 | 1000 |
| 10 | 1.48 | 50,000 | 300 |

Column: 100 x 4.6mm  Eluent: $CH_3OH – H_2O$ (85:15)  Flow rate: 1.0ml/min  Temperature: 22°C

### Column Back Pressure

For columns with smaller particles the back pressure will increase inversely proportional to the square of the particle size. Table 1 illustrates that as particle size is reduced the column pressure is seen to increase more rapidly than resolution. For instance theoretically, when reducing the particle size from 5µm to 2µm, the efficiency will more than double. This results in a resolution increase of nearly 50%, but pressure can be expected to increase by a factor of 5. At higher operating pressures, frictional heating of the eluent can become a significant factor, with a corresponding reduction of column efficiency and change of peak resolution and selectivity.

### Selectivity

From the resolution equation above it can be deduced that selectivity (α) has a greater influence on a separation than attempting to improve efficiency alone. Therefore, it is strongly recommended that when a decrease in analysis time is required, selectivity effects are always investigated in addition to efficiency effects. Please contact us for further advice and support.

### Instrument Requirements

Generally sub 2µm columns are available in short column lengths and small column i.d.s, leading to low column volumes. To maximise performance, the dead volume of the HPLC system has to be carefully considered and optimised irrespective of whether or not the columns are being operated at ultra high pressure. The use of high pressure, low dead volume column connectors is recommended (see page 66, for example), alongside low volume tubing and flow cells. In addition, fast data recording is required to ensure that the chromatographic performance is accurately recorded.

### UHPLC Phases[1]

| Brand | Phase | Manufacturer | Particle Size (µm) | Page |
|---|---|---|---|---|
| ACE Excel | C18, C18-AR, C18-PFP, C8, C4, CN, Phenyl, AQ, SIL, SuperC18, C18-Amide, CN-ES | Advanced Chromatography Technologies | 2 | 58, 65, 66, 71 |
| Endeavorsil | C18 | Dikma | 1.8 | 82, 83 |
| Epic | C18, C18-MS, C18-SD, C8, C4-SD, Phenyl-Hexyl, HILIC-HC, PFP-LB, SCX | ES Industries | 1.8 | 85 |
| Hypersil GOLD | GOLD, C8, C4, aQ, CN, PFP, Phenyl, Amino, AX, Polar, SAX, Silica, HILIC | Thermo Scientific | 1.9 | 150 |
| Inertsil | ODS-4, C8-4, ODS-3, C8-3, Phenyl-3 | GL Sciences | 2 | 87 |
| InertSustain | C18 | | 2 | 87 |
| NUCLEODUR | C18 Gravity, C8 Gravity, C18 Pyramid, C18 Isis, Sphinx RP, HILIC, PolarTec, C18 PAH, C18 HTec, PFP | Macherey-Nagel | 1.8 | 102 |
| ProntoPEARL sub2 TPP | C18 ace-EPS, C8 ace-EPS, NH2 | Bischoff | 1.8 | - |
| Purospher STAR | RP-18e, RP-8e | Merck | 2 | 110 |
| Syncronis | C18, C8, aQ, Phenyl, Amino, Silica, HILIC | Thermo Scientific | 1.7 | 151 |
| VisionHT | C18-HL, C18-B, C18, C18-P, HILIC, Silica | Grace | 1.5 | 90 |
| YMC-UltraHT | ProC18, Hydrosphere C18 | YMC | 2 | - |
| YMC-Triart | C18, C8, Diol-HILIC, Phenyl, PFP | YMC | 1.9 | - |

[1] For superficially porous phases please see page 16

# SUPERFICIALLY POROUS COLUMNS

Superficially porous particles (also called Fused-Core®, core shell or Core Enhanced Technology™ particles) consist of a solid silica core with a porous silica outer shell. These particles typically have diameters of 2.5 to 5µm. Columns packed with 2.5 to 2.7µm phases typically provide the efficiency and separation speed of sub 2µm UHPLC particles but at considerably lower back pressure. Columns packed with 5µm phases typically show comparable efficiencies to conventional porous 3µm columns.

## Benefits of Superficially Porous Phases

### High efficiency

Since diffusion only occurs in the porous outer shell and not the solid core, efficiency is increased compared to a totally porous particle of the same size. Resistance to mass transfer (C term in van Deemter equation) in superficially porous particles is reduced due to the limited diffusional path of the analytes (see Figure 1). For fast LC this results in high flow velocity without peak broadening. In addition, the tight control of particle diameter in superficially porous materials leads to highly uniform packed beds with minimised eddy diffusion, which also contributes to high efficiencies.

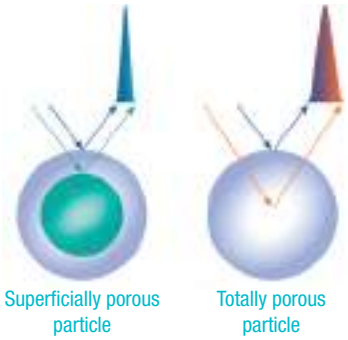



Figure 1. Mass transfer

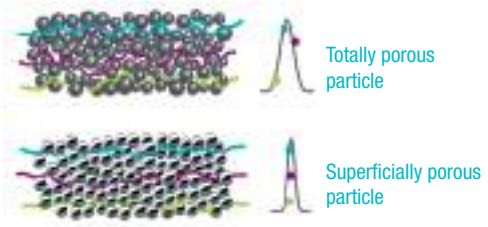



Figure 2. Eddy diffusion

### Low back pressure

Superficially porous columns generate significantly lower back pressure compared to other UHPLC columns, facilitating rugged and reliable performance. Operating at these lower pressures avoids frictional heating of the eluent that could have negative effects on column efficiency and unpredictable changes in peak retention and column selectivity. Whereas sub 2µm phases require specialised UHPLC instrumentation to cope with the high pressures generated, superficially porous phases can be used with either UHPLC or conventional HPLC systems.

### Robustness

The narrow particle size distribution of superficially porous materials enables the production of more uniformly packed column beds than found in totally porous particles. This leads to robust columns with long lifetimes. In addition, most superficially porous material columns use 2µm porosity column inlet frits, which reduces the inconvenience caused by pressure increases from plugged frits, which can occur with sub 2µm particles.

### Columns for biomolecules

Wider pore superficially porous phases (e.g. 150, 160Å or 400Å) have been designed specifically to provide an optimum combination of retention and resolution for peptides and small proteins.

### Method conversion from fully porous phases

The larger 4 and 5µm superficially porous phases can be used to directly replace conventional methods developed on the same chemistry columns using standard HPLC instruments, without any changes to instrument configuration or method conditions. Higher efficiencies and higher sensitivities can be generated using these 4 and 5µm superficially porous phases. In addition to reproducing established conventional methods, these 4 and 5µm phases enable methods to be modified to include reduced analysis times and hence increased productivity.

## Superficially Porous Phases

| Brand | Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Page |
|---|---|---|---|---|---|
| Accucore | RP-MS, C18, aQ, C8, Phenyl-Hexyl, PFP, Phenyl-X, HILIC, Urea-HILIC | Thermo Scientific | 2.6 | 80 | 149 |
| | 150-C18, 150-C4, 150-Amide-HILIC, Polar Premium, C30 | Thermo Scientific | 2.6 | 150 | 149 |
| Accucore XL | C18, C8 | Thermo Scientific | 4 | 80 | 149 |
| ACE UltraCore | SuperC18, SuperPhenylHexyl | Advanced Chromatography Technologies | 2.5, 5 | 95 | 1 |
| Brownlee SPP | C18, C8, HILIC, PFP, Phenyl-Hexyl, RP-Amide | Perkin Elmer | 2.7 | 90 | - |
| | Peptide ES-C18 | Perkin Elmer | 2.7 | 160 | - |
| HALO | C18, C8, HILIC (Silica), RP-Amide, Phenyl-Hexyl, PFP, ES-CN, Penta-HILIC | Advanced Materials Technology | 2.7 | 90 | 91 |
| | Peptide ES-C18, Peptide ES-CN | Advanced Materials Technology | 2.7 | 160 | 91 |
| HALO-5 | C18, C8, PFP, Phenyl-Hexyl, ES-CN, HILIC, Penta-HILIC, Peptide ES-C18, Peptide ES-CN | Advanced Materials Technology | 4.6 | 90 | 91 |
| HALO | Protein C4 | Advanced Materials Technology | 3.4 | 400 | 91 |
| NUCLEOSHELL | RP 18, HILIC, PFP, Phenyl-Hexyl | Macherey-Nagel | 2.7 | 90 | 102 |

# METHOD TRANSFER FROM HPLC TO UHPLC



The use of UHPLC (Ultra High Performance Liquid Chromatography) and superficially porous particles enables columns with smaller i.d. and shorter lengths to be used on low dispersion, high pressure instrumentation. This results in faster analyses and increased sample throughput. Frequently, these faster methods are derived from the conversion of an established method on a conventional HPLC column (e.g. 3 or 5µm) to small format columns packed with 2 or sub 2µm particles or superficially porous particles. It should be noted that this section looks at the benefits resulting from decreasing particle size only and the benefits of adjusting selectivity should not be ignored (see page 30). In order to realise the full benefits of these faster methods, care must be taken to ensure that operating flow rate, injection volume and gradient profile are adjusted appropriately, without exceeding the pressure limitations of the instrument.

In addition, since peaks elute from ultra-fast columns faster and with much smaller volumes than conventional columns, modifications may have to be made to conventional HPLC equipment (see page 19) to obtain the full benefits and avoid problems. Other factors such as column connections and tubing also become highly important.

In the sections below it is assumed that the column chemistry of the original and ultra-fast column is the same, preferably the same brand, with the same pore size and carbon load. This will ensure that the selectivity of the original separation is preserved, with only minor changes occurring. This is often referred to as the scalability of the material. The equations below are given as guidelines for the conversion from fully porous HPLC to fully porous UHPLC columns. For superficially porous columns, column void volumes will be lower than for the equivalent dimension fully porous column, as a higher percentage of the column is taken up by stationary phase and additional criteria need to be considered. Please contact Hichrom for additional information and advice on this.

## Isocratic Method Transfer

If particle size only is reduced, whilst maintaining column dimensions, an improvement in efficiency is observed. However, for a reduction in analysis time, column dimensions also need to be reduced.

### Adjustment of column dimensions

When converting a conventional separation to an ultra-fast separation, the shortest ultra-fast column should be chosen that will provide resolving power equal to or better than the conventional column it is replacing. This will allow run time to be minimised whilst maintaining acceptable resolution. This is accomplished by using columns that have the same ratio of column length to particle size. Equation 1 shows the relationship between column length and particle size.

$$L_2 = L_1 \times \frac{d_{p2}}{d_{p1}}$$

where $L_1$ = length of conventional column, $L_2$ = length of UHPLC column, $d_{p1}$ = particle size of conventional column, $d_{p2}$ = particle size of UHPLC column

Equation 1. Adjustment of column dimensions

For example, using this equation, it can be seen that a 2µm, 100mm length column will maintain the same separation (efficiency and resolution) as a 3µm, 150mm length column.

### Adjustment of flow rate

In order to maintain an equivalent separation when transferring a method, the linear velocity must be kept constant between the original and new method. This is achieved by decreasing the flow rate proportionally to the column internal diameter. Equation 2 shows the relationship between flow rate and column internal diameter. It is assumed that the stationary phase and the column length are kept the same.

$$F_2 = F_1 \times \left(\frac{d_{c2}}{d_{c1}}\right)^2$$

where $F_1$ = original flow rate, $F_2$ = new flow rate, $d_{c1}$ = i.d. of original column, $d_{c2}$ = i.d. of new column

Equation 2. Adjustment of flow rate

For example, if a method is converted from a 5µm, 100 x 4.6mm column run at 1ml/min to a 2µm, 100 x 2.1mm column, the flow rate giving the same linear velocity is 0.21ml/min.

Since the optimum flow velocity of an ultra-fast column is faster than for a conventional column, it may be possible to reduce run time, with no or negligible loss in resolution, by operating at a higher flow rate. However, care must be taken not to exceed the pressure limitation of the system or column.

## METHOD TRANSFER FROM HPLC TO UHPLC (continued)

### Estimation of back pressure

Back pressure is directly proportional to column length and inversely proportional to the square of the column diameter. Such pressure is inversely related to the square of the particle size and directly affected by flow rate. These changes can be represented by equation 3.

$$P_2 = P_1 \times \frac{L_2}{L_1} \times \left(\frac{d_{c1}}{d_{c2}}\right)^2 \times \left(\frac{d_{p1}}{d_{p2}}\right)^2 \times \frac{F_2}{F_1}$$

Equation 3. Estimation of back pressure

where $P_1$ = original column pressure
$L_1$ = original column length
$d_{c1}$ = i.d. of original column
$d_{p1}$ = particle size of original column
$F_1$ = original flow rate
$P_2$ = new column pressure
$L_2$ = new column length
$d_{c2}$ = i.d. of new column
$d_{p2}$ = particle size of new column
$F_2$ = new flow rate

This equation can be used to check that the back pressure due to the increased flow rate of the new method does not exceed the limitations of your system.

### Adjust injection volume

When the column length and internal diameter are decreased, the overall column volume and sample capacity are also decreased. If the same sample injection volume is used, this will fill a larger proportion of the ultra-fast column volume which could result in band broadening and column overload. The injection volume must therefore be scaled down according to the decrease in column volume. The injection volume in UHPLC can be calculated from Equation 4. In practical terms, the injection volume should be between 1-5% of the column volume in order to avoid overload.

$$V_{i2} = V_{i1} \times \left(\frac{d_{c2}}{d_{c1}}\right)^2 \times \frac{L_2}{L_1}$$

Equation 4. Adjustment of injection volume

where $V_{i1}$ = original injection volume
$d_{c1}$ = i.d. of original column
$L_1$ = length of original column
$V_{i2}$ = new injection volume
$d_{c2}$ = i.d. of new column
$L_2$ = length of new column

Figure 1 illustrates the considerable reduction in analysis time achieved when transferring from a 5µm, 150 x 4.6mm i.d. column to a 2µm, 50 x 2.1mm i.d. column. A further reduction in analysis time is achieved by increasing the flow rate by a factor of 4. The pressure increase is within the limits of the UHPLC system.

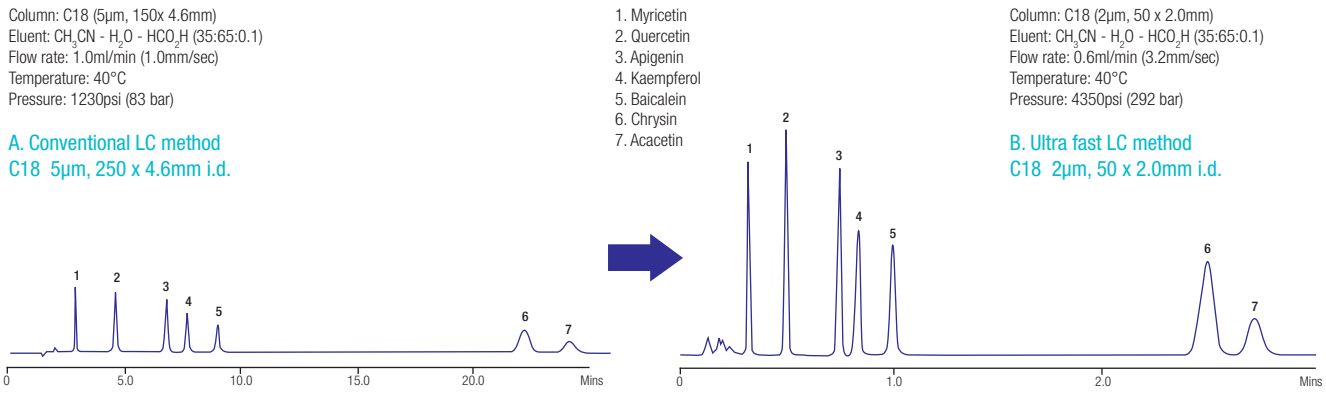



Figure 1. Conversion of HPLC to UHPLC method

## Gradient Method Transfer

Following adjustment of column dimensions, flow rate and injection volume, as discussed above for isocratic separations, the gradient time can then be adjusted for each step of the gradient. The new calculated gradient should take place over the same number of column volumes of eluent as in the gradient with the initial column. The initial and final solvent compositions are kept the same. If the dwell volumes of the original and new UHPLC/HPLC systems are different, it may be necessary to introduce an isocratic hold into the method to allow for this.

$$t_{g2} = t_{g1} \times \frac{F_1}{F_2} \times \left(\frac{d_{c2}}{d_{c1}}\right)^2 \times \frac{L_2}{L_1}$$

Equation 5. Adjustment of gradient profile

where $t_{g1}$ = gradient time in original method
$F_1$ = original flow rate
$d_{c1}$ = i.d. of original column
$L_1$ = length of original column
$t_{g2}$ = gradient time in new method
$F_2$ = new flow rate
$d_{c2}$ = i.d. of new column
$L_2$ = length of new column

## METHOD TRANSFER FROM HPLC TO UHPLC (continued)

### Instrument Considerations

In order to obtain the best data using fast chromatography, it is essential that the LC instrument is optimised for the conditions to be used. Extra column volume is the volume in an HPLC system external to the column that contributes to the total peak volume and therefore band broadening. Extra column effects are far more significant for scaled down separations because of the smaller column volumes involved.

#### Minimising system volume

In particular, system volume must be minimised. Connecting tubing i.d. and length, injection volume, volume of UV flow cell and any volume added by heat exchangers, fittings, connectors and inline filters must be kept as small as possible. In general, in order to get 90% of the maximum resolution from a column, the extra column volume should be less than half of the total peak volume.

#### Making good UHPLC connections

When installing any UHPLC column, it is important to ensure that the high pressure inlet tubing is fitted into the column port to the correct depth to avoid the introduction of any extra column volume. Incorrect positioning of the inlet tubing, ferrule and nut can result in a significant loss in efficiency and deterioration in asymmetry. Figures 2 and 3 illustrate correctly and incorrectly fitted tubing. The tubing that is incorrectly fitted shows a gap between the tubing and the column frit which will lead to the introduction of extra column volume. This gap may result from either poor initial connection, possibly as a result of using connectors pre-swaged to the tubing, or tubing slippage subsequent to connection. It is recommended that a new connection is made every time a new UHPLC column is installed to help avoid this problem.

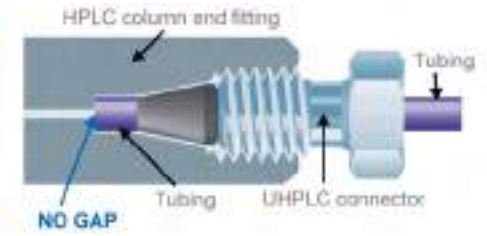



Figure 2. Tubing correctly fitted

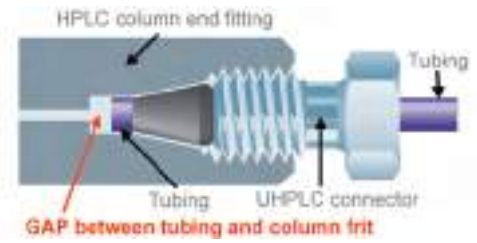



Figure 3. Tubing incorrectly fitted

#### Dwell volume

For gradient separations the pump dwell volume is of particular importance, as it affects the time taken for the gradient to reach the head of the column, i.e. it effectively adds an isocratic hold to the beginning of the gradient. This will have more of an effect on early eluting peaks. To minimise the effect on a separation, the dwell volume should be kept as small as possible, by using micro gradient mixers and keeping the system tubing volume to a minimum.

#### Filtering of solvents and samples

The frits used in UHPLC columns are generally of smaller pore size than those of conventional HPLC columns and therefore more prone to clogging. As a result, it is critical to ensure that any potential small particles are removed from the eluent. In order to ensure the absence of insoluble particles it is recommended that high grade organic solvents and ultra pure water are used. Eluents used should always be freshly prepared in order to avoid microbiological growth.

#### Detector flow cell and sampling rate

When short narrow UHPLC columns are used, the peaks generated are narrower than in conventional HPLC. Smaller, lower dispersion flow cells with volumes of <4µl or even <1µl are preferred for UHPLC. In addition, the detector time constant and data collection rate should be set fast enough for sufficient peak information to be collected, otherwise resolution, efficiency and analytical accuracy will be compromised.

### Method Transfer from UHPLC to HPLC

Similar considerations need to be made when transferring methods from UHPLC to HPLC as for HPLC to UHPLC. Please contact Hichrom for additional information and advice on this.

### Superficially Porous Columns

Please contact Hichrom for additional information and advice on transferring methods from HPLC to superficially porous type particles.

# HICHROM GUARD CARTRIDGES

- Protection for columns from 1.0 - 30mm i.d.
- No loss in column performance or selectivity
- Significantly extends column lifetime
- Packed with the same high performance silica used in main column
- Stand alone or integral design
- Tested to ensure consistent high level performance
- Readily disposable and cost effective

## Introduction

Guard cartridges are designed to protect valuable analytical and preparative HPLC columns from contamination with impurity particles and irreversibly adsorbed solutes. By placing a guard cartridge between the column and the injector valve, contaminants which would otherwise damage the column are trapped on the disposable cartridge. This procedure significantly extends column lifetime without affecting performance or selectivity (see Figure 1). Without column protection, column fouling can lead to increased back pressure and peak splitting and/or severe peak tailing.

## Silica

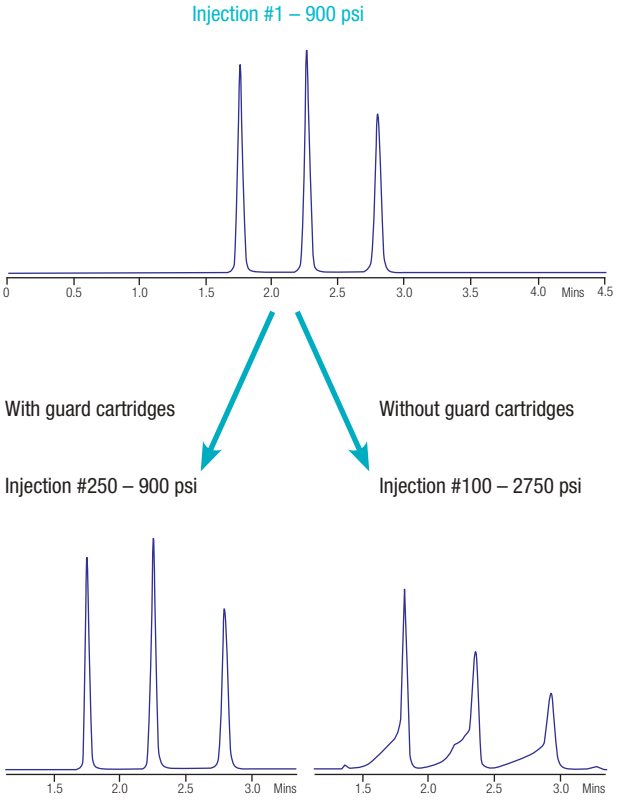
It is recommended that guard cartridges are packed with the same silica and bonded phase as used in the HPLC column to be protected (this eliminates the possibility of any loss of performance or selectivity). All Hichrom cartridges conform to this requirement. As Hichrom is able to supply cartridges packed with any commercially available silica, virtually all analytical and preparative columns can be suitably protected.

## Guard Cartridge Holder System

For traditional analytical columns (with compression end fittings) a fingertight column coupler (HI-081) is required to connect the stand alone holder (HI-161) to the column (see Figure 2). Similar arrangements are available for semi-preparative (7.75 - 21.2mm i.d.) and preparative (30mm i.d.) columns, as detailed on the following page.

## Guard Cartridge Replacement

It is generally recommended that for effective column protection, guard cartridges should be replaced when the column back pressure increases by 10% or column efficiency or resolution decreases by 10%.




The use of guard cartridges leads to a significant increase in column lifetime due to prevention of column fouling

Figure 1. Increased column lifetime

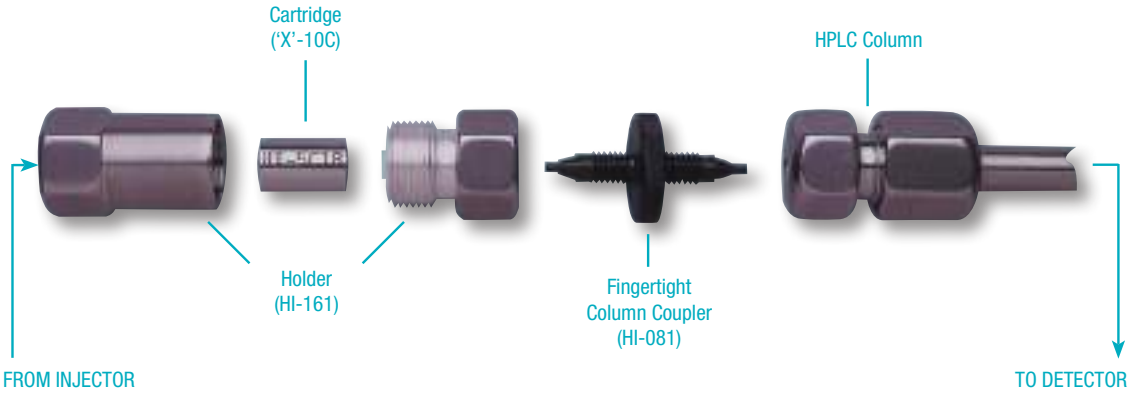



Figure 2. Stand alone guard cartridge system for traditional analytical columns

## HICHROM GUARD CARTRIDGES (continued)

### Ordering Information - Hichrom Manufactured Guard Cartridges

| Guard Cartridge[3] | Catalogue No.[1] | Pack Quantity | Holder | Coupler |
|---|---|---|---|---|
| For 1.0mm i.d. columns | **X**-10CE5 | 5 | HI-161 | HI-081 |
| For 2.1mm i.d. columns | **X**-10CM5 | 5 | HI-161 | HI-081 |
| For 3.2 - 4.6mm i.d. columns | **X**-10C5 | 5 | HI-161 | HI-081 |
| For 7.75 - 21.2mm i.d. columns | **X**-10CP3 | 3 | HI-150 | HI-081 |
| For 30mm i.d. columns | **X**-20CP | 1 | HI-183[2] | HI-083 |

[1] When ordering replace '**X**' with the appropriate silica code - see column listings or contact Hichrom for details.
Example: For a 5 pack of Hichrom 5 C18 guard cartridges for 3.2 - 4.6mm i.d. columns, Catalogue No. = HI-5C18-10C5

[2] A new improved holder HI-655 for use with new guards **X**-10CB3 (3/pk) is now available

[3] Guard cartridges for 50mm i.d. columns are also available – please enquire

### Starter Kits

Starter kits (see Figure 3) contain five guard cartridges packed with any chosen silica, a free-standing holder (HI-161) and a fingertight column coupler (HI-081).

| Starter Kit | Catalogue No.[1] |
|---|---|
| For 1.0mm i.d. columns | **X**-10CE5-SK |
| For 2.1mm i.d. columns | **X**-10CM5-SK |
| For 3.2 - 4.6mm i.d. columns | **X**-10C5-SK |

[1] When ordering replace '**X**' with the appropriate silica code - see column listings or contact Hichrom for details.
Example: For a Hichrom 5 C18 starter kit for 3.2 - 4.6mm i.d. columns,
Catalogue No. = HI-5C18-10C5-SK

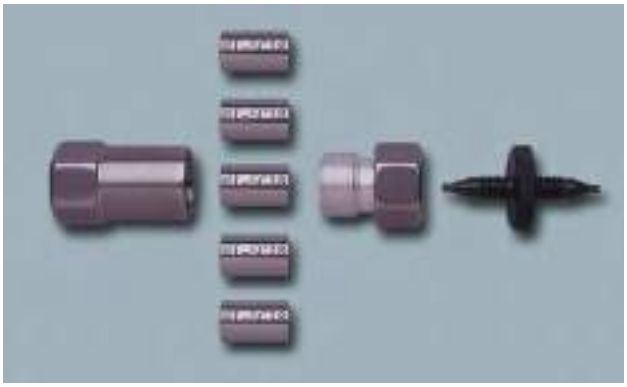


Figure 3. Guard cartridge starter kit

## ColumnSaver Pre-Column Filters

- Economical column protection
- Protect columns from particulates
- Compatible with all HPLC columns
- Ultra-low dead volume

Placed immediately before the column, pre-column filters trap sample particulates and provide a convenient, lower cost solution than the use of guard cartridges (see p.20/21). However, for samples that may irreversibly adsorb onto the column, guard cartridges remain the preferred choice for maximum column protection.

The ColumnSaver pre-column filter is simply hand tightened into the column inlet (no tools are required), and is leak-proof to over 5000psi. Both 2µm and 0.5µm versions are available, for protection of 5µm and 3µm columns respectively.

ColumnSaver pre-column filters are universally compatible with all column manufacturers' end fittings, and may be used with either stainless steel or PEEK tubing and nuts (see Figure 4). It is recommended that pre-column filters are replaced when a 10-20% increase in back pressure is detected.

Please see page 194 for column filter protection for UHPLC and superficially porous columns.


Figure 4. Column with pre-column filter

| Description | Catalogue No. |
|---|---|
| 2µm ColumnSaver (10/pk) | HI-685 |
| 0.5µm ColumnSaver (10/pk) | HI-686 |

# PREPARATIVE AND PROCESS SCALE COLUMNS



- Wide range of bulk silicas
- Particle sizes 5 - 50µm
- Column internal diameters 10 - 100mm (4˝)
- Analytical matched test columns
- High purity products
- Good recoveries

## Introduction

Preparative HPLC is used to isolate and purify milligram to kilogram amounts of compound. The technique uses larger particle size silica materials and wider internal diameter columns than in analytical HPLC. Column efficiency can be preserved on scale-up from analytical to preparative separations. However, broader lower efficiency chromatographic peaks are more often observed when the column is used in an overload state.

## Separation Criteria

The criteria governing preparative separations are very similar to those influencing analytical HPLC. However, economic considerations become more important. They are governed by four factors.

**1) Resolution –** By optimising the separation between the peak of interest and the nearest contaminant, high sample loads can be achieved without compromising product purity.

**2) Loadability –** Loadability is controlled by the silica's pore size and available surface area. The smaller the pore size the larger the surface area and the higher the potential loadability. The comparative loadability of different pore size silicas is shown in Figure 1. However, application of the smaller pore size silicas is limited by the range of molecular weight materials they can separate.

**3) Chemical stability –** The lifetime of a column is often dependent on the silica's chemical stability. Conditions of use are very important.

**4) Physical stability –** Larger preparative and process scale columns are often repacked during their lifetime. The robustness of a silica will determine how many times a material can be successfully repacked into a column.

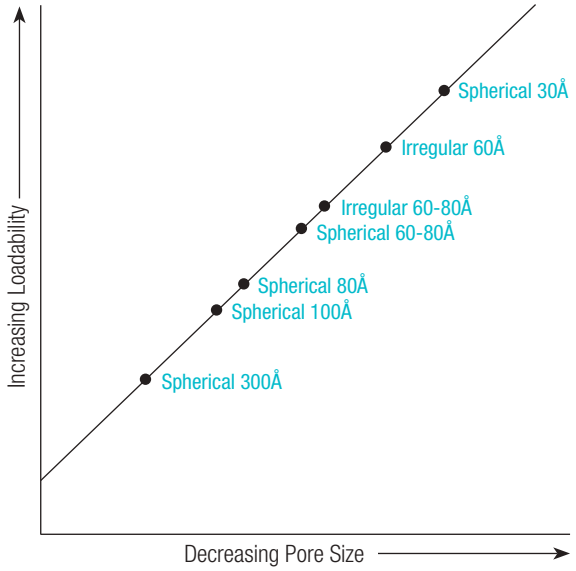



Figure 1. Comparative loadability of different pore size silicas

## Separation Strategy

Reversed-phase is the dominant technique used in analytical HPLC. However, normal-phase HPLC is still often used in preparative separations due to the high cost of reversed-phase materials and the easier recovery of solute from the organic solvents used.

Two strategies dominate the approach to preparative HPLC. In the 'scale-up' approach, a method developed for analytical purposes is directly applied to a larger i.d. column. Although typical 3 - 5µm particles may be replaced with 10µm material of identical selectivity, high preparative efficiencies are maintained. Such an approach is particularly suitable for purifying gram quantities of material with low k values.

In the alternative 'overload' approach, resolution is sacrificed by operating the column in an overload situation. Resolution needs only to be maintained between the peak of interest and the nearest contaminant. Such high loadings maximise column capacity. Separations are poorer but gram to kilogram amounts of material may be purified.

Figure 2 illustrates a typical preparative method development strategy. The extent to which the analytical column can be overloaded, whilst still maintaining adequate resolution and peak shape, is first determined (Figure 2(b)). The injection volume is then scaled up in line with the flow rate increase, used to maintain the same linear velocity through the preparative column (Figure 2(c)).

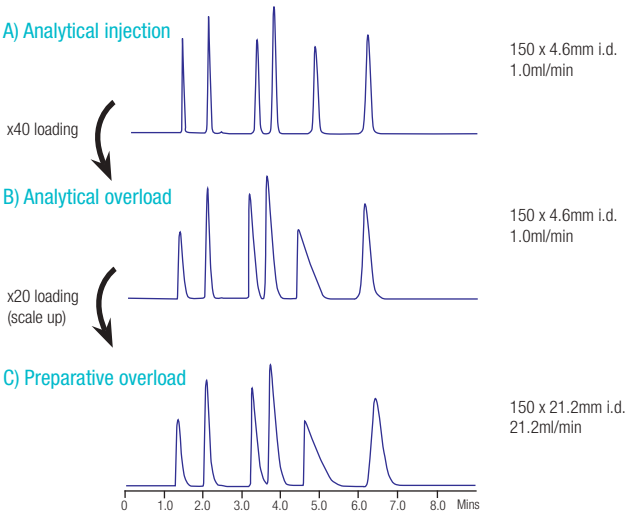



Figure 2. Scale-up strategy using C18 column

## PREPARATIVE AND PROCESS SCALE COLUMNS (continued)

A comparison of scale-up parameters is highlighted in Table 1.

### Table 1. Typical sample capacities

| Column Size | Column i.d. (mm) | Relative Flow Rate (ml/min) | Volume of 250mm Length Column (ml) | Weight of Phase[1] (g) | Typical Injection Volume (µl) | Maximum Column Capacity per Injection | |
|---|---|---|---|---|---|---|---|
| | | | | | | Optimum | Overload |
| Analytical | 4.6 | 1.0 | 4.2 | 2.5 | 10 | 2mg | 85mg |
| Semi-preparative | 7.75 | 2.8 | 12 | 7 | 30 | 6mg | 240mg |
| Semi-preparative | 10 | 4.7 | 20 | 12 | 50 | 10mg | 400mg |
| Preparative | 21.2 | 21 | 90 | 53 | 200 | 45mg | 1.8g |
| Preparative | 30 | 42.5 | 177 | 106 | 400 | 90mg | 3.6g |
| Process | 50 | 118 | 490 | 295 | 1200 | 250mg | 10g |
| Process | 100 | 473 | 1964 | 1182 | 4800 | 1g | 40g |

[1] Assumes 250 x 4.6mm column contains 2.5g material

## Bulk Preparative Materials

Hichrom distributes a large number of commercially available preparative HPLC bulk materials. The physical properties of a selection of these are listed below. Please note that for a given brand not all chemistries are available in all particle sizes and pore sizes.

| Material[1] | Manufacturer | Particle Size (µm) | Particle Shape[2] | Pore Size (Å) | Surface Area ($m^2/g$) | Chemistry | Page |
|---|---|---|---|---|---|---|---|
| Daisogel | Daiso Co. | 7, 10, 15, 20, 50 | S | 60, 100, 120, 200, 300, 1000, 2000 | 450, 450, 300, 200, 100, 25, 15 | Sil, C18, C8, C4, C1, $NH_2$ | - |
| Develosil | Nomura Chemical Co. | 10, 10-20, 15-30 | S | 30, 60, 100 | 760, 500, 320 | Sil, C18 | 80 |
| Impaq | Silicycle | 5, 10, 20, 40 | I | 60, 100 | 500, 400 | Sil, C18 | - |
| Kromasil | Akzo Nobel | 7, 10, 13, 16 | S | 60 | 540 | Sil, CN, Diol | - |
| | | 7, 10, 13, 16 | S | 100 | 320 | Sil, C18, C8, C4, $NH_2$, Phenyl | - |
| | | 10, 16 | S | 300 | 110 | Sil, C18, C8, C4 | - |
| LiChroprep | Merck | 15-25, 25-40, 40-63 | I | 60,100 | 500, 300 | Sil, C18, C8, $NH_2$, CN, Diol | 114 |
| MCI GEL | Mitsubishi Chemical Corp. | 10, 30 | S | 120, 250, 600 | Polyhydroxymethacrylate based resins | | 108 |
| | | Various | S | 250, 450 | Non-functionalised styrene-divinylbenzene copolymer for RP | | 108 |
| | | Various | S | - | Styrene-divinylbenzene copolymer ion-exchange resins | | 108 |
| SiliaSphere PC | Silicycle | 20-45, 40-75, 75-200 | S | 70, 100, 300, 1000 | 500, 280, 100, 50 | Sil, C18 | - |
| Vydac | Grace | 10-15, 15-20, 20-30 | S | 300 | - | C18, C8, C4, Phenyl | 90 |
| YMC ProC18 | YMC | 10 | S | 120 | - | C18 | - |
| YMC HG Series | | 10, 15, 20, 50 | S | 120, 200, 300 | 330, 175, 100 | Sil, C18, C8, C4, C1, Phenyl, $NH_2$, CN, Diol | - |
| YMC Triart Prep | | 10, 15, 20, 50 | S | 120, 200 | - | C18, C8 | - |
| YMC BioPro | | 10, 30, 75 | S | 1000 | - | QA, SP | - |

[1] Not all chemistries available in all particle sizes and pore sizes

[2] S=Spherical, I=Irregular

A large range of bulk resins from Tosoh Bioscience is also available – please contact Hichrom for further details.

# BUFFER SELECTION



## Introduction

In reversed-phase HPLC, the pH of the eluent can significantly influence the separation of components. Buffers are required when the sample contains ionic or ionisable analytes. Without a buffer, poor peak shape and variable retention may result.

In general, for acids, increasing eluent pH leads to increased ionisation and a decrease in retention (see Figure 1). For bases, decreasing the pH results in greater ionisation and decreased retention. For robust methods, separations should be developed at a pH where retention is least affected by pH change (see robust pH zones in Figure 1).

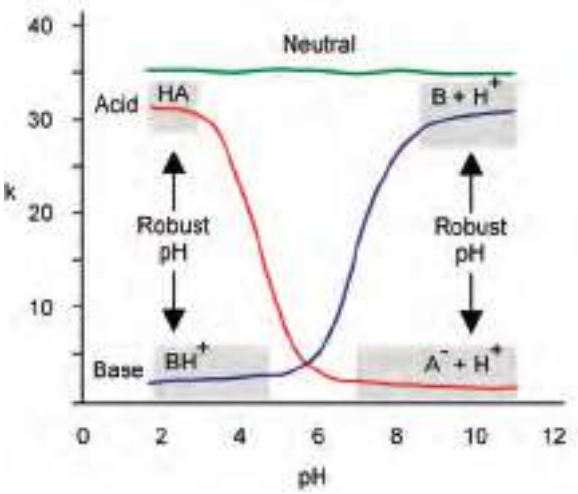



Figure 1. pH Effect on retention

## Choice of Buffer pH

Optimum buffering capacity occurs at a pH equal to the $pK_a$ of the buffer. In general, the effective pH range for a buffer is within ± 1 pH unit of its $pK_a$. Table 1 indicates the pH range of some common buffers. The buffer pH should be selected so that it is at least ± 1 pH unit from the $pK_a$ of the analyte. This will ensure that the analytes are 100% ionised or 100% non-ionised, for reproducible retention.

## Further Considerations

At pH >7, phosphate buffers accelerate the dissolution of silica. For optimum column lifetime, organic buffers (e.g. pyrrolidine) should be used for pH above 8. Buffer concentrations in the 10 – 25mM range are recommended (maximum 50mM) to prolong column lifetime and reduce the possibility of system wear and tear. Fresh buffer solutions should be prepared regularly and pre-column filters should be used to protect columns. Buffers should be flushed from the column prior to storage.

## Buffers for LC-MS

For LC-MS analyses, volatile buffers or additives are preferred, in order to minimise MS ion suppression and maintain sensitivity. In addition, non-volatile buffers such as phosphate may lead to contamination of the ion source. Buffer concentrations should be as low as possible e.g. 10 to 20mM. Ammonium salts are more volatile than those of $Na^+$ or $K^+$. TFA should be avoided with electrospray LC-MS as it reduces sensitivity. Formic acid or acetic acid (0.01 to 1% v/v) are preferred. For higher pH applications, ammonium hydroxide is recommended.

## Table 1. Common Buffers and Additives for HPLC

| Buffer or Additive | $pK_a$ | pH Range | UV Cut-off (nm) | MS Compatible |
|---|---|---|---|---|
| TFA | 0.3 | - | 210 (0.1%) | Yes – low levels |
| Phosphate | 2.1<br>7.2<br>12.3 | 1.1 – 3.1<br>6.2 – 8.2<br>11.3 – 13.3 | 200 | No |
| Formic acid | 3.8 | - | 210 | Yes |
| Formate | 3.8 | 2.8 – 4.8 | 210 (10mM) | Yes |
| Acetic acid | 4.8 | - | 210 | Yes |
| Acetate | 4.8 | 3.8 – 5.8 | 210 (10mM) | Yes |
| Citrate | 3.1<br>4.7<br>5.4 | 2.1 – 4.1<br>3.7 – 5.7<br>4.4 – 6.4 | 230 (10mM) | No |
| Bicarbonate | 6.4<br>10.3 | 5.4 – 7.4<br>9.3 – 11.3 | 200 (10mM) | Yes |
| Ammonia / ammonium hydroxide | 9.2 | 8.2 – 10.2 | 200 (10mM) | Yes |
| Borate | 9.2 | 8.2 – 10.2 | | No |
| Tris | 8.3 | 7.3 – 9.3 | 205 (10mM) | No |
| 1-Methylpiperidine | 10.1 | 9.1 – 11.1 | 215 (10mM) | Yes |
| Diethylamine | 10.5 | 9.5 – 11.5 | | Yes |
| Pyrrolidine | 11.3 | 10.3 – 12.3 | 200 | Yes |

# PROTEOMICS



## Introduction

Proteomics is the analysis of the protein complement present in a cell, organ or organism at any given time. The application of proteomic technologies is key to the understanding of healthy and diseased conditions. Proteomics involves the identification of all the proteins made in a given cell, tissue or organism and understanding how these proteins function and the determination of their 3D structures. This information can be used to help identify biomarkers and target sites for drug interaction. In addition to applications in biochemistry and medicine, proteomics can also be applied in plant and crop biotechnology research.

Many proteins are subjected to a wide variety of post-translational chemical modifications. These all contribute to the composition and functioning of the proteome. Typical modifications include phosphorylation, glycosylation, oxidation, acetylation etc.

## Techniques

A typical cell produces hundreds of thousands of different proteins. Enzymatic digestion (e.g. with trypsin) of extracted proteins, or those separated by 2D gel electrophoresis, generates a range of peptides in the digest. Multidimensional LC-MS/MS can be used to separate and sequence these peptides. The 2D HPLC approach increases the resolving power of the separation, by combining reversed-phase with ion-exchange, HILIC or affinity techniques. This reduces the complexity of the peptide mixture being analysed, improving detection chances for less abundant species.

Due to the limited amounts of sample generally available, microbore and capillary/nano columns are now preferred. Experimental electrospray MS/MS data is matched against sequence-analysis identification software.

Many products listed in this catalogue can be used for proteomic studies.

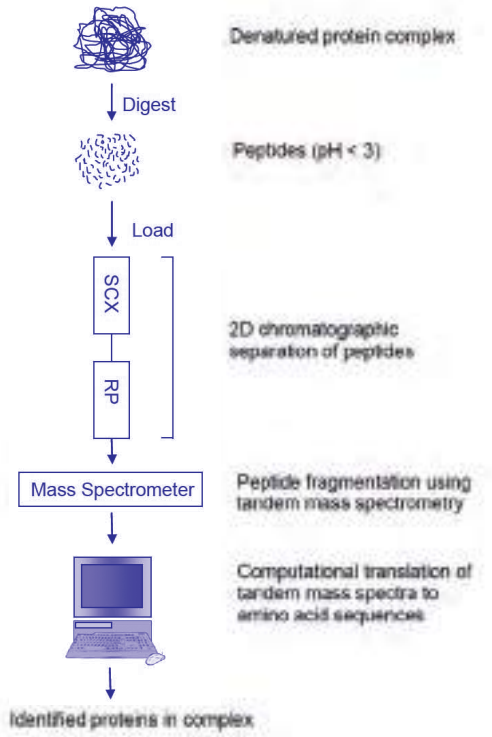



Figure 1. Proteomics by 2D LC-MS/MS

## HPLC Columns

Capillary and LC-MS columns are discussed on pages 13 and 14 respectively. In addition to reversed-phase columns, ion-exchange (p.46), HILIC (p.44) and affinity columns (p.50) are also used in 2D analysis.

A selection of suitable columns can be found on the following pages.

**Reversed-phase columns:** Acclaim PepMap (p.153), ACE (p.58), Cogent (p.115), HALO (p.91), Inertsil (p.87), NUCLEOSIL (p.102 ), Vydac (p.90).

**Ion-exchange columns:** BioBasic (p.153), MCI GEL (p.107), PolyLC (p.127), Shodex (p.140), TSKgel (p.155).

**HILIC columns:** PolyLC (p.127), TSKgel (p.155), ZIC-HILIC (p.109).

**Affinity columns:** ProPac (p.153), Shodex (p.142), TSKgel (p.155).

**Speciality columns:** Titansphere (p.89) – especially effective in the isolation of phosphopeptides
Cogent Diamond Hydride (p.116) – silicon-hydride bonding
Hypercarb (p.150).

## Sample Preparation

In addition to conventional SPE products (see page 175), more specialised products have applications for sample pre-treatment of low level proteins and peptides.

Pipette and syringe tips can separate very low sample volumes, prior to HPLC, MALDI and electrophoresis (see page 177). Tips packed with the following materials are available: PolyLC, titania, monolithic and others. Please contact us for further details.

**Titansphere Phos-TiO kits:** Kits containing titania packed tips, waste and collection tubes for enrichment of phosphopeptides. Please contact us for further details.

**MonoSpin TiO:** Monolithic SPE centrifugal spin columns for enrichment of phosphopeptides. Please contact us for further details.

# METABOLOMICS



Metabolomics is defined as the quantitative measurement of all low molecular mass metabolites in an organism's cells at a specific time under specific environmental conditions. A metabolome represents the complete set of small molecule metabolites, which are the intermediates and products of metabolism, found in a biological cell, tissue, organ or organism. In general, for metabolomics, a metabolite is usually defined as any molecule less than 1kDa in size.

## Metabolomic Techniques

In targeted metabolomic analysis, the compounds in a given biofluid or tissue extract are identified and quantified by comparing the spectrum of interest to a library of reference spectra. This is generally used for the determination of a few specific known metabolites.

Metabolic profiling involves the qualitative or quantitative determination of a particular class of metabolites or compounds from a specific metabolic pathway.

Metabolic fingerprinting compares the patterns of metabolites and helps to distinguish between samples based on the metabolites characterised.

## Separation and Detection Methods

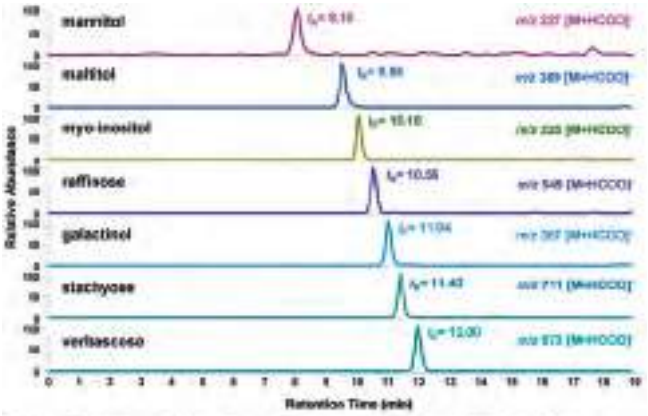
Due to the chemical diversity of small molecule metabolites, it is not possible to study the entire metabolome using a single analytical technique or technology. Therefore, a variety of targeted and non-targeted methods are applied and the data integrated in order to obtain as much information as possible regarding metabolite content.

**GC** and **GC-MS** are able to detect a wide range of compounds. Volatile components can be analysed by headspace GC-MS. For some less volatile metabolites, compounds are derivatized prior to GC analysis.

**HPLC** and **LC-MS** are the most common separation techniques used for targeted analysis and for metabolic profiling of individual classes. The simultaneous measurement of hundreds of secondary metabolites in samples can be achieved using gradient HPLC. Reversed-phase HPLC columns are most widely used, but HILIC separations of polar metabolites have also been described. Figure 1 shows the extracted ion chromatograms from a mixture of sugars and sugar alcohols, found in plants, by HILIC-MS.

**Direct injection MS** may be used to obtain metabolite mass profiles, without any chromatographic separation. Using electrospray ionisation, mainly protonated or deprotonated ions are formed for each species, with very little fragmentation. A fingerprint spectrum of metabolites is obtained, with metabolites being separated by accurate molecular mass.

**Multivariate analysis.** Due to the complex nature of a metabolome, it is difficult to make a visual comparison of the large numbers of spectra and chromatograms. As a result, data analysis and interpretation is often accomplished using a chemometric approach, including principal component analysis (PCA).




C. Antonio, T. Larson, A. Gilday, I. Graham, E. Bergström, J. Thomas-Oates, Rapid Commun. Mass Spectrom., 22 (2008) 1399-1407

Column: ZIC-HILIC (3.5µm, 150 x 2.1mm)
Eluent: A: $CH_3CN$ – 0.1% formic acid
B: 5mM ammonium acetate, 0.1% formic acid, pH 4
Gradient: 10% to 90% B in 19mins
Detection: MS

Figure 1. Analysis of plant metabolites by HILIC-MS

## Application Areas

**Clinical –** Metabolomics is of interest to medical science as it may lead to improvements in the diagnosis and treatments of human diseases. Finding unique patterns of metabolites could aid the identification of a target enzyme or protein (biomarker) for the disease, resulting in faster drug development.

**Toxicology –** Metabolomic profiling of urine and blood samples can detect physiological changes caused by toxic chemicals. This is used in the pharmaceutical industry for toxicity testing of potential drug candidates.

**Nutrition –** Metabolomics can be used for physiological monitoring in food intervention or diet challenge studies.

**Food –** Metabolomics is used as an aid to developing high performing crop varieties, e.g. it provides biomarkers of flavour in tomatoes and other fruit and vegetables. It is also used in food quality testing and the detection of food adulteration.

**Environmental –** Metabolomics is used to study the interactions of organisms with their environment and has applications in the fields of ecology and ecophysiology.

## Column Availability

A wide range of columns suitable for metabolomic analyses are supplied by Hichrom:

GC columns – see p.167-170
RP HPLC columns – see p.33-43
HILIC columns – e.g. ZIC-HILIC (p.109), TSKgel Amide-80 (p.155), PolyLC (p.127), also see p.44
Speciality columns – e.g. Cogent Diamond Hydride (p.116)

# SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)



- Fast analyses
- Reduced solvent consumption
- High flow rates possible
- Lower cost per sample
- Compatible with MS
- Excellent for preparative separations

## Introduction

Supercritical fluid chromatography (SFC) is a chromatographic technique which uses a supercritical fluid as the mobile phase. Although SFC has been around for some time, its adaptation as an orthogonal technique to HPLC, particularly in the pharmaceutical industry, has seen an increase over the last few years. This interest has been fuelled by the increasing requirement for high throughput and a desire for 'greener' techniques. Large reductions in the use of solvents have significant benefits in terms of decreased sample processing and drying-down times, as well as providing cost and safety benefits.

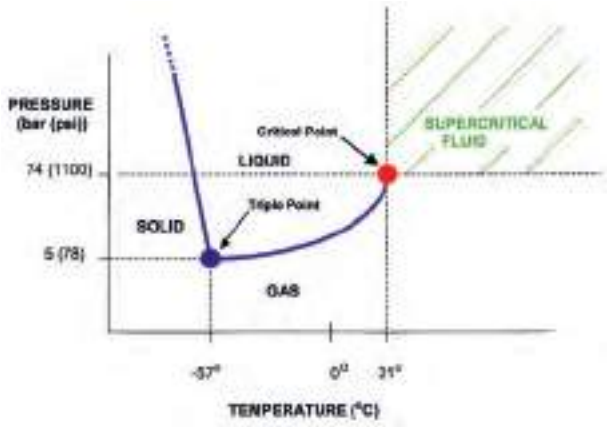



Figure 1.$CO_2$ Phase Diagram

In SFC the mobile phase consists of a 'fluid', either a gas or a liquid above its critical temperature and pressure. Liquefied $CO_2$ is most commonly used as the main fluid, with the addition of a modifier fluid such as methanol to aid elution of very polar or ionic compounds. The modifier improves the solvating power of the supercritical fluid and enhances the selectivity of the separation. Supercritical fluids can have solvating powers similar to organic solvents but with higher diffusivity, lower viscosity and lower surface tension. The lower viscosity allows higher flow rates compared with HPLC. The solvating power can be adjusted by changing the pressure. Any solute soluble in methanol or a less polar organic solvent will elute in SFC.

Packed column SFC is based on HPLC instrumentation and columns. The mobile phase is kept supercritical by an electronically controlled variable pressure restrictor positioned after the detector.

## Advantages of SFC

- **Faster diffusion of mobile phase.** This leads to higher speed and throughput enabling more samples per day to be run. Typically SFC will allow a fivefold improvement in throughput and also saves time in post-chromatographic processing.
- **Lower viscosity of mobile phase.** The lower pressure drop enables higher flow rates or longer columns to be used.
- **Improved chromatographic resolution** will give better analyses and high yield and purity during purification.

## Preparative SFC

SFC is an ideal preparative chromatography technique due to the speed of analysis and the vaporization at the end of the preparative process, which reduces solvent removal costs.

## Chiral SFC

SFC has been shown to be particularly useful for chiral analyses and is used in enantioselective phase screening, followed by optimisation of separation conditions on the chosen column. This leads on to preparative purification of a drug in mg to kg quantities. Daicel and Regis chiral columns have been extensively used for SFC and preparative scale-up. Please contact us for further details on any of these column ranges.

## Achiral SFC

For achiral SFC analyses normal-phase materials are generally used, typically silica, cyano and diol (see pages 45, 42 and 43 respectively for a selection of suitable columns). More recently, specialised bonded phases for SFC, including 2-Ethylpyridine, Pyridylamide and many others have been developed. Figure 2 shows the SFC separation of non-steroidal anti-inflammatories on a COSMOSIL 3-Hydroxyphenyl column. Column ranges specifically designed for achiral SFC can be found on the following pages:

COSMOSIL from Nacalai Tesque – see p.79
GreenSep from ES Industries – see p.85
PrincetonSFC from Princeton Chromatography – see p.131-133

Column: COSMOSIL 3-Hydroxyphenyl (5µm, 150 x 4.6mm)
Eluent: $CO_2$ – $CH_3OH$ (95:5 to 65:35 over 6 mins)
Flow rate: 5ml/min
Temperature: 30°C
Detection: UV, 230nm

1. Ibuprofen
2. Fenoprofen
3. Flurbiprofen
4. Ketoprofen
5. Indoprofen

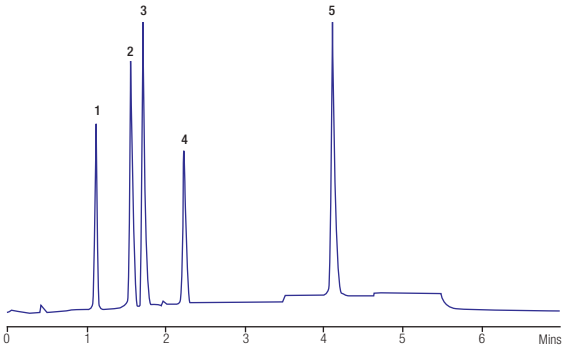



Figure 2. SFC separation of anti-inflammatory drugs

# CAPILLARY ELECTROPHORESIS (CE)

Separation in capillary electrophoresis (CE) is achieved by the differential migration of solutes in a narrow fused silica capillary by the application of an electric field. The technique developed from a combination of various electrophoresis and chromatographic techniques. The separation mechanism is mainly based on differences in solute size and charge at a given pH. Different modes of capillary electrophoretic separations can be performed using a standard CE instrument.

## CE Instrumentation

Figure 1 shows a simplified schematic diagram of a typical CE instrument. This consists of a high voltage power supply, two buffer reservoirs, a capillary, detector and output device. Each side of the high voltage power supply is connected to an electrode. The capillary is made of fused silica (typically 25 - 75µm i.d. and 0.5 to 1.5m in length) and is usually coated externally with polyimide. Each end of the capillary is dipped in a vial containing the electrode and aqueous buffer. For UV detection, the capillary has a small window near the cathodic end which allows UV-VIS light to pass through the analyte and measure absorbance. Capillaries used for MS detection do not require this window. Other common detection modes include MS and fluorescence.

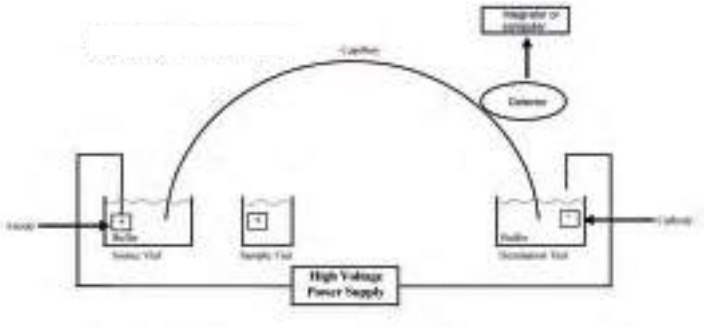


Figure 1. CE instrumentation

## Principle

CE separates ions according to their electrophoretic mobility, which takes into account the charge and hydrodynamic size of the molecule and eluent viscosity. The actual migration of an ion is also affected by the level of voltage applied. The most important parameter in CE is the electro-osmotic flow (EOF) which is the bulk flow of liquid in the capillary as a consequence of the surface charge on the interior capillary wall. EOF forms the mobile phase 'pump'. An unbonded fused silica capillary (at pH >3) contains deprotonated silanol ($SiO^-$) ions on the interior surface. The capillary wall then develops a double layer of cations attracted to it. The inner cation layer is stationary, whilst the diffuse outer layer can move along the capillary. Under the applied electric field, the cations move towards the cathode, creating a bulk flow, due to the EOF. Anions in solution, although attracted to the anode, get swept along to the cathode as well. In general, cations will separate first, followed by neutrals and then anions. By chemically coating the internal surface of the fused silica tubing by cationic groups (e.g. surfactant), the direction of flow is reversed and is independent of pH.

## CE vs HPLC

- CE has a flat flow profile due to the EOF, which does not contribute significantly to band broadening and results in narrower peaks. HPLC has a parabolically shaped pressure-driven flow profile (see Figure 2).
- CE has greater peak capacity compared with HPLC
- HPLC has more complex instrumentation
- CE requires smaller injection volumes, typically 1-50nl
- HPLC has a wider range of column lengths and packing materials

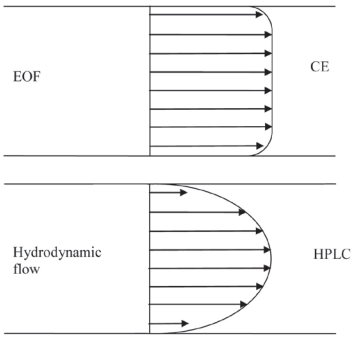



Figure 2. Comparison of CE and HPLC flow profiles

## Applications

Typical applications of CE include the analysis of proteins, peptides, amino acids, nucleic acids, inorganic ions, organic bases and organic acids.

Figure 3 shows the separation of inorganic ions on a Funcap-CE/Type A capillary.

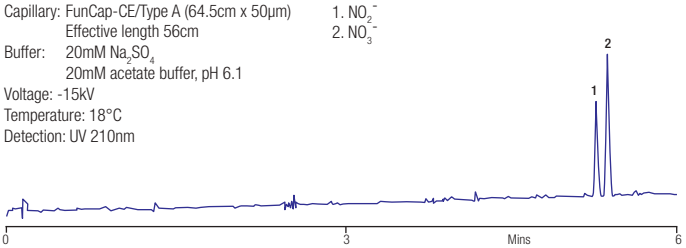



Figure 3. Analysis of inorganic ions

A selection of capillaries and accessories for CE is offered by the following manufacturers:

GL Sciences (FunCap) – see p.174
MicroSolv (Simplus, Celerity etc) – see p.174

# COLUMN SELECTION OVERVIEW

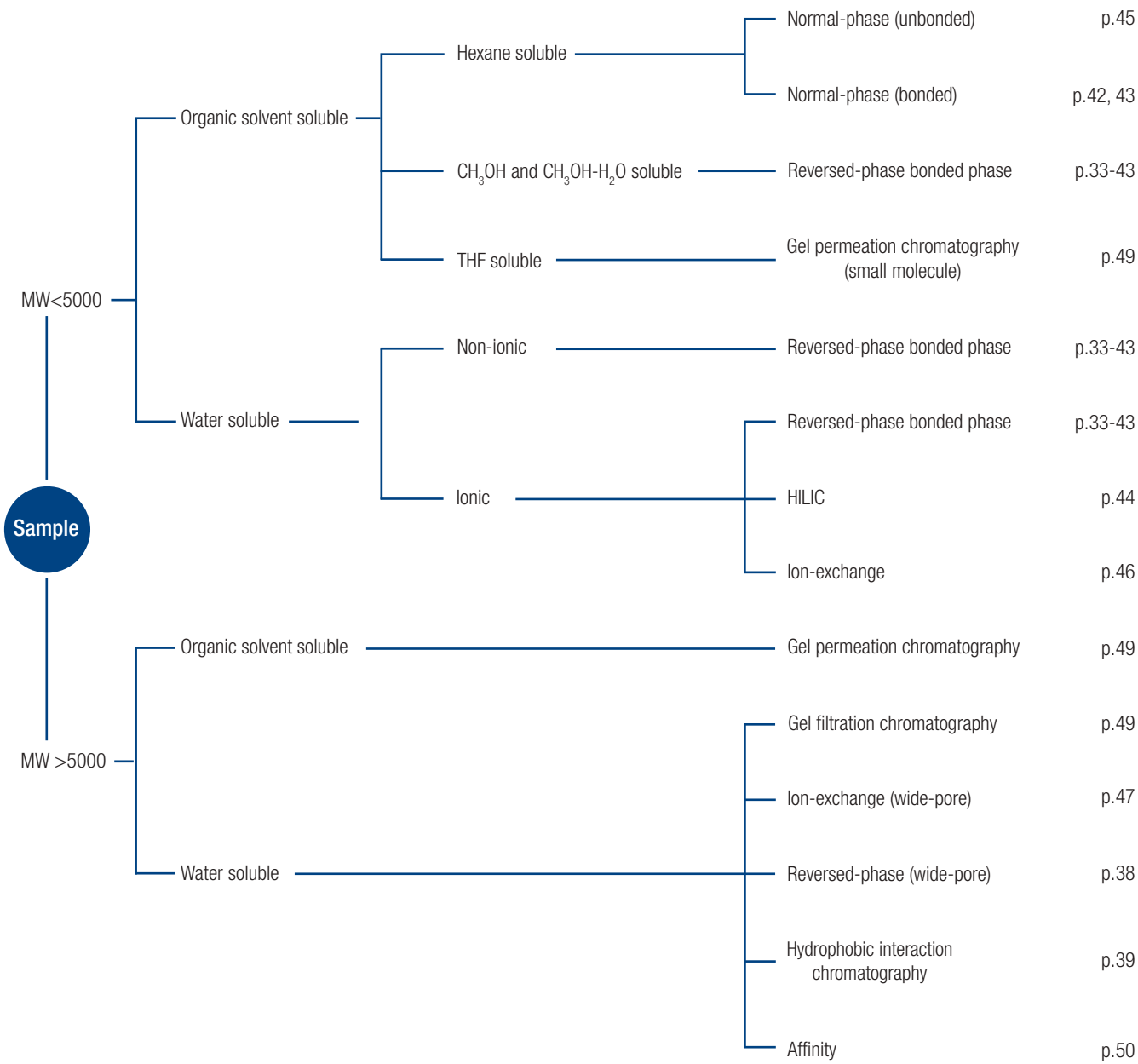


## Base Material

- **Silica** is the most popular base material. It has a high physical strength and a surface which is easily chemically modified to give phases suitable for use in a broad range of HPLC modes. However, silica dissolves in water at pH ≥6.5, whilst bonded silicas are prone to be unstable at pH ≤2.5. Newer bonded silicas may have an extended pH range of 2 -10 or higher. More recent innovations include the TYPE-C™ Silica range in which the silica surface is modified with a layer of silicon hydride (see page 115 for further details).
- **Polymeric** materials have minimal pH restrictions but are less physically stable and exhibit lower separation efficiencies than silica for small molecules. For large molecules such as proteins or synthetic polymers, their performance is comparable to that of silica based materials.
- **Graphitised carbon** has high strength and excellent pH stability but cannot be modified. It can be expensive and is best used in unique selectivity applications (see page 150).
- **Zirconia** ($ZrO_2$) has the advantage of unique selectivity combined with extreme chemical and thermal stability (up to 200°C) (see pages 162-164).
- **Titania** ($TiO_2$) is stable over a wide pH range and at elevated temperatures. In contrast to silica, the surface of titania is alkaline, which can be beneficial in the analysis of basic drugs, but separation efficiencies are generally lower (see pages 89 and 163).
- **Alumina** has greater pH stability than silica but cannot be easily chemically modified (see pages 85 and 110).

## Separation Mode

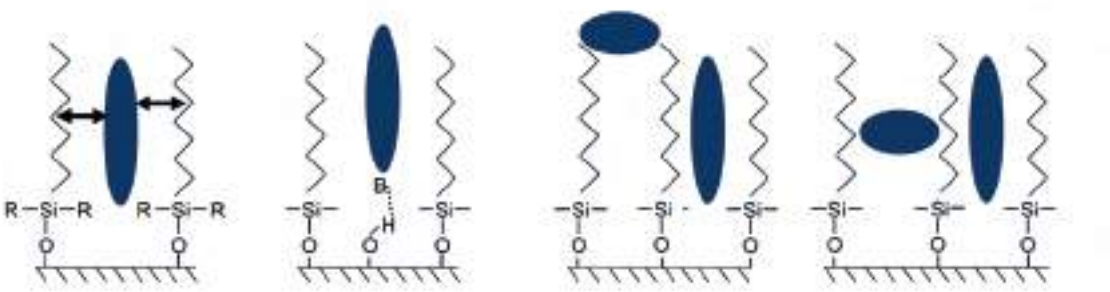
In simple terms, selection of the appropriate chromatographic separation mode is guided by the solute's molecular size and polarity. An outline selection guide is given below. For some molecules more than one technique may be appropriate.



# REVERSED-PHASE COLUMN SELECTIVITY

Selectivity is the most powerful tool for modifying solute resolution. Different bonded phases for reversed-phase separations will exhibit different combinations of possible solute-stationary phase interactions. For any given bonded phase, a combination of separation mechanisms will influence the overall selectivity of the phase. The predominance of each mechanism will also depend on the properties of the analyte and the chromatographic conditions applied. The most significant interactions between solute and stationary phase contributing towards column selectivity are summarised below.

- **Hydrophobic interaction** is a dominant retention mechanism for all reversed-phase columns and the most significant interaction of alkyl phases. For a given phase, retention time is proportional to the hydrophobicity of the molecule.
- **Hydrogen-bonding capacity** of a phase generally involves the interaction of a basic solute group with an acidic group within the stationary phase, possibly from unbonded silanol groups.
- **π-π interactions** are observed between an aromatic or unsaturated solute and an aromatic stationary phase.
- **Steric selectivity** is a measure of the accessibility of solutes to the stationary phase. Larger solute molecules may be excluded from the stationary phase.
- **Dipole-dipole interactions,** between a dipolar solute group and a dipolar group in the stationary phase, are most important in the case of cyano and PFP bonded columns.
- **Cation-exchange interactions** may occur between a cationic solute and an ionised silanol within the stationary phase.




Hydrophobic interactions | H-bonding interaction | Steric selectivity

A general summary of these interactions for typical reversed-phase bonded phases is given below. Different interactions may be dominant for different analytes and interaction strengths will vary amongst different manufacturers' bonded phases. However, this table gives a useful indication of likely interaction strengths.

| Bonded Phase | USP Listing | Hydrophobic | H-Bonding | π-π | Steric | Dipole-dipole | Cation-exchange |
|---|---|---|---|---|---|---|---|
| C18[1] | L1 | Very strong | Weak | No | Weak | No | Weak |
| C8 | L7 | Strong | Weak | No | Weak | No | Weak |
| C4 | L26 | Weak | Weak | No | Weak | No | Weak |
| Phenyl | L11 | Strong | Weak | Strong donor | Moderate | Weak | Weak |
| PFP | L43 | Moderate | Moderate | Strong acceptor | Moderate | Strong | Moderate |
| Cyano | L10 | Weak | Weak | Weak | Weak | Strong | Weak |

[1] For new generation high purity type B phases. Properties may vary for older generation type A phases

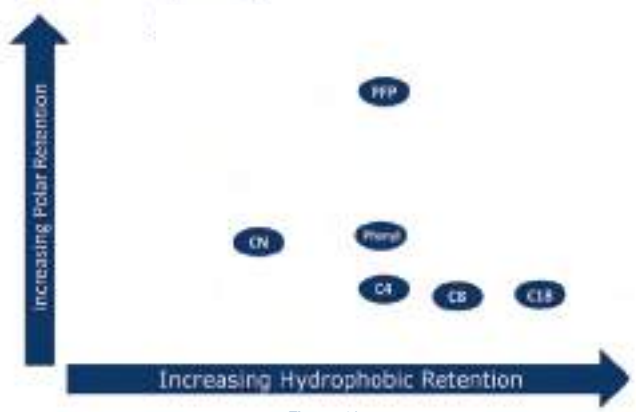



Figure 1*

Figure 1 shows a schematic representation of the relative selectivity expected of typical bonded phases with regards to hydrophobic and polar retention for specific test probes. This may not be representative of all phases in each category and for all analytes, but gives a useful general guideline.

** Based on the PQRI database (http://www.usp.org/app/USPNF/columnsDB.html) Plot of hydrophobicity (H) and cation exchange capacity at pH 7 (C @ pH 7.0)*

# CHARACTERISATION OF C18 PHASES



Hydrophobicity is the primary mechanism of analyte interaction with C18 and other alkyl-bonded stationary phases (see page 30). In addition, the polarity of the phase will also contribute to the overall selectivity observed.

## Hydrophobicity

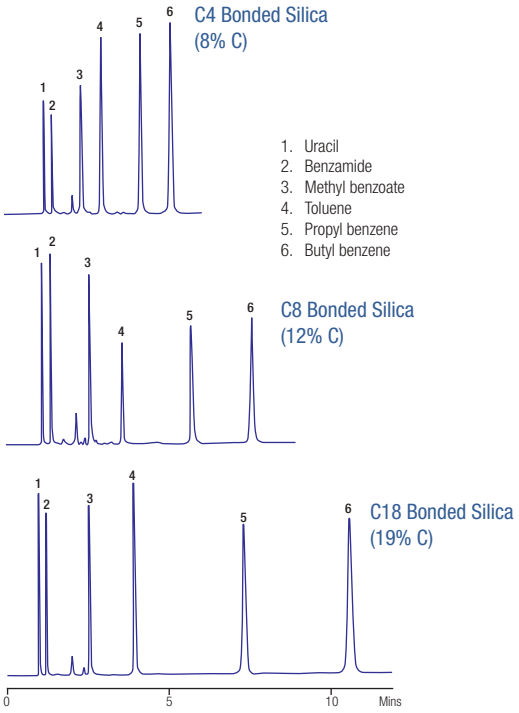
The strength of hydrophobic interaction can be measured by the retention of neutral (non-polar) molecules. The k values (retention factors) for a neutral species, for a given C18 phase, will give an indication of the surface area and surface coverage (ligand density) of the silica.

The percentage of carbon in the material is a simplistic but useful guide to the hydrophobic retention characteristics of a column. In Figure 1 this loose correlation is demonstrated by the increase in retention observed when alkyl chain length (i.e. carbon load) is increased. This increase results from an increase in hydrophobicity of the stationary phase. Similarly an increase in retention would be expected in going from a C18 phase with low carbon load to one of high carbon load.

Hydrophobic selectivity can be determined from the retention factor ratio between two neutral species. This is a better measure of surface coverage than carbon content, as surface area and porosity may vary from silica to silica.

## Polarity

The second key property of C18 materials is their silanol activity, often discussed in terms of polarity. This can be determined by measuring the retention factor ratio between a basic and an acidic compound. At pH >7 the total ion-exchange capacity will correspond to a measure of the total silanol activity. At acidic pH (e.g. pH 2.7) an indication of the acidic activity of the silanol groups can be obtained. The presence of metal ions in the base silica increases the level of silanol activity. Older generation silicas have higher and less tightly controlled levels of metal ions, and hence higher silanol activity compared to newer generation alkyl bonded phases. For this and other reasons, it is strongly recommended that new method development should be approached using newer generation higher purity silicas.




Column: 250 x 4.6 mm
Eluent: $CH_3CN$ - $H_2O$ (70:30)
Flow rate: 2ml/min.

Figure 1. Increase in retention with alkyl chain length

## High Purity Base Deactivated Phases

Modern alkyl bonded phases have very low cumulative metal ion levels within the base silica (<10ppm), resulting in the number of isolated silanol groups, and hence the polarity of the silica surface, also being reduced. Combined with more effective and reproducible bonding processes, these newer generation reversed-phase materials lead to significantly improved chromatography for the more basic polar solute molecules. Use of bonded alkyl groups containing hydrophilic substituents (i.e. polar embedded) can either enhance this effect and/or offer alternative selectivity.

## Optimising Selectivity

Figure 2 illustrates the relationship between the change in polarity and hydrophobicity for typical C18, C8 and C4 materials, showing a decrease in hydrophobicity on reducing alkyl chain length. Greater ligand density, and hence lower polarity, is also seen as the length of the alkyl chain is reduced. However, changing the alkyl chain length may reduce analysis time but will not significantly affect selectivity. Changing the chemistry to an alternative bonded phase is a more powerful tool to achieve this.

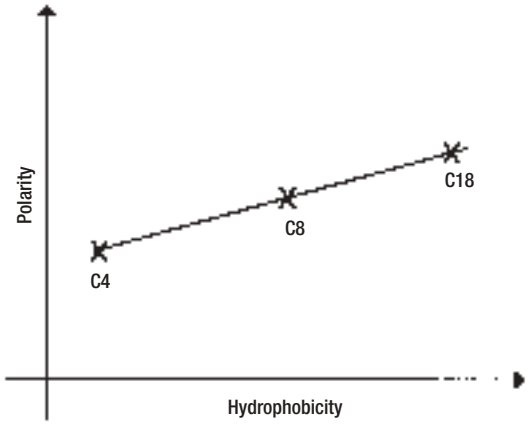


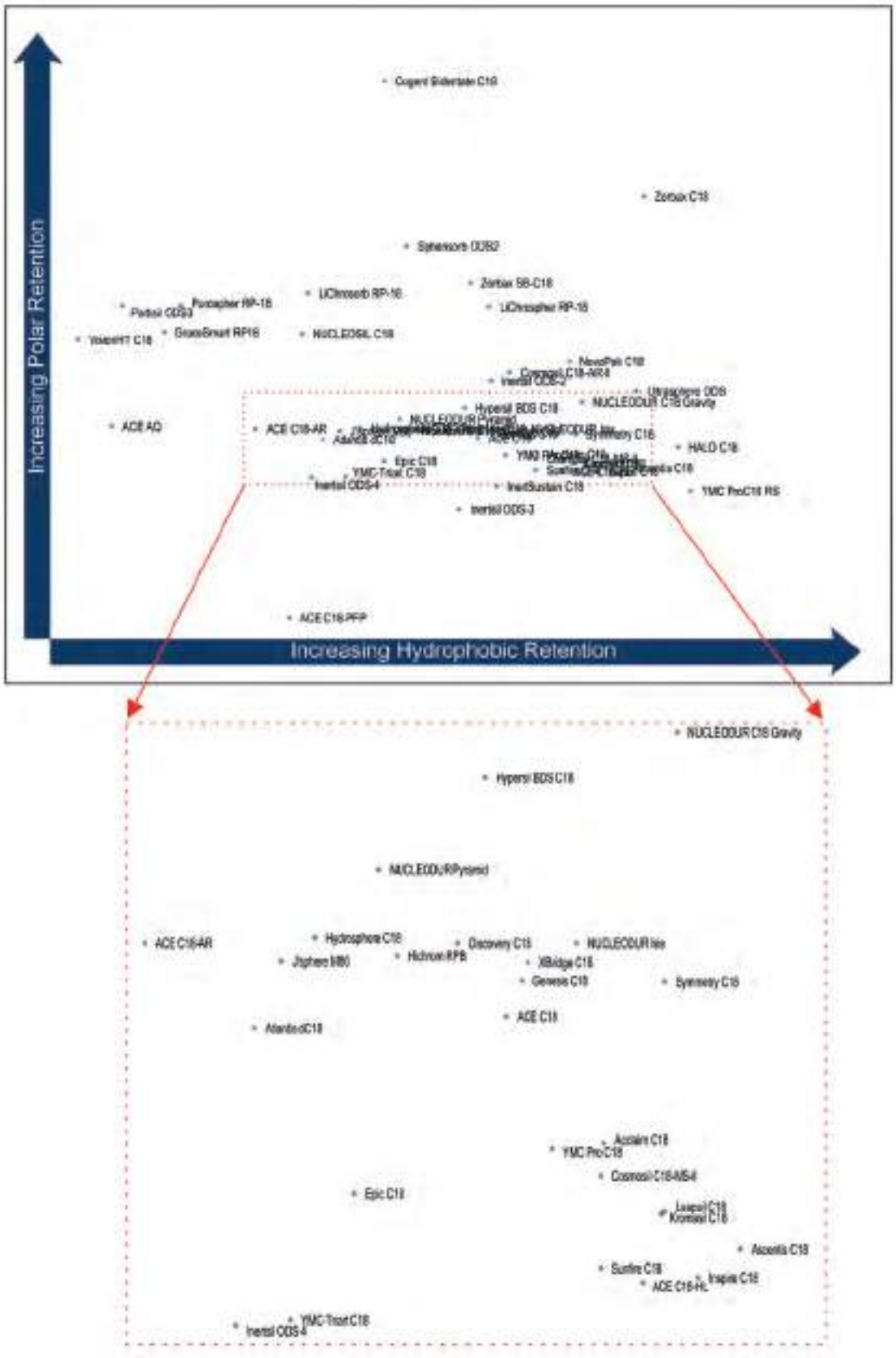

Figure 2. Variation of phase polarity with change in hydrophobicity

## Older Generation 'Traditional' Phases

The older 'traditional' C18 phases are hydrophobic and have a high polarity due to the lower purity silica containing a higher level of acidic silanol groups on which they are based. Use of the newer high purity silicas reduces the resultant phases' silanol activity and improves reproducibility. Employing a polar embedded functionality may also result in a reduced polarity material. For basic solutes that will interact strongly with surface silanols, lower polarity phases are generally recommended. However, for certain analyses, the additional interactions provided by the surface silanols of a 'traditional' C18 material may be beneficial to the overall separation.

## Characterisation of C18 Phases (continued)

Figure 3 is a plot showing the relative selectivity comparison for a number of C18 bonded columns, in which the hydrophobicity is plotted versus increasing polar retention.




**Based on the PQRI database (http://www.usp.org/app/USPNF/columnsDB.html)*
*Plot of hydrophobicity (H) and cation exchange capacity at pH 7 (C @ pH 7.0)*

Figure 3*

# SPECIFICATIONS OF C18 BONDED RP MATERIALS



Octadecyl (ODS) or C18 bonded phases are the most widely used reversed-phase materials. Table 1 lists the physical characteristics of a range of C18 bonded small pore silica phases.

## Table 1. Octadecylsilyl-bonded silica phases

| Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | Page |
|---|---|---|---|---|---|---|
| Acclaim C18 | 2.2, 3, 5 | 120 | 300 | 18 | Yes | 152 |
| Accucore C18[1] | 2.6 | 80 | 130 | 9 | Yes | 149 |
| Accucore XL C18[1] | 4 | 80 | 90 | 7 | Yes | 149 |
| ACE C18[2] | 2, 3, 5, 10 | 100 | 300 | 15.5 | Yes | 58, 71, 72 |
| ACE C18-HL | 3, 5, 10, 15 | 90 | 400 | 20 | Yes | 58, 64, 71, 72 |
| ACE C18-AR[2] | 2, 3, 5, 10 | 100 | 300 | 15.5 | Yes | 58, 60, 71, 72 |
| ACE C18-PFP[2] | 2, 3, 5, 10 | 100 | 300 | 14.3 | Yes | 58, 61, 71, 72 |
| ACE SuperC18[2,4] | 2, 3, 5, 10 | 90 | 400 | 14.8 | Yes | 58, 59, 71, 72 |
| Brownlee Spheri RP-18 | 5, 10 | 80 | 180 | 11 | Yes | 126 |
| Brownlee Spheri ODS | 5 | 80 | 180 | 14 | Yes | 126 |
| Brownlee SPP C18[1] | 2.7 | 90 | 150 | 8 | Yes | - |
| CAPCELL PAK ACR | 3, 5 | 80 | 340 | 18 | Yes | - |
| CAPCELL PAK AG C18 | 5 | 120 | 300 | 15 | Yes | - |
| CAPCELL PAK MG III C18 | 3, 5 | 100 | 260 | 15 | Yes | - |
| CAPCELL PAK SG C18 | 5 | 120 | 300 | 14 | Yes | - |
| CAPCELL PAK UG C18 | 3, 5 | 120 | 300 | 15 | Yes | - |
| Chromegabond BAS-C18 | 5 | 120 | 180 | 12 | No | 85 |
| Chromegabond C18 | 5 | 100 | 300 | 16 | No | 85 |
| Chromegabond MC-18 | 5 | 60 | 475 | - | Yes | 85 |
| Chromolith RP-18e | - | - | 300 | 18 | Yes | 110 |
| Cogent Bidentate C18 | 4 | 100 | 350 | 16.5 | No | 115, 117, 120 |
| COSMOSIL C18-AR-II | 3, 5 | 120 | 300 | 17 | Yes | 76, 79 |
| COSMOSIL C18-MS-II | 3, 5 | 120 | 300 | 16 | Yes | 76,79 |
| Develosil ODS-UG | 3, 5 | 140 | 300 | 18 | Yes | 80, 81 |
| Develosil ODS-MG | 3, 5 | 100 | 450 | 15 | Yes | 80, 81 |
| Develosil ODS-HG | 3, 5 | 140 | 300 | 18 | Yes | 80, 81 |
| Develosil ODS-SR | 3, 5 | 80 | - | 18 | Yes | 80, 81 |
| Endeavorsil C18 | 1.8 | 120 | 300 | 20 | Yes | 82, 83 |
| Epic C18 | 1.8, 3, 5, 10 | 120 | 230 | 18 | Yes | 85 |
| Epic C18-MS | 1.8, 3, 5, 10 | 120 | 350 | 22 | Yes | 85 |
| Epic C18-SD | 1.8, 3, 5, 10 | 120 | 350 | 24 | Yes | 85 |
| Exsil ODS | 3, 5, 10 | 100 | 200 | 11 | Yes | 86 |
| Exsil ODS1 | 3, 5 | 100 | 200 | 7 | No | 86 |
| Exsil ODSB | 3, 5 | 100 | 200 | 12 | Yes | 86 |
| Genesis C18 | 3, 4, 7 | 120 | 300 | 18 | Yes | 90 |
| GraceSmart C18 | 3, 5 | 120 | 220 | 10 | Yes | 90 |
| HALO C18[1] | 2.7 | 90 | 150 | 8 | Yes | 91 |
| HALO-5 C18[1] | 5 | 90 | 90 | 5.5 | Yes | 91 |
| HALO Peptide ES-C18[1] | 2.7 | 160 | 80 | 4.6 | Yes | 91 |
| HECTOR-M C18 | 3, 5, 10 | 100 | 320 | 17 | Yes | 2 |
| Hichrom C18 | 3.5, 5 | 150 | 250 | 15 | Yes | 92-96 |
| Hichrom RPB[3] | 3.5, 5, 10 | 110 | 340 | 14 | Yes | 97, 98 |
| Hydrosphere C18 | 2, 3, 5 | 120 | 340 | 12 | Yes | - |
| Hypersil ODS | 3, 5 | 120 | 170 | 10 | Yes | 151 |
| Hypersil BDS C18 | 2.4, 3, 5 | 130 | 170 | 7 | Yes | 151 |
| Inertsil ODS | 5 | 100 | 350 | 14 | Yes | 88 |
| Inertsil ODS-2 | 5 | 150 | 320 | 18.5 | Yes | 88, 89 |
| Inertsil ODS-3 | 2, 3, 4, 5 | 100 | 450 | 15 | Yes | 87, 88 |
| Inertsil ODS-4 | 2, 3, 5 | 100 | 450 | 11 | Yes | 87 |
| Inertsil ODS-P | 3, 5 | 100 | 450 | 29 | No | 87 |
| Inertsil Peptide C18 | 5 | 100 | 450 | 15 | Yes | 87 |
| Inertsil ODS-Sprint | 3, 5 | 100 | 450 | 8.5 | Yes | 87 |
| Inertsil Sulfa C18 | 3, 5 | 100 | 450 | 15 | Yes | 87 |
| InertSustain C18 | 2, 3, 5 | 100 | 350 | 14 | Yes | 87 |
| Inspire C18 | 3, 5, 10 | 100 | 440 | 27 | Yes | - |
| Kromasil C18 | 2.5, 3.5, 5, 10 | 100 | 320 | 20 | Yes | - |
| Kromasil Eternity C18 | 2.5, 5 | 100 | 330 | 14 | Yes | - |
| L-column ODS | 3, 5 | 120 | 340 | 17 | Yes | 101 |
| L-column2 ODS | 2, 3, 5 | 120 | 340 | 17 | Yes | 101 |

[1] Superficially porous phase [2] UHPLC compatible columns available as ACE Excel [3] Mixed alkyl mode C18/C8 [4] Superficially porous 2.5µm and 5µm also available

## Specifications of C18 Bonded RP Materials (continued)

### Table 1. Octadecylsilyl-bonded silicas (continued)

| Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | Page |
|---|---|---|---|---|---|---|
| Leapsil C18 | 2.7 | 100 | 440 | 27 | Yes | 82, 83 |
| LiChrosorb RP-18 | 5, 10 | 100 | 300 | 16.2 | No | 111 |
| LiChrospher RP-18 | 5 | 100 | 350 | 21.0 | No | 112, 113 |
| LiChrospher RP-18e | 5 | 100 | 350 | 21.6 | Yes | 112, 113 |
| NUCLEODUR C18 Gravity | 1.8, 3, 5 | 110 | 340 | 18 | Yes | 102 |
| NUCLEODUR C18 ec | 3, 5 | 110 | 340 | 17.5 | Yes | 102 |
| NUCLEODUR C18 Isis | 1.8, 3, 5 | 110 | 340 | 20 | Yes | 102 |
| NUCLEODUR C18 PAH | 1.8, 3 | 110 | 340 | proprietary | Yes | 102 |
| NUCLEODUR C18 HTec | 1.8, 3, 5, 7, 10 | 110 | 340 | 18 | Yes | 102 |
| NUCLEOSHELL RP 18[1] | 2.7 | 90 | 130 | 7.5 | Yes | 102 |
| NUCLEOSIL C18 | 3, 5, 7, 10 | 100 | 350 | 15 | Yes | 103, 104 |
| NUCLEOSIL C18 | 3, 5, 7, 10 | 120 | 200 | 11 | Yes | 103, 105 |
| NUCLEOSIL C18 AB | 5 | 100 | 350 | 25 | Yes | 103, 104 |
| NUCLEOSIL C18 HD | 3, 5 | 100 | - | 20 | Yes | - |
| Partisil ODS | 10 | - | - | - | - | 121-125 |
| Partisil ODS2 | 10 | - | - | - | - | 121-125 |
| Partisil ODS3 | 5, 10 | - | - | - | - | 121-125 |
| Partisphere C18 | 5 | - | - | - | - | 122, 123 |
| PrincetonSPHER C18 | 3, 5, 10 | 60, 100 | 500, 325 | 23, 19 | Yes | 134, 135 |
| Purospher RP-18 | 5 | 90 | 500 | 18.5 | Yes | 110 |
| Purospher RP-18e | 5 | 120 | 350 | 18 | Yes | 110 |
| Purospher STAR RP-18e | 2, 3, 5 | 120 | 330 | 17 | Yes | 110 |
| Spursil C18 | 3, 5, 10 | 100 | 440 | 25 | Yes | 82, 83 |
| Spursil C18-EP | 3, 5, 10 | 100 | 440 | 24 | Yes | 82, 83 |
| Symmetry C18 | 3.5, 5 | 100 | 335 | 19 | Yes | - |
| Syncronis C18 | 1.7, 3, 5 | 100 | 320 | 16 | Yes | 151 |
| TSKgel ODS-140HTP | 2.3 | 140 | - | 8 | Yes | 154 |
| TSKgel ODS-100V | 3, 5 | 100 | - | 15 | Yes | 154 |
| TSKgel ODS-100Z | 3, 5 | 100 | - | 20 | Yes | 154 |
| TSKgel ODS-80TM | 5, 10 | 80 | - | 15 | Yes | - |
| TSKgel ODS-80TS | 5, 10 | 80 | - | 15 | Yes | - |
| TSKgel Super-ODS | 2.3 | 110 | - | 8 | Yes | 154 |
| TSKgel ODS-120T | 5, 10 | 120 | - | 22 | Yes | - |
| TSKgel ODS-120A | 5, 10 | 120 | - | 20 | Yes | - |
| Ultrasphere ODS | 3, 5 | 80 | - | - | Yes | 156, 157 |
| Vydac Denali | 3, 5, 10 | 120 | - | 20 | Yes | - |
| Waters µBondapak C18 | 10 | 125 | 330 | 10 | Yes | - |
| Waters Nova-Pak C18 | 4 | 60 | 120 | 7 | Yes | - |
| Waters Spherisorb ODS1 | 3, 5, 10 | 80 | 220 | 6.2 | No | 160, 161 |
| Waters Spherisorb ODS2 | 3, 5, 10 | 80 | 220 | 11.5 | Yes | 160, 161 |
| Waters Spherisorb ODSB | 5 | 80 | 220 | 11.5 | Yes | 160, 161 |
| YMC J-sphere ODS-L80 | 4 | 80 | 510 | 9 | Yes | - |
| YMC J-sphere ODS-M80 | 4 | 80 | 510 | 14 | Yes | - |
| YMC J-sphere ODS-H80 | 4 | 80 | 510 | 22 | Yes | - |
| YMC ODS-A | 3, 5 | 120 | 330 | 17 | Yes | - |
| YMC ODS-AL | 3, 5 | 120 | 330 | 17 | No | - |
| YMC ODS-AM | 3, 5 | 120 | 330 | 17 | Yes | - |
| YMC ODS-AQ | 3, 5 | 120 | 330 | 14 | Yes | - |
| YMC ProC18 | 2, 3, 5 | 120 | 330 | 17 | Yes | - |
| YMC ProC18RS | 3, 5 | 80 | 510 | 22 | Yes | - |
| YMC-Triart C18 | 1.9, 3, 5 | 120 | - | 16 | Yes | - |
| ZORBAX ODS | 5 | 70 | 330 | 20 | Yes | 165, 166 |
| ZORBAX SB-C18 | 1.8, 3.5, 5, 7 | 80 | 180 | 10 | No | - |
| ZORBAX Extend-C18 | 1.8, 3.5, 5 | 80 | 180 | 12.5 | Yes | - |
| ZORBAX Eclipse XDB-C18 | 1.8, 3.5, 5 | 80 | 180 | 10 | Yes | - |
| ZORBAX Eclipse Plus C18 | 1.8, 3.5, 5 | 95 | 160 | 8 | Yes | - |

[1] Superficially porous phase

# SPECIFICATIONS OF C1 TO C8 & C30 BONDED REVERSED-PHASE MATERIALS

Octyl-bonded phases are the most common medium polarity alternative to C18 bonded phases. Very short chain alkyl-bonded phases are less stable. The shorter the alkyl chain the greater the vulnerability of the material to aqueous dissolution at high pH or loss of bonded phase at low pH. For wide pore silicas the C4 chemistry retains high popularity. Table 1 lists the physical characteristics of a range of C1 to C8 bonded and C30 bonded narrow pore silica phases. For wide pore (300Å) phases see page 38.

## Table 1. Short chain alkyl (C1 to C8) and C30 bonded silica phases

| Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | Page |
|---|---|---|---|---|---|---|
| **C1 Bonded** | | | | | | |
| CAPCELL PAK C1 UG | 5 | 120 | 300 | 7 | No | - |
| Chromegabond TMS | 5 | 60 | 475 | - | No | - |
| Develosil TMS-UG | 3, 5 | 140 | 300 | 4.5 | Yes | 80, 81 |
| Exsil C1 | 3, 5 | 100 | 200 | 3 | No | 86 |
| Hypersil SAS | 5 | 120 | 170 | 2.5 | No | 151 |
| Kromasil C1 | 5 | 100 | 320 | 4.7 | Yes | - |
| ProntoSIL C1 | 3, 5 | 120 | 300 | 2 | No | - |
| Waters Spherisorb C1 | 3, 5 | 80 | 220 | 2.2 | No | 160, 161 |
| YMC TMS | 3, 5 | 120 | 330 | 4 | No | - |
| ZORBAX TMS | 5 | 70 | 330 | 4 | Yes | 165, 166 |
| **C2 Bonded** | | | | | | |
| Chromegabond C2 | 5 | 60 | 480 | - | No | 85 |
| NUCLEOSIL C2 | 7 | 100 | 350 | 3.5 | No | 103, 104 |
| **C3 Bonded** | | | | | | |
| ZORBAX SB-C3 | 5 | 80 | 180 | 4 | No | - |
| **C4 Bonded** | | | | | | |
| ACE C4 | 2[2], 3, 5, 10 | 100 | 300 | 5.5 | Yes | 58, 71, 72 |
| Epic C4-SD | 1.8, 3, 5, 10 | 120 | 350 | 8 | Yes | 85 |
| HECTOR-M C4 | 3, 5, 10 | 100 | 320 | 3 | Yes | 2 |
| Inertsil C4 | 5 | 150 | 320 | 7.5 | Yes | 88, 89 |
| Kromasil C4 | 2.5, 3.5, 5, 7, 10 | 100 | 320 | 8 | Yes | - |
| PrincetonSPHER C4 | 3, 5, 10 | 60, 100 | 500, 325 | 8, 6 | No | 134, 135 |
| ProntoSIL C4 | 3, 5 | 120 | 300 | 4 | No | - |
| YMC C4 | 3, 5 | 120 | 330 | 7 | Yes | - |
| YMC ProC4 | 3, 5 | 120 | 340 | 7 | Yes | - |
| **C6 Bonded** | | | | | | |
| Chromegabond C6 | 5 | 60 | 220 | 6 | No | 85 |
| Chromegabond MC-CC6 | 5 | 60 | 475 | 7 | Yes | - |
| PrincetonSPHER C6 | 3, 5, 10 | 60, 100 | 500, 325 | 10, 8 | Yes | 134, 135 |
| Waters Spherisorb C6 | 3, 5 | 80 | 220 | 4.7 | Yes | 160, 161 |
| **C8 Bonded** | | | | | | |
| Acclaim C8 | 2.2, 3, 5 | 120 | 300 | 11 | Yes | 152 |
| Accucore C8[1] | 2.6 | 80 | 130 | 5 | Yes | 149 |
| ACE C8 | 2[2], 3, 5, 10 | 100 | 300 | 9.0 | Yes | 58, 71, 72 |
| AquaSep | 3, 5 | 100 | 450 | 16 | - | 85 |
| Brownlee Spheri RP-8 | 5, 10 | 80 | 180 | 6 | Yes | 126 |
| Brownlee SPP[1] | 2.7 | 90 | 150 | 7.7 | Yes | - |
| CAPCELL PAK C8 UG | 5 | 120 | 300 | 10 | Yes | - |
| CAPCELL PAK C8 AG | 5 | 120 | 300 | 10 | Yes | - |
| CAPCELL PAK C8 DD | 5 | 80 | 300 | 11 | Yes | - |
| CAPCELL PAK C8 SG | 5 | 120 | 300 | 10 | Yes | - |
| Chromegabond BAS-C8 | 5 | 100 | 300 | 8 | No | - |
| Chromegabond C8 | 5 | 100 | 300 | 8 | No | 85 |
| Chromegabond C8-BD | 5 | 100 | 475 | 12 | No | - |
| Chromolith RP-8e | - | - | 300 | 11 | Yes | 110 |
| Cogent Bidentate C8 | 4 | 100 | 350 | 7 | No | 115, 117, 120 |
| Develosil UG C8 | 5 | 140 | 300 | 11 | Yes | 80, 81 |
| Epic C8 | 1.8, 3, 5, 10 | 120 | 230 | 10 | Yes | 85 |
| Exsil C8 | 3, 5 | 100 | 200 | 6 | Yes | 86 |
| Genesis C8 | 3, 4, 7 | 120 | 300 | 11 | No | 90 |
| Genesis C8 e/c | 3, 4, 7 | 120 | 300 | 11 | Yes | 90 |
| HALO C8[1] | 2.7 | 90 | 150 | 5.4 | Yes | 91 |
| HALO-5 C8[1] | 5 | 90 | 90 | 3.7 | Yes | 91 |
| HECTOR-M C8 | 3, 5, 10 | 100 | 320 | 10 | Yes | 2 |

[1] Superficially porous phases [2] As ACE Excel column

## Specifications of C1 to C8 & C30 Bonded Reversed-Phase Materials (continued)

### Table 1. Short chain alkyl (C1 to C8) and C30 bonded silica phases (continued)

| Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | Page |
|---|---|---|---|---|---|---|
| **C8 Bonded** | | | | | | |
| Hichrom C8 | 3.5, 5 | 150 | 250 | 8 | Yes | 92-96 |
| Hypersil MOS | 3, 5 | 120 | 170 | 6.5 | No | 151 |
| Hypersil MOS-2 | 5 | 120 | 170 | 6.5 | Yes | 151 |
| Hypersil BDS C8 | 2.4, 3, 5 | 130 | 170 | 11 | Yes | 151 |
| Hypersil GOLD C8 | 1.9, 3, 5 | 175 | 220 | 8 | Yes | 150 |
| Inertsil C8 | 5 | 150 | 320 | 10.5 | Yes | 88 |
| Inertsil C8-3 | 2, 3, 5 | 100 | 450 | 9 | Yes | 87, 88 |
| Inertsil C8-4 | 2, 3, 5 | 100 | 450 | 5 | Yes | 87 |
| InertSustain C8 | 2, 3, 5 | 100 | 350 | 8 | Yes | 87 |
| Inspire C8 | 3, 5, 10 | 100 | 440 | 17 | Yes | 82, 83 |
| Kromasil C8 | 2.5, 3.5, 5, 10 | 100 | 320 | 12 | Yes | - |
| L-column C8 | 5 | 120 | 340 | 10 | Yes | 101 |
| L-column2 C8 | 5 | 120 | 340 | 10 | Yes | 101 |
| LiChrosorb RP-8 | 5, 10 | 100 | 300 | 9.5 | No | 111 |
| LiChrospher RP-8 | 5 | 100 | 350 | 12.5 | No | 112, 113 |
| LiChrospher RP-8e | 5 | 100 | 350 | 13 | Yes | 112, 113 |
| Nova-Pak C8 | 4 | 60 | 120 | 4 | Yes | - |
| NUCLEODUR C8 Gravity | 1.8, 5 | 110 | 340 | 11 | Yes | 102 |
| NUCLEODUR C8 ec | 3, 5 | 110 | 340 | 10.5 | Yes | 102 |
| NUCLEOSIL C8 | 5, 7, 10 | 100 | 350 | 8.5 | No | 103, 104 |
| NUCLEOSIL C8 | 3, 5, 7, 10 | 120 | 200 | 6.5 | No | 103, 105 |
| NUCLEOSIL C8 HD | 5 | 100 | - | 13 | Yes | - |
| Partisil C8 | 5, 10 | - | - | - | - | 121-125 |
| Partisphere C8 | 5 | - | - | - | - | 122, 123 |
| PrincetonSPHER C8 | 3, 5, 10 | 60, 100 | 500, 325 | 15, 11 | Yes | 134, 135 |
| Symmetry C8 | 3.5, 5 | 100 | 335 | 12 | Yes | - |
| Syncronis C8 | 1.7, 3, 5 | 100 | 320 | 10 | Yes | 151 |
| TSKgel Octyl-80Ts | 5 | 80 | - | 11 | Yes | - |
| TSKgel Super-Octyl | 2.3 | 110 | - | 5 | Yes | 154 |
| Ultrasphere C8 | 3, 5 | 80 | - | - | Yes | 156, 157 |
| Waters Spherisorb C8 | 3, 5, 10 | 80 | 220 | 5.8 | Yes | 160, 161 |
| YMC Basic | 3, 5 | proprietary | proprietary | 8 | Yes | - |
| YMC C8 | 3, 5 | 120 | 330 | 10 | Yes | - |
| YMC ProC8 | 3, 5 | 120 | 340 | 10 | Yes | - |
| YMC-Triart C8 | 1.9, 3, 5 | 120 | - | 7 | Yes | - |
| ZORBAX C8 | 5 | 70 | 330 | 12 | Yes | 165, 166 |
| ZORBAX Eclipse Plus C8 | 1.8, 3.5, 5 | 95 | 160 | 7 | Yes | - |
| ZORBAX Eclipse XDB-C8 | 1.8, 3.5, 5 | 80 | 180 | 7.6 | Yes | - |
| ZORBAX Rx-C8 | 5 | 80 | 180 | 5.5 | No | - |
| ZORBAX SB-C8 | 1.8, 3.5, 5 | 80 | 180 | 5.5 | No | - |
| **C30 Bonded** | | | | | | |
| Acclaim C30 | 3, 5 | 200 | 200 | 13 | Yes | 152 |
| Accucore C30[1] | 2.6 | 150 | 80 | 5 | Yes | 149 |
| Cogent C30[2] | 3, 5 | 200 | - | 18 | No | 119 |
| Develosil RPAQUEOUS | 3, 5 | 140 | 300 | 18 | Yes | 80, 81 |
| Develosil RPAQUEOUS-AR | 3, 5 | 140 | 300 | 18 | Yes | 80, 81 |
| Develosil XG-C30 | 3, 5 | 140 | 300 | 19.5 | Yes | 80, 81 |
| ProntoSil C30 | 3, 5, 10 | 200 | 200 | 20 | No | - |
| PrincetonSPHER C30[2] | 3, 5, 10 | 200 | 200 | 19 | No | 134, 135 |
| YMC Carotenoid | 3, 5 | proprietary | proprietary | - | - | - |

[1] Superficially porous phases

[2] C27 phase also available

# POLAR EMBEDDED AND OTHER 'AQ' TYPE PHASES



## Introduction

When separating very polar, water-soluble compounds, eluents containing less than 5% organic modifier are commonly used to achieve sufficient retention. However, operation under such highly aqueous conditions can lead to poor chromatographic reproducibility and decreasing retention times. Conventional C8 and C18 phases undergo dewetting or 'phase collapse' under these conditions, resulting in a reduction of accessible bonded phase. This phenomenon may either occur very quickly or more gradually.

## 'High Aqueous' Phases

Approaches to address this problem include embedding a polar group in the alkyl chain or using hydrophilic (polar) endcapping reagents (see Figure 1). Both these approaches, or the use of a C30 phase, have the effect of maintaining the phase surface under fully wetted conditions, even when using 100% aqueous eluent. Polar embedded phases are also used to obtain different selectivity from conventional C18 phases.


Figure 1. Polar embedded (A) and hydrophilic endcapped (B) phases

## Good Retention and Resolution for Polar Compounds

Compared to traditional alkyl phases these 'high aqueous' phases are resistant to retention loss when using highly aqueous eluents, even after several days or weeks. Reproducible retention times and improved peak shapes are achieved for acidic, basic and zwitterionic analytes.

## Alternative Selectivity

Conventional C18 phases depend primarily on differing hydrophobic interactions between analytes and the stationary phase to provide separation. 'AQ' type phases may also show hydrophilic (polar) interactions via H-bonding and dipole-dipole forces. This can influence retention time and improve selectivity for polar analytes.

## Eliminate Need for Ion-pair Additives

Many separations of very polar analytes are performed using ion-pair chromatography in order to provide adequate retention. The use of an 'AQ' phase generally enables reproducible results to be obtained using conventional aqueous/organic eluents.

## Typical Applications

Typical applications of these 'AQ' type phases include carboxylic acids, water soluble vitamins, catecholamines, nucleic acid bases and various polar pharmaceuticals.

## 'AQ' Type Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Comments | Page |
|---|---|---|---|---|---|
| Acclaim PolarAdvantage | Thermo Scientific | 2.2, 3, 5 | 120 | Embedded sulphonamide group | 152 |
| Acclaim PolarAdvantage II | | 2.2, 3, 5 | 120 | Embedded amide group | 152 |
| Accucore AQ[1] | | 2.6 | 80 | C18 with polar endcapping | 149 |
| Accucore Polar Premium[1] | | 2.6 | 150 | Amide embedded C18 | 149 |
| ACE AQ | Advanced Chromatography Technologies (ACT) | 2[2], 3, 5, 10 | 100 | C18 with integral polar functionality | 58, 64, 71, 72 |
| ACE C18-Amide | | 2[2], 3, 5, 10 | 100 | C18 with integral amide polar group | 58, 62, 71, 72 |
| ACE C18-AR | | 2[2], 3, 5, 10 | 100 | C18 with integral phenyl group | 58, 60, 71, 72 |
| AquaSep | ES Industries | 3, 5 | 100 | C8 with embedded ether group | 85 |
| Chromegabond ODS-PI | | 3, 5 | 120 | Ureide embedded polar group | 85 |
| CAPCELL PAK C18 AQ | Shiseido | 3, 5 | 80 | C18 | - |
| COSMOSIL C18-PAQ | Nacalai Tesque | 5 | 120 | C18 phase with polymeric linkage | 76, 79 |
| Develosil RPAQUEOUS | Nomura | 3, 5 | 140 | C30, monofunctional | 80, 81 |
| Develosil RPAQUEOUS-AR | | 3, 5 | 140 | C30, trifunctional | 80, 81 |
| Epic Polar | ES Industries | 1.8, 3, 5, 10 | 120 | Embedded ether group | 85 |
| HALO RP-Amide[1] | Advanced Materials Technology | 2.7 | 90 | Polar embedded amide | 91 |
| Hydrosphere C18 | YMC | 2, 3, 5 | 120 | Hydrophilic C18 surface | - |
| Hypersil GOLD AQ | Thermo Scientific | 1.9, 3, 5, 8 | 175 | Alkyl chain with polar endcapping | 150 |
| Inertsil ODS-EP | GL Sciences | 5 | 100 | C18 phase with polar embedded group | 87 |
| NUCLEODUR C18 Pyramid | Macherey-Nagel | 1.8, 3, 5 | 110 | C18 with hydrophilic endcapping | 102 |
| NUCLEODUR PolarTec | | 1.8, 3, 5 | 110 | Polar embedded group | 102 |
| NUCLEOSIL Nautilus | | 3, 5 | 100 | C18 with polar embedded group | - |
| NUCLEOSIL Protect 1 | | 5 | 100 | Protective polar group | - |
| Princeton ULTIMA C18, C8 & Phenyl | Princeton Chromatography | 3, 5, 10 | - | Embedded polar amide functionality | 134, 135 |
| ProntoSil C18 (or C8) ace-EPS | Bischoff | 3, 5 | 120 | C18 or C8 with embedded amide group | - |
| ProTec-RP | ES Industries | 3, 5 | 100 | C8, C18 or Phenyl with embedded amide group | - |
| Spursil C18 and C18-EP | Dikma Technologies | 3, 5, 10 | 100 | C18 with proprietary polar modification | 82, 83 |
| SymmetryShield | Waters | 3.5, 5 | 100 | C18 or C8 with polar embedded group | - |
| Syncronis aQ | Thermo Scientific | 1.7, 3, 5 | 100 | C18 with polar endcapping | 151 |
| YMC ODS-AQ | YMC | 3, 5 | 120 | C18 with hydrophilic endcapping | - |
| ZORBAX Bonus-RP | Agilent Technologies | 1.8, 3.5, 5 | 80 | C14 chain with embedded amide group | - |
| ZORBAX SB-Aq | | 1.8, 3.5, 5 | 80 | Proprietary | - |

[1] Superficially porous phases [2] As ACE Excel column

# WIDE PORE (300Å) REVERSED-PHASE MATERIALS



## Introduction

In order for a sample molecule to freely access the interior of the pores of the packing material, its diameter must be smaller than the average pore diameter. For high molecular weight solutes, the use of lower pore size materials of 60-120Å may result in frictional drag within the pore, leading to restricted diffusion and reduced column efficiency.

The use of larger pore silica-based bonded phases therefore leads to improvements in resolution, capacity and recovery of proteins and other biomolecules, due to a reduction in size exclusion mechanism and enhanced molecular diffusion rates. A pore size of 300Å has become the accepted standard for wide pore silicas, and has been found to be suitable for a broad range of molecular weight proteins, peptides and oligonucleotides. In general, peptides exceeding approximately 50 amino acids and oligonucleotides greater than 25 residues are preferentially analysed on 300Å materials. Separations of very large biomolecules (MW >100,000Da) may require larger pore size packings (500 to 4000Å).

## Bonded Phases

Alkyl-bonded silica phases are the most commonly used materials for the reversed-phase separation of biomolecules. The shorter C4 phases are generally recommended for large hydrophobic peptides and most proteins. Peptide maps, natural and synthetic peptides and small hydrophilic proteins are best chromatographed on C8 columns. C18 columns are often chosen for the analysis of small peptides. Other bonded wide pore phases, including cyano and phenyl, are available in some brands. The table below summarises a range of wide pore alkyl-bonded reversed-phase silica materials. Ion-exchange and size exclusion packings are also available as wider pore materials (please contact us for details).

## Column Dimensions

Wide pore silica phases are available in a range of column dimensions from rapid analysis to preparative and process scale. Increased column capacity favours these wide pore materials for preparative separations of samples with molecular weight >5,000Da.

## Separation Mechanism

In reversed-phase chromatography, proteins are retained by adsorption of the face of the protein (hydrophobic foot) to the hydrophobic surface of the packing material. The adsorption/desorption mechanism differs from that of small molecules, in that small changes in organic solvent composition can rapidly change the protein retention, thereby requiring use of shallow gradients. Proteins adsorb near the top of the column (Figure 1A) and remain adsorbed until the organic concentration reaches a high enough level for the protein to desorb (Figure 1B) and elute from the column.

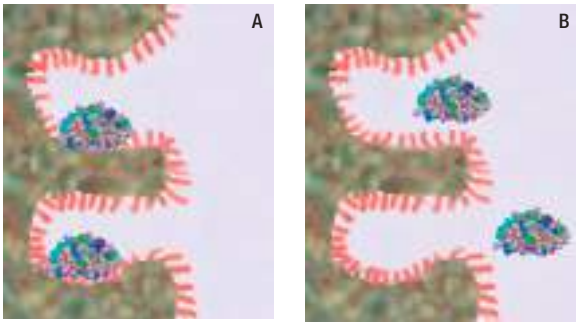



Figure 1. Adsorption and desorption of protein molecules

## 300Å Reversed-Phase Alkyl-Bonded Silica Phases

| Phase | Manufacturer | Particle Size (µm) | Surface Area ($m^2/g$) | Carbon Load (%) | Page |
|---|---|---|---|---|---|
| Acclaim C18 | Thermo Scientific | 3 | 100 | 8 | 152 |
| ACE[1] C4-300, C8-300, C18-300 | ACT | 3, 5, 10 | 100 | 2.6, 5, 9 | 58, 67, 73 |
| Aquapore Butyl, Octyl, ODS | Perkin Elmer | 7 | 100 | 3, 5, 10 | 126 |
| BioBasic 4, 8, 18 | Thermo Scientific | 5 | 100 | 4, 5, 9 | 153 |
| Bio-Bond C4, C8, C18 | Dikma Technologies | 3, 5, 10 | 100 | 3, 5, 8 | 82, 83 |
| Cogent Bidentate C8 300 | MicroSolv | 5 | 150 | 5 | 115, 117, 120 |
| COSMOSIL C18-AR-300, C8-AR-300, C4-AR-300 | Nacalai Tesque | 5 | 150 | 12, 7, 6 | 79 |
| Eprogen RP8 | Eprogen | 5 | - | - | 84 |
| HECTOR-W C-18, C8, C4, $NH_2$ | RStech Corporation | 3, 5, 10 | - | 7, 4, 3, - | 2 |
| Inertsil[1] WP300-C4, C8, C18 | GL Sciences | 5 | 150 | 3, 8, 9 | - |
| Kromasil[1] C4, C8, C18 | Akzo Nobel | 5, 10, 16 | 110 | 2.9, 4.7, 8.7 | - |
| NUCLEOSIL 300 C4, C8, C18 | Macherey-Nagel | 5, 7, 10 | 100 | 2, 3, 6.5 | 103, 106 |
| PrincetonSPHER-300 C18, C8, C4, Phenyl, CN, $NH_2$, Diol, Silica | Princeton Chromatography | 5, 10 | 100 | - | 134, 135 |
| TSKgel Protein C4-300 | Tosoh Bioscience | 3 | 100 | 3 | 154 |
| Vydac 201TP | Grace | 3, 5, 10 | - | 8 | 90 |
| Vydac 202TP | | 3, 5, 10 | - | 9 | 90 |
| Vydac[1] 208TP, 208MS, 214TP, 214MS, 218TP, 218MS | | 3, 5, 10 | - | - | 90 |
| Vydac Everest C18 | | 5, 10 | - | 6 | 90 |
| YMC[1] C4, C8, ODS-A | YMC | 5 | 100 | 3, 4, 7 | - |
| ZORBAX 300SB-C3, C8, C18 | Agilent Technologies | 3.5, 5, 7 | 45 | 1.1, 1.5, 2.8 | - |
| ZORBAX 300-Extend | | 3.5, 5 | 45 | 4 | - |
| ZORBAX Poroshell 300SB-C3, C8, C18 | | 5 | - | - | - |

[1] Other wide pore bonded phases available

# HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC) PHASES

## Introduction

Hydrophobic Interaction Chromatography (HIC) is a powerful technique for the separation and purification of proteins and peptides. Separations are based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. Although the separation mechanism of HIC has similarities with that of standard reversed-phase HPLC, the density of the bonded phase ligands on the surface of the HIC packing material is much lower. HIC therefore involves weaker interactions and weaker eluents can be used. Samples are adsorbed to the HIC resin at relatively high salt concentrations and eluted by applying a linear or stepwise decreasing salt gradient. The mild conditions used in HIC typically maintain tertiary protein structure and thus biological activity (ie. no denaturation).

## Selectivity

An optimum HIC separation will combine high dynamic binding capacity (DBC), adequate selectivity, good mass recovery and retention of biological activity. Proteins show varying degrees of hydrophobicity depending on their amino acid composition, structure and size. Separation can be optimised by varying the nature of the HIC phase or by varying the eluent. Very hydrophilic proteins are generally purified using highly hydrophobic stationary phases, whereas very hydrophobic proteins are separated using the least hydrophobic phases.

## Method Development

In addition to the hydrophobicity of the phase ligand, several parameters affect HIC separations. These include salt type, pH, buffer concentration, temperature and gradient. Ammonium sulphate (1 or 2M) or sodium chloride (3M) salts are most commonly used for HIC applications. The pH of the salt solution will influence retention; pH 7.0 is a good starting point. Figure 1 shows the influence of pH for various salts on the DBCs of lysozyme.

## Applications

Hydrophobic interaction chromatography is suitable for the separation and purification of a wide range of biomolecules. In addition to proteins, antibody fragments, RNAs, antibiotics etc. can be analysed by HIC. HIC can also be used for protein desalting. Figure 2 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column.

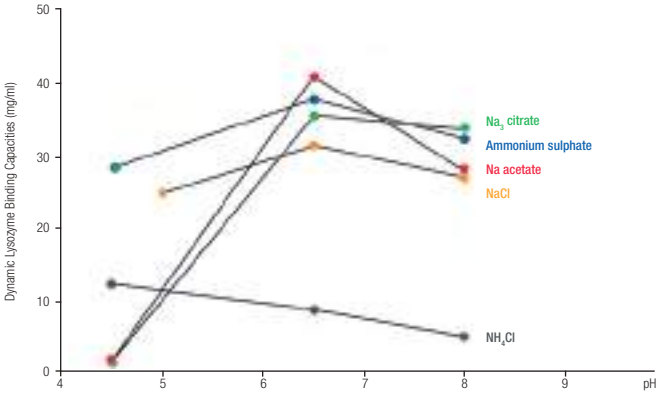
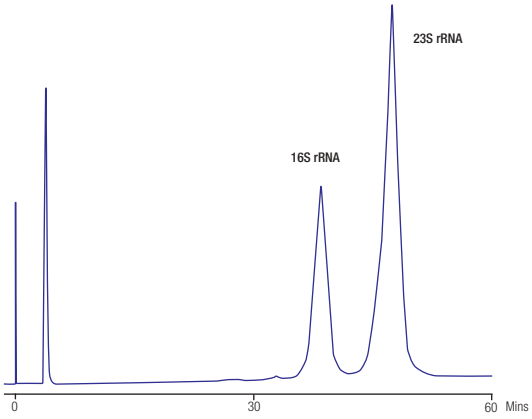



Figure 1. Influence of pH




Column: TSKgel Phenyl-5PW (75 x 7.5mm)
Eluent: Linear gradient from 2mol/l to 0mol/l $(NH_4)_2SO_4$ in 0.1mol/l phosphate buffer, pH 7.0
Flow rate: 0.5ml/min
Detection: UV, 280nm

Figure 2. Separation of RNAs on TSKgel Phenyl-5PW

## HIC Phases

| Phase | Manufacturer | Base material | Bonding | Particle Size (µm) | Pore Size (Å) | Page |
|---|---|---|---|---|---|---|
| COSMOSIL HIC | Nacalai Tesque | Silica | Diol | 5 | 300 | 76, 79 |
| HIC PH-814 | Shodex | Polyhydroxymethacrylate | Phenyl | 10 | 2,000 | 142 |
| MCI GEL CQH Series | Mitsubishi Chemicals | Polyhydroxymethacrylate | Ether, Butyl, Phenyl | 10 | 600 | 108 |
| PolyPROPYL A | PolyLC | Silica | Propylaspartamide | 3, 5, 12 | 300, 1000, 1500 | 127, 130 |
| PolyETHYL A | | | Ethylaspartamide | | | 127, 130 |
| PolyMETHYL A | | | Methylaspartamide | | | 127, 130 |
| ProPac HIC-10 | Thermo Scientific | Silica | Amide/ethyl | 5 | 300 | 153 |
| TSKgel | Tosoh Bioscience | Methacrylate | Ether, Phenyl | 10, 13, 20 | 1,000 | 155 |
| | | | Butyl-NPR | 2.5 | - | 155 |

# PHENYL BONDED PHASES

Phenyl bonded silica phases offer an alternative reversed-phase selectivity to alkyl bonded phases. They show lower hydrophobic retention than their C18 counterparts, with similar retention characteristics to C8-bonded phases. Phenyl stationary phases interact with compounds containing aromatic groups or unsaturated bonds through the involvement of $\pi$-$\pi$ interactions. For aromatic solutes containing an electronegative atom or group (e.g. F, $NO_2$), the degree of $\pi$-$\pi$ interactions with the phenyl phase will increase.

Due to the rigid nature of the phenyl ring, solute shape can also influence selectivity.

Traditional phenyl phases tend to be less stable than the corresponding C8 or C18 reversed-phases. Additionally, the larger steric size of the phenyl group reduces surface coverage, leaving a greater number of exposed silanol sites. More recently introduced phenyl phases show greater stability. The use of a purer silica base, more effective and reproducible bonding procedures and the availability of a sterically protected phenylsilane all contribute to greater phase robustness and reduced column bleed.

Conventional phenyl phases are bonded to the silica through a propyl spacer. The incorporation of the longer chain hexyl spacer results in increased hydrophobic retention and aromatic selectivity. Phenyl bonded (mainly with propyl linker) and Phenyl-Hexyl bonded phases are listed in separate tables below.

## Phenyl Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| Acclaim Phenyl-1 | Thermo Scientific | 3 | 120 | 300 | 152 |
| Accucore Phenyl-X[1] | | 2.6 | 80 | 130 | 149 |
| ACE C18-AR[2] | Advanced Chromatography Technologies (ACT) | 2[3], 3, 5, 10 | 100 | 300 | 58, 60, 71, 72 |
| ACE Phenyl | | 2[3], 3, 5, 10 | 100 | 300 | 58, 71, 72 |
| CAPCELL PAK UG Phenyl | Shiseido | 5 | 120 | 300 | - |
| Chromegabond Alkyl Phenyl | ES Industries | 3, 5, 10 | 60, 80, 100 | 475, 200, 190 | 85 |
| Cogent Phenyl Hydride | MicroSolv | 4 | 100 | 350 | 115, 118, 120 |
| Develosil Phenyl-UG | Nomura | 3, 5 | 140 | 300 | 80, 81 |
| Genesis Phenyl | Grace | 4 | 120 | 300 | 90 |
| HECTOR-M Phenyl | RStech Corporation | 3, 5, 10 | 100 | 320 | 2 |
| Hypersil GOLD Phenyl | Thermo Scientific | 1.9, 3, 5 | 175 | 220 | 150 |
| Hypersil Phenyl | | 5 | 120 | 170 | 151 |
| Hypersil Phenyl-2 | | 5 | 120 | 170 | 151 |
| Hypersil BDS Phenyl | | 3, 5 | 130 | 170 | 151 |
| Inertsil Phenyl | GL Sciences | 5 | 150 | 320 | 88, 89 |
| Inertsil Phenyl-3 | | 2, 3, 5 | 100 | 450 | 87, 88 |
| InertSustain Phenyl | | 3, 5 | 100 | 350 | 87 |
| Kromasil Phenyl | Akzo Nobel | 5, 10, 16 | 100 | 320 | - |
| NUCLEOSIL Phenyl | Macherey-Nagel | 5, 7 | 100, 120 | 350, 200 | 103-105 |
| PrincetonSPHER Phenyl | Princeton Chromatography | 3, 5, 10 | 60, 100 | 500, 325 | 134, 135 |
| ProntoSIL Phenyl | Bischoff | 3, 5 | 120 | 300 | - |
| Syncronis Phenyl | Thermo Scientific | 1.7, 3, 5 | 100 | 320 | 151 |
| TSKgel Super-Phenyl | Tosoh Bioscience | 2.3 | 110 | - | 154 |
| Vydac 219MS[4] | Grace | 5 | 300 | - | 90 |
| Waters µBondapak Phenyl | Waters | 10 | 125 | 330 | - |
| Waters Nova-Pak Phenyl | | 4 | 60 | 120 | - |
| Waters Spherisorb Phenyl | | 3, 5 | 80 | 220 | 160, 161 |
| YMC Phenyl | YMC | 3, 5 | 120 | 330 | - |
| YMC-Triart Phenyl | | 1.9, 3, 5 | 120 | - | - |
| ZORBAX Phenyl | Agilent Technologies | 5 | 70 | 330 | 165, 166 |
| ZORBAX SB-Phenyl | | 3.5, 5 | 80 | 180 | - |
| ZORBAX Eclipse XDB-Phenyl | | 3.5, 5 | 80 | 180 | - |

[1] Superficially porous phase [2] C18 with integral Phenyl, classed as L1 [3] As ACE Excel column [4] Diphenyl phase

## Phenyl-Hexyl Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| Accucore Phenyl-Hexyl[1] | Thermo Scientific | 2.6 | 80 | 130 | 149 |
| ACE UltraCore SuperPhenylHexyl[1] | Advanced Chromatography Technologies (ACT) | 2.5, 5 | 95 | 130, 100 | 1 |
| Brownlee SPP Phenyl-Hexyl[1] | Perkin Elmer | 2.7 | 90 | 150 | - |
| Epic Phenyl-Hexyl | ES Industries | 1.8, 3, 5, 10 | 120 | 350 | 85 |
| HALO Phenyl-Hexyl[1] | Advanced Materials Technology | 2.7 | 90 | 150 | 91 |
| HALO-5 Phenyl-Hexyl[1] | | 5 | 90 | 90 | 91 |
| Kromasil Eternity Phenyl-Hexyl | Akzo Nobel | 2.5, 5 | 100 | 330 | - |
| NUCLEODUR Phenyl-Hexyl | Macherey-Nagel | 1.8, 3, 5 | 110 | 340 | 102 |
| NUCLEOSHELL Phenyl-Hexyl[1] | | 2.7 | 90 | 130 | 102 |

[1] Superficially porous phase

# PENTAFLUOROPHENYL (PFP) BONDED PHASES

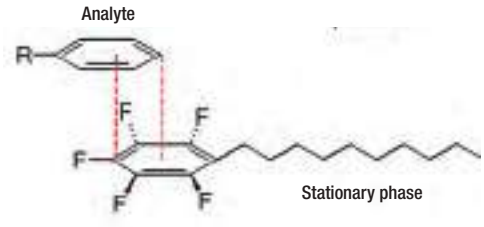


Fluorinated stationary phases have shown novel selectivity for several classes of compounds and in many cases have proved useful as an alternative to traditional C18 and C8 phases. In particular, pentafluorophenyl (PFP) bonded phases are becoming increasingly popular when alternative selectivity is required. Details of these PFP phases are listed on this page. For other fluorinated phases please see individual manufacturer's pages (eg. Fluophase page 151, Wakopak Fluofix page 159, PrincetonSPHER Fluoropropyl and Fluorooctyl page 135).

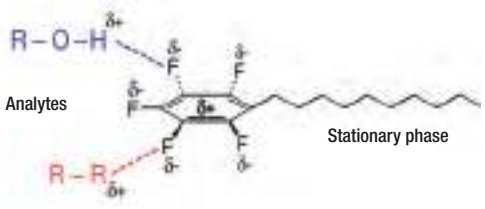
## Separation Mechanisms

PFP-bonded phases use multiple retention mechanisms for separation of challenging compounds. These interactions include hydrophobic, π-π interaction, dipole-dipole, H-bonding and shape selectivity. The predominance of each retention mechanism will be influenced by the solute's physicochemical properties, its structure and the chromatographic conditions utilised.

The electronegative fluorine atoms produce an electron deficient phenyl ring, so that the PFP phase acts as a Lewis acid or electron acceptor. This is the opposite of phenyl phases, which contain an electron rich aromatic ring. π-π interaction can occur with solutes that are rich in electrons (Lewis bases). The carbon-fluorine bonds of the PFP ring are very polar, thus enabling analytes to also be retained by dipole-dipole and H-bonding interactions, resulting in increased analyte retention.




π–π interaction



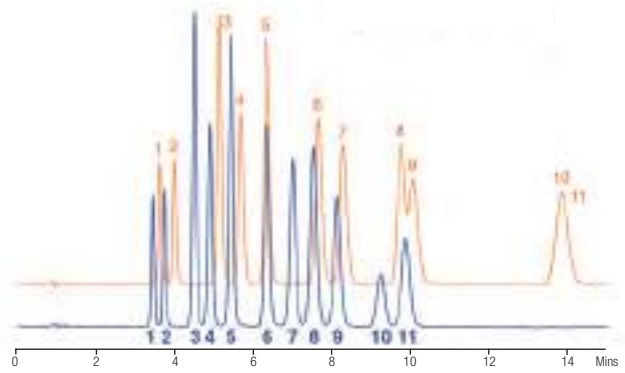

Dipole-dipole and H-bonding

## Applications

PFP phases show excellent selectivity especially for highly polar analytes, aromatic and unsaturated compounds, phenols, halogenated compounds and taxanes. In addition, positional isomers show increased separation on PFP phases.

Due to the low bleed characteristics of many of the newer PFP phases, they are ideally suited for low UV wavelength and LC-MS applications. PFP phases are generally resistant to dewetting and can be used under highly aqueous conditions.

Figure 1 illustrates the orthogonal selectivity shown by a PFP compared to a C18 phase for the separation of phenol isomers.




1. o-Cresol
2. m-Cresol
3. 3,4-Dimethylphenol
4. 3,5-Dimethylphenol
5. 2,5-Dimethylphenol
6. 2,6-Dichlorophenol
7. 2,3-Dichlorophenol
8. 2,4-Dichlorophenol
9. 3,4-Dichlorophenol
10. 2,4-Dibromophenol
11. 3,5-Dibromophenol

Columns: NUCLEODUR PFP (blue) and C18 HTec (red)
Dimensions: 5µm, 125 x 4mm
Eluent: 0.1% formic acid in $CH_3CN$ – 0.1% formic acid in $H_2O$ (35:65)
Flow rate: 1ml/min
Temperature: 35°C
Detection: UV, 280nm

Figure 1. Separation of phenol isomers

## PFP Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| Accucore PFP[1] | Thermo Scientific | 2.6 | 80 | 130 | 149 |
| ACE C18-PFP[2] | ACT | 2[3], 3, 5, 10 | 100 | 300 | 58, 61, 70, 71 |
| Epic PFP-LB | ES Industries | 1.8, 3, 5, 10 | 120 | 230 | 85 |
| FluoroSep-RP Phenyl (FSP) | | 3, 5 | 60 | 350 | 85 |
| HALO PFP[1] | Advanced Materials Technology | 2.7 | 90 | 150 | 91 |
| HALO-5 PFP[1] | | 5 | 90 | 90 | 91 |
| Hypersil GOLD PFP | Thermo Scientific | 1.9, 3, 5, 8, 12 | 175 | 220 | 150 |
| NUCLEODUR PFP | Macherey-Nagel | 1.8, 3, 5 | 110 | 340 | 102 |
| NUCLEOSHELL PFP[1] | | 2.7 | 90 | 130 | 102 |
| Partisphere TAC-1 | Hichrom | 5 | - | - | 122, 123 |
| PrincetonSPHER PFP | Princeton | 3, 5 | 60, 100 | - | 134, 135 |
| YMC-Triart PFP | YMC | 1.9, 3, 5 | 120 | - | - |

[1] Superficially porous phase [2] C18 with integral PFP, classed as USP L1 [3] As ACE Excel column

# POLAR BONDED PHASES



## Introduction

Polar bonded silica phases offer an alternative selectivity to alkyl bonded materials (see p.31-36). In general they have a lower hydrophobicity but higher polarity. Cyano, amino and diol bonded phases can be used in both normal- and reversed-phase modes. In normal-phase they equilibrate more rapidly with the eluent than silica itself and are not deactivated by traces of water.

## Availability

Cyano bonded phases show unique selectivity for polar compounds and are more suitable than bare silica for normal-phase gradient separations. The cyano functional group is a strong dipole that can interact with other dipoles or induce dipoles on solutes. These phases also exhibit moderate hydrophobicity due to the alkyl linker.

Amino bonded phases show alternative normal-phase selectivity to unbonded silica, especially for aromatics. Amino columns are also used in the HILIC mode for carbohydrate analysis and for other polar compounds. Their weak anion-exchange properties can be used in the analysis of anions and organic acids.

Diol bonded phases are a versatile alternative to unbonded silica for normal-phase separations. The hydroxyl groups provide good selectivity without excessive retention, since H-bonding with the diol layer is weaker than with silanols. Some diol bonded phases have been developed specifically for HILIC applications. Differing pore size materials are used in size-exclusion separations.

## Cyano Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| ACE CN | Advanced Chromatography Technologies (ACT) | 2[1], 3, 5, 10 | 100 | 300 | 58, 71, 72 |
| ACE CN-ES | | 2[1], 3, 5, 10 | 100 | 300 | 58, 63, 71, 72 |
| CAPCELL PAK CN UG | Shiseido | 5 | 120 | 300 | - |
| Chromegabond BAS-CN | ES Industries | 3, 5, 10 | 120 | 180 | 85 |
| Chromegabond CN-BD | | 3, 5, 10 | 100 | 475 | 85 |
| Chromegabond CN-HS | | 3, 5, 10 | 60 | 550 | 85 |
| COSMOSIL CN-MS | Nacalai Tesque | 5 | 120 | 300 | 76 |
| Develosil CN-UG | Nomura | 5 | 140 | 300 | 80, 81 |
| Develosil XG-CN | | 3, 5 | 140 | 300 | 80 |
| Exsil CN | Grace | 3, 5 | 100 | 200 | 86 |
| Genesis CN | | 4 | 120 | 300 | 90 |
| HALO ES-CN[2] | Advanced Materials Technology | 2.7 | 90 | 150 | 91 |
| HALO-5 ES-CN[2] | | 5 | 90 | 90 | 91 |
| HECTOR-M CN | RStech Corporation | 3, 5, 10 | 100 | 320 | 2 |
| Hypersil GOLD CN | Thermo Scientific | 1.9, 3, 5 | 175 | 220 | 150 |
| Hypersil CPS | | 3, 5 | 120 | 170 | 151 |
| Hypersil CPS-2 | | 5 | 120 | 170 | 151 |
| Hypersil BDS CPS | | 3, 5 | 130 | 170 | 151 |
| Inertsil CN-3 | GL Sciences | 3, 5 | 100 | 450 | 87, 88 |
| Kromasil CN | Akzo Nobel | 5, 10, 16 | 60 | 540 | - |
| LiChrosorb CN | Merck | 5 | 100 | 300 | 111 |
| LiChrospher CN | | 5 | 100 | 350 | 112, 113 |
| NUCLEODUR CN and CN-RP | Macherey-Nagel | 3, 5 | 110 | 340 | 102 |
| NUCLEOSIL CN | | 5, 10 | 100 | 350 | 103, 104 |
| | | 7 | 120 | 200 | 103, 105 |
| PrincetonSPHER CN | Princeton Chromatography | 3, 5, 10 | 60, 100 | 500, 325 | 134, 135 |
| ProntoSIL CN | Bischoff | 3, 5 | 120 | 300 | - |
| TSKgel CN-80Ts | Tosoh Bioscience | 5 | 80 | - | - |
| Ultrasphere CN | Hichrom | 3, 5 | 80 | - | 156, 157 |
| Waters µBondapak CN | Waters | 10 | 125 | - | - |
| Waters Nova-Pak CN HP | | 4 | 60 | - | - |
| Waters Spherisorb CN | | 3, 5 | 80 | 220 | 160, 161 |
| YMC CN | YMC | 3, 5 | 120 | 330 | - |
| ZORBAX CN | Agilent Technologies | 5 | 70 | 330 | 165, 166 |

[1] As ACE Excel column [2] Superficially porous phase

## Polar Bonded Phases (continued)

### Amino Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| CAPCELL PAK $NH_2$ UG | Shiseido | 5 | 80 | - | - |
| Chromegabond A/RP | ES Industries | 3, 5, 10 | 60, 100 | 475, 330 | - |
| Chromolith $NH_2$ | Merck | - | - | 300 | 110 |
| COSMOSIL NH2-MS | Nacalai Tesque | 5 | 120 | 300 | 76 |
| Exsil $NH_2$ | Grace | 3, 5 | 100 | 200 | 86 |
| Genesis $NH_2$ | | 3 | 120 | 300 | 90 |
| HECTOR-M $NH_2$ | RStech Corporation | 3, 5, 10 | 100 | 320 | 2 |
| Hypersil GOLD Amino | Thermo Scientific | 1.9, 3, 5 | 175 | 220 | 150 |
| Hypersil APS-2 | | 3, 5 | 120 | 170 | 151 |
| Inertsil $NH_2$ | GL Sciences | 3, 5 | 100 | 450 | 87 |
| InertSustain $NH_2$ | | 3, 5 | 100 | 350 | 87 |
| Kromasil $NH_2$ | Akzo Nobel | 3.5, 5, 7, 10 | 100 | 320 | - |
| LiChrosorb $NH_2$ | Merck | 5, 10 | 100 | 300 | 111 |
| LiChrospher $NH_2$ | | 5 | 100 | 350 | 112, 113 |
| NUCLEODUR $NH_2$ and $NH_2$-RP | Macherey-Nagel | 3, 5, 7 | 110 | 340 | 102 |
| NUCLEOSIL $NH_2$ | | 5 | 100 | 350 | 103, 104 |
| | | 7 | 120 | 200 | 103, 105 |
| PrincetonSPHER $NH_2$ | Princeton Chromatography | 3, 5, 10 | 60, 100 | 500, 300 | 134, 135 |
| Purospher STAR $NH_2$ | Merck | 5 | 120 | 330 | 110 |
| Syncronis $NH_2$ | Thermo Scientific | 1.7, 3, 5 | 100 | 320 | 151 |
| TSKgel NH2-100 | Tosoh Bioscience | 3 | 100 | 450 | 155 |
| Waters µBondapak $NH_2$ | Waters | 10 | 125 | 330 | - |
| Waters Spherisorb $NH_2$ | | 3, 5, 10 | 80 | 220 | 160, 161 |
| YMC $NH_2$ | YMC | 3, 5 | 120 | 330 | - |
| ZORBAX $NH_2$ | Agilent Technologies | 5 | 70 | 330 | 165, 166 |

### Diol Bonded Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| Chromegabond D/RP | ES Industries | 3, 5 | 60, 100 | 475, 330 | 85 |
| COSMOSIL Diol | Nacalai Tesque | 5 | 120 | 300 | 76 |
| HECTOR-M Diol | RStech Corporation | 3, 5, 10 | 100 | 320 | 2 |
| Inertsil Diol | GL Sciences | 3, 5 | 100 | 450 | 88 |
| Inertsil WP Diol | | 5 | 300 | 150 | - |
| Kromasil Diol | Akzo Nobel | 5, 10, 16 | 60 | 540 | - |
| Kromasil HILIC-D | | 5 | 60 | 540 | - |
| LiChrosorb Diol | Merck | 5, 10 | 100 | 300 | 111 |
| LiChrospher Diol | | 5 | 100 | 350 | 112, 113 |
| NUCLEOSIL Diol | Macherey-Nagel | 5, 7 | 100 | 350 | 103, 104 |
| PrincetonSPHER Diol | Princeton Chromatography | 3, 5, 10 | 60, 100 | 500, 325 | 134, 135 |
| ProntoSIL Diol | Bischoff | 3, 5 | 120 | 300 | - |
| YMC Diol | YMC | 5 | 120 | 330 | - |
| YMC-Triart Diol-HILIC | | 1.9, 3, 5 | 120 | - | - |

Figures 1 and 2 show typical applications on amino and diol bonded columns respectively.

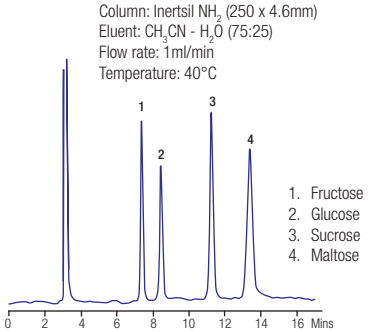



Figure 1. Separation of sugars on amino column

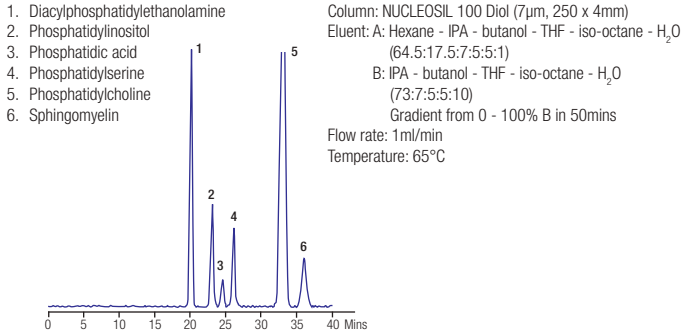



Figure 2. Separation of phospholipids on diol column

# HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) PHASES

## Introduction

Hydrophilic Interaction Chromatography (HILIC) is a variant of normal-phase chromatography which is performed using polar stationary phases with partially aqueous eluents. The technique combines the characteristics of 3 major liquid chromatography techniques – reversed-phase, normal-phase and ion chromatography. HILIC is an alternative approach to reversed-phase for the effective separation of polar compounds. Solutes elute in the order of increasing hydrophilicity (polarity), the opposite of reversed-phase, thus providing an orthogonal selectivity.

## Mode of Operation

Retention in HILIC is proportional to the amount of organic solvent in the eluent. Typical HILIC eluents contain 65-90% acetonitrile or methanol. The low proportion of water in the eluent generates a water-rich layer on the surface of the polar stationary phase. This enables solutes to partition between the eluent and this water-rich layer (Figure 1). In addition, weak electrostatic interactions between solute and stationary phase contribute to overall selectivity. Gradient elution may be performed either with a decreasing organic or increasing salt gradient. Salt is not required for uncharged solutes such as carbohydrates, but typically 10mM salt is necessary with charged solutes such as peptides. Ammonium formate and acetate are suitable volatile buffers for LC-MS.

Several types of HILIC phases have been developed, including unbonded silica, neutral bonded ligands (eg. amide, diol), charged ligands (eg. amino), zwitterionic phases and mixed reversed-phase/HILIC phases. A wide selection of HILIC phases is summarised in the table below.

ERLIC, also referred to as eHILIC, is a subset of HILIC separations which employs charged interactions and their subsequent orientation effects (see PolyLC section for further details).

Aqueous normal-phase (ANP) is a further technique related to HILIC (see pages 115-120 for further details).

## Applications

HILIC phases are particularly useful for compounds that are weakly retained by reversed-phase columns. Typical application areas include carbohydrates, oligonucleotides, peptides and proteins, amino acids and natural products.

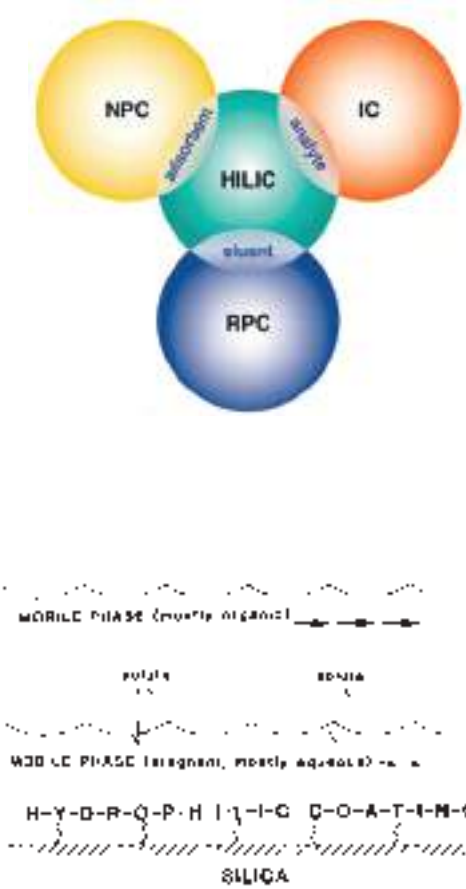



Figure 1. Hypothetical partition mechanism of hydrophilic interaction chromatography (HILIC)

## HILIC Phases

| Phase | Manufacturer | Functional Group | Particle Size (µm) | Pore Size (Å) | Page |
|---|---|---|---|---|---|
| Acclaim HILIC-10 | Thermo Scientific | Proprietary | 3 | 120 | 152 |
| Accucore HILIC[1] | | Proprietary | 2.6 | 80 | 149 |
| Accucore Urea-HILIC[1] | | Urea | 2.6 | 80 | 149 |
| Accucore 150-Amide-HILIC[1] | | Amide | 2.6 | 80 | 149 |
| BioBasic AX | | Polyethyleneimine | 5 | 300 | 153 |
| Brownlee SPP HILIC[1] | Perkin Elmer | Unbonded silica | 2.7 | 90 | - |
| COSMOSIL HILIC | Nacalai Tesque | Triazole | 2.5, 5 | 120 | 76, 78, 79 |
| Epic HILIC-HC | ES Industries | Polyhydroxylated polymer | 1.8, 3, 5, 10 | 120 | 85 |
| HALO and HALO-5 HILIC[1] | Advanced Materials Technology | Unbonded silica | 2.7, 5 | 90 | 91 |
| HALO and HALO-5 Penta-HILIC[1] | | Penta-hydroxy | 2.7, 5 | 90 | 91 |
| Hypersil GOLD HILIC | Thermo Scientific | Polyethyleneimine | 1.9, 3, 5 | 175 | 150 |
| Inertsil HILIC | GL Sciences | Propyl alcohol | 3, 5 | 100 | 87, 88 |
| Kromasil HILIC-D | Akzo Nobel | Diol | 5 | 60 | - |
| NUCLEODUR HILIC | Macherey-Nagel | Zwitterionic ammonium sulphonic acid | 1.8, 3, 5 | 110 | 102 |
| NUCLEOSHELL HILIC[1] | | | 2.7 | 90 | 102 |
| Obelisc N | SIELC | Proprietary | 5 | 100 | 147 |
| PolyGLYCOPLEX | PolyLC | - | 5, 12 | - | 127, 128, 130 |
| Syncronis HILIC | Thermo Scientific | Zwitterionic | 1.7, 3, 5 | 100 | 151 |
| TSKgel Amide-80 | Tosoh Bioscience | Carbamoyl | 3, 5 | 100 | 155 |
| TSKgel NH2-100 | | Ethylamino | 3 | 100 | 155 |
| VisionHT HILIC | Grace | - | 1.5, 3, 5, 10 | 120 | 90 |
| YMC-Triart Diol-HILIC | YMC | Diol | 1.9, 3, 5 | 120 | - |
| ZIC-HILIC | Merck | Zwitterionic sulphobetaine | 3.5, 5 | 100, 200 | 109 |
| ZIC-*p*HILIC | | Zwitterionic sulphobetaine | 5 | - | 109 |
| ZIC-cHILIC | | Zwitterionic phosphorylcholine | 3 | 100 | 109 |

[1] Superficially porous phase

# SILICA PHASES



## Introduction

Despite its porosity, spherical porous HPLC silica exhibits a high mechanical strength compared with other materials. Additionally, it is readily chemically modified. A wide range of porous silicas is available for normal-phase HPLC, characterised by surface area, pore size and particle size measurements.

The use of normal-phase HPLC has not been limited by the silica dissolution or peak tailing problems associated with reversed-phase HPLC. Hence traditional silicas are still commonly used. As an alternative to normal-phase HPLC, some unbonded silica phases are promoted for use as HILIC phases (see page 44).

## Particle Size

For analytical work, as the quality and reproducibility of porous silica improves, the use of 3, 3.5 or 4µm particle size materials increases. 10µm particles are less commonly used but remain a key particle size for preparative applications. For economic reasons irregular silicas still share some of this latter market.

## Physical Characteristics

The physical characteristics of the newer silica particles have been improved in several ways. However, a number of them are not readily available as they are principally used as a base material for the manufacture of new reversed-phase silicas.

- **Surface Activity**
  A lower level of the unwanted, free and isolated silanol groups is observed. The lower metal ion contaminant level partly contributes to this drop in surface activity. Basic compounds interact less strongly with the silica surface resulting in improved chromatography.
- **Physical Properties**
  Improved control of physical properties such as surface area, pore volume, mean pore diameter and particle size have given the new silicas better lot-to-lot reproducibility.
- **Purity**
  The level of metal ion impurities has in some cases been reduced to cumulative figures < 10ppm. Undesirable chelation of metal ion and solute has been minimised.

## Silica Phases

| Phase | Manufacturer | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Page |
|---|---|---|---|---|---|
| ACE SIL | ACT | 2[1], 3, 5, 10 | 100 | 300 | 58, 71, 72 |
| Chromolith Si | Merck | - | - | 300 | 110 |
| Cogent Silica-C | MicroSolv | 4 | 100 | 350 | 115, 119, 120 |
| COSMOSIL SL-II | Nacalai Tesque | 3, 5 | 120 | 300 | 76, 79 |
| Exsil Silica | Grace | 5, 10 | 100 | 200 | 86 |
| Genesis Silica | | 4, 7, 15 | 120 | 300 | 90 |
| HECTOR-M Sil | RStech Corporation | 3, 5, 10 | 100 | 320 | 2 |
| Hypersil Silica | Thermo Scientific | 3, 5 | 120 | 170 | 151 |
| Hypersil GOLD Silica | | 1.9, 3, 5 | 175 | 220 | 150 |
| Inertsil SIL | GL Sciences | 3, 5 | 100 | 450 | 87 |
| Kromasil Silica | Akzo Nobel | 3.5, 5, 7, 10 | 60, 100 | 540, 320 | - |
| LiChrosorb Silica | Merck | 5, 10 | 60, 100 | 500, 300 | 111 |
| LiChrospher Si | | 5 | 60, 100 | 700, 400 | 112, 113 |
| NUCLEODUR Silica | Macherey-Nagel | 3, 5 | 110 | 340 | 102 |
| NUCLEOSIL Silica | | 3, 5, 7, 10 | 100, 120 | 350, 200 | 103-105 |
| Partisil Silica | Hichrom | 5, 10 | - | - | 121-125 |
| Partisphere Silica | | 5 | - | - | 122, 123 |
| PrincetonSPHER Silica | Princeton Chromatography | 3, 5, 10 | 60, 100 | 500, 325 | 134, 135 |
| ProntoSIL Si | Bischoff | 3, 5, 10 | 120 | 300 | - |
| Purospher STAR Si | Merck | 5 | 120 | 330 | 110 |
| Syncronis Silica | Thermo Scientific | 1.7, 3, 5 | 100 | 320 | 151 |
| Ultrasphere Silica | Hichrom | 3, 5 | 80 | - | 156, 157 |
| VisionHT Silica | Grace | 1.5, 3, 5, 10 | 120 | 220 | 90 |
| Waters Nova-Pak Silica | Waters | 4 | 60 | 120 | - |
| Waters Spherisorb Silica | | 3, 5, 10 | 80 | 220 | 160, 161 |
| YMC Silica | YMC | 3, 5 | 120 | 300 | - |
| ZORBAX Silica | Agilent Technologies | 5 | 70 | 330 | 165, 166 |

[1] As ACE Excel column

# ION-EXCHANGE PHASES

## Introduction

Ion-exchange phases separate solutes on the basis of differences in ionic charge. Retention in ion-exchange chromatography is determined by the pH of the eluent, the nature and ionic strength of the buffer and temperature. Column efficiencies are lower than in reversed-phase HPLC. Eluents are normally aqueous but can contain some organic component.

## Base Material

Both silica based and polymer based ion-exchangers are available. For the former, ionic species are attached to the silica surface, whereas for the latter the ion-exchange groups are distributed throughout the matrix. Silica based materials maintain a mechanical strength and higher efficiency advantage, whereas the polymer based materials have greater pH stability.

## Applications

Ion-exchange is used for the analysis of small ions but the key application area of the technique is the separation of biomolecules such as peptides, proteins and oligonucleotides. Weak ion-exchangers are used for the analysis of inorganic ions, a technique more specifically termed ion chromatography (see page 48).

## Ion-Exchange Capacity

The exchange capacity of an ion-exchanger is an important measure of its retentivity (typically measured in milliequivalents per gram material). For any one column the packing density of the phase must also be taken into account. Wide pore materials will typically have lower ion-exchange capacities.

Cation-exchange phases contain negatively charged functional groups and retain positively charged cations. Conversely, anion-exchange phases retain negatively charged analytes by their positively charged functional groups. In the schematics below, the ion strength of the counter ions can be adjusted to shift the equilibrium position and thus the retention times of the analytes.

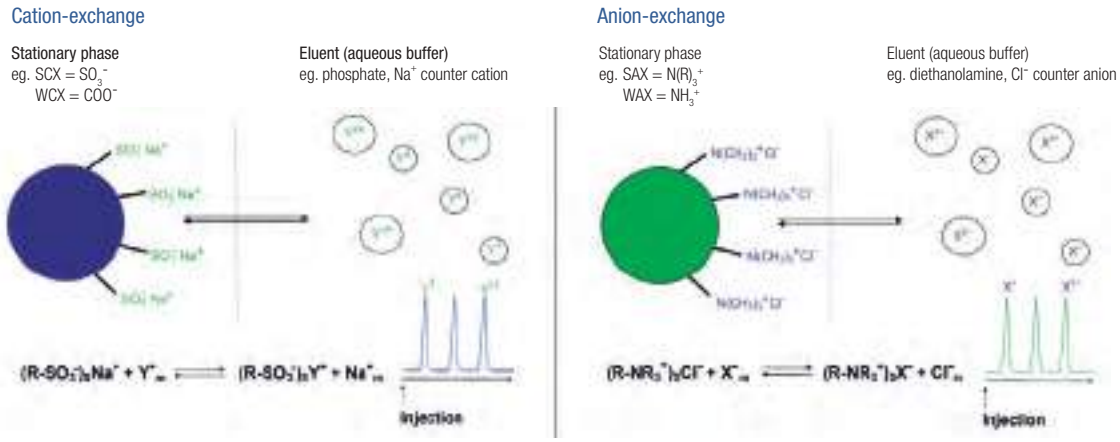



Figure 1. Mechanisms of ion-exchange

## Classification

| | Type | Strength | Nomenclature | Typical Functionality | pH Ionisation Range |
|---|---|---|---|---|---|
| Ion-Exchange | Anion | Weak | WAX | Amine | Ionised at specific pH |
| | | Strong | SAX | Quaternary Ammonium | Ionised over complete pH range |
| | Cation | Strong | SCX | Sulphonic Acid | |
| | | Weak | WCX | Carboxylic Acid | Ionised at specific pH |

## Ion-Exchange Phases

| Phase | Manufacturer | Base Material | Classification | Particle Size (µm) | Pore Size (Å) | Applications and Features | Page |
|---|---|---|---|---|---|---|---|
| BioBasic AX, SCX | Thermo Scientific | Silica | SAX, SCX | 5 | 300 | Proteins, peptides, nucleic acids | 153 |
| CAPCELL PAK UG SCX | Shiseido | Silica | SCX | 5 | 80 | Small molecules | - |
| COSMOGEL IEX Type Q, Type S | Nacalai Tesque | Polymer | SAX, SCX | 5 | 1000 | Proteins, DNA | 79 |
| COSMOGEL IEX Type Q-N, Type S-N | | | SAX, SCX | 5 | Non-porous | | |

## Ion-Exchange Phases (continued)

### Ion-Exchange Phases (continued)

| Phase | Manufacturer | Base Material | Classification | Particle Size (µm) | Pore Size (Å) | Applications and Features | Page |
|---|---|---|---|---|---|---|---|
| Epic SCX | ES Industries | Silica | SCX | 1.8, 3, 5, 10 | 120 | Small molecules | 85 |
| Eprogen AX300, CM300 | Eprogen | Silica | WAX, WCX | 6 | 300 | Small molecules | 84 |
| Eprogen Q300, S300 | | | SAX, SCX | 6 | 300 | | 84 |
| Exsil SAX, SCX | Grace | Silica | SAX, SCX | 5 | 100 | Small molecules | 86 |
| Hamilton PRP-X100, PRP-X200 | Hamilton | Polymer | WAX, WCX | 10 | 100 | Inorganic ion analysis | - |
| Hamilton PRP-X500, PRP-X600 | | Polymer | SAX, WAX | 7 | - | Proteins, DNA oligomers | - |
| HECTOR-ACD WCX, SCX | RStech Corporation | Silica | WCX, SCX | 3, 5, 10 | 100 | Separation of acidic compounds | 2 |
| Hypersil GOLD AX, SAX | Thermo Scientific | Silica | WAX, SAX | 1.9, 3, 5 | 175 | AX – small proteins and peptides SAX- small molecules | 150 |
| Inertsil AX, CX | GL Sciences | Silica | SAX, SCX | 5 | 100 | Small molecules | 87, 88 |
| MCI GEL ProtEx-DEAE, -SP | Mitsubishi Chemicals | Polymer | WAX, SCX | 5 | - | Proteins | - |
| MCI GEL CQA Series | | | SAX, WAX | 10 | | | 108 |
| MCI GEL CQK Series | | | SCX, WCX | 10 | | | 108 |
| NUCLEOSIL SA, SB | Macherey-Nagel | Silica | SCX, SAX | 5, 10 | 100 | Small molecule analysis | 103, 104 |
| NUCLEOGEN DEAE | | | WAX | 7 | 60, 500, 4000 | Bioanalytical | 102 |
| Partisil SAX, SCX | Hichrom | Silica | SAX, SCX | 5, 10 | - | Small molecule analysis | 121-125 |
| Partisphere SAX, SCX | | | SAX, SCX | 5 | - | | 122, 123 |
| PolyCAT A | PolyLC | Silica | WCX | 3, 5, 12 | 300, 1000, 1500 | Aspartic acid functionality | 127, 128, 130 |
| PolySULFOETHYL A | | | SCX | | 200, 300, 1000 | Sulfoethylaspartamide | 127, 129, 130 |
| PolyWAX | | | WAX | | 100, 300, 1000, 1500 | Proteins with isoelectric point <6.0 | 127-130 |
| PL-SAX, PL-SCX | Agilent Technologies | Polymer | SAX, SCX | 8, 10 | 1000 | Protein applications | - |
| ProPac WCX-10, SCX-10, WAX-10, SAX-10 | Thermo Scientific | Polymer | WCX, SCX, WAX, SAX | 10 | Non-porous | Proteins variants | 153 |
| Asahipak ES-502N, ES-502C | Shodex | Polymer | WAX, WCX | 9 | - | Proteins, peptides, oligonucleotides | 140 |
| Shodex IEC QA | | | SAX | 12 | - | | 140 |
| Shodex IEC DEAE, SP, CM | | | WAX, SCX, WCX | 8 | - | | 140 |
| TSKgel DEAE-2SW, CM-2SW | Tosoh Bioscience | Silica | WAX, WCX | 5 | 125 | Nucleotides, drug molecules, catecholamines | 155 |
| TSKgel DEAE-3SW, CM-3SW | | | WAX, WCX | 10 | 250 | | 155 |
| TSKgel SuperQ-5PW, DEAE-5PW, SP-5PW, CM-5PW | | Polymer | SAX, WAX, SCX, WCX | 10, 13 | 1000 | Enzymes, proteins, DNA, nucleic acids | 155 |
| TSKgel BioAssist Q | | Polymer | SAX | 10, 13 | ~4000 | Plasmids, antibodies and other large proteins | 155 |
| TSKgel BioAssist S | | Polymer | SCX | 7, 13 | ~1300 | | 155 |
| TSKgel Q-STAT, CM-STAT, SP-STAT | | Non-porous resin | SAX, WCX, SCX | 7, 10 | Non-porous | Nucleic acids, mAb variants, protein aggregates | 155 |
| TSKgel DNA-STAT | | | SAX | 5 | | | 155 |
| Waters Spherisorb SAX, SCX | Waters | Silica | SAX, SCX | 5 | 80 | Small molecules | 160, 161 |
| YMC-BioPro QA, SP | YMC | Hydrophilic polymer | SAX, SCX | 5 | 1000 | Peptides, proteins, nucleic acids, other biomolecules | - |
| YMC-BioPro QA-F, SP-F | | | SAX, SCX | 5 | Non-porous | | - |
| ZirChrom SAX, WAX | ZirChrom Separations | Zirconia | SAX, WAX | 3, 5 | 300 | Inorganic and organic anions, biomolecules | 162-164 |
| ZirChrom SHAX, WCX | | | SAX, WCX | | | Proteins | 162-164 |

# ION CHROMATOGRAPHY PHASES

## Introduction

Ion chromatography (IC) is a special form of ion-exchange chromatography developed as a means of separating the ions of strong acids and bases. The most important form of ion chromatography involves a combination of specific ion-exchange phases with conductivity detection. It is a sensitive technique, in some cases being able to detect ppb levels of ions.

## Suppressed or Non-Suppressed Detection

Eluents used in IC contain a relatively high level of salt ions and therefore exhibit high conductivity. This leads to a high background signal which could inhibit the detection of low level analytes. Suppression of eluent conductivity post column is necessary for efficient detection of sample ions and is the most common method for anion analyses. Although isocratic elution is more commonly used, the use of suppressors enables gradient elution to be used for complex samples.

## Phases

Silica and polymer based phases are available for anion and cation analyses. Silica based columns, although showing better efficiency, have a limited pH range. As a result, they are not compatible with anion IC methods requiring suppressed detection, due to the high pH of the eluents required. Polymer based materials are stable over a wider pH range and have higher capacities.

Tables 1 and 2 show typical base materials and bonding for anion and cation chromatography phases respectively. The majority of phases for anion chromatography are bonded with quaternary ammonium groups, with a permanent cationic charge. For cation chromatography phases, sulphonate is the most common functionality. Columns optimised for non-suppressed IC generally have lower capacity than those for suppressed detection, in order to achieve a relatively low background conductivity. They are therefore not suitable for suppressed detection.

## Eluents

The choice of eluent for IC depends on whether the method is suppressed or non-suppressed. For suppressed anion IC, carbonate/bicarbonate or hydroxide are the most common eluents. Hydroxide eluents have the advantage of producing water only in the suppressor and thereby giving a very low background conductivity. However, some phases are not stable at the high pH (12) of this eluent. For non-suppressed anion IC analyses, typical eluents include p-hydroxybenzoic acid and phthalic acid. Typical eluents for suppressed and non-suppressed cation IC include HCl, $HNO_3$, tartaric acid and succinic acid.

## Applications

High sensitivity ion analyses are important in a wide spectrum of industries including pharmaceutical, food, water, semiconductor etc. In addition to the common inorganic anions eg. $F^-$, $Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$, acid salts can also be analysed by IC eg. formate, acetate. Quantitative analysis of anions at the ppb level can be achieved. Cation chromatography is used for the separation and detection of Group I and II metal ions, in addition to some transition metal ions, ammonium ions and ethanolamines. Small ions are generally eluted before larger ions and monovalent ions before di- and trivalent ions.

### Table 1. Anion Chromatography Phases

| Base Material | Functional Group | Typical pH Range |
|---|---|---|
| Silica | Quaternary ammonium | 2 – 5.5 |
| Polystyrene-divinylbenzene | Quaternary ammonium | 2 – 12 |
| Polymethacrylate | Quaternary ammonium | 2 – 10 |
| Polyvinyl alcohol | Quaternary ammonium | 3 – 12 |

### Table 2. Cation Chromatography Phases

| Base Material | Functional Group | Typical pH Range |
|---|---|---|
| Silica | Carboxylate, sulphonate | 2 – 7 |
| Silica | Polybutadiene/maleic acid coating | 2 – 7 |
| Polystyrene-divinylbenzene | Sulphonate | 2 – 12 |

A selection of ion chromatography columns can be found in the following sections:

MCI GEL page 107
Shodex page 140

Please contact Hichrom for advice on further IC column selection.

# SIZE EXCLUSION CHROMATOGRAPHY (SEC) PHASES



## Introduction

SEC columns separate components according to their molecular size in solution, larger molecules eluting first. Separation is achieved by the differential exclusion or inclusion of components within the packing material particles. In addition to the separation of discrete components, the technique is used for characterising the molecular weight distribution of polymers.

## Base Material

Silica based SEC materials generally exhibit higher resolving power than polymer based materials. However, polymer based materials show greater stability for use with high pH eluents. Polymeric packing materials are generally available in larger particle sizes, which may be more practical for large-scale preparative separations.

## Modes of Operation

Gel permeation chromatography (GPC) refers to the SEC separation of organic soluble polymers using an organic solvent as the eluent. Gel filtration chromatography (GFC) refers to the SEC separation of water soluble polymers in aqueous eluents. SEC separations exhibit lower resolving power and capacity compared with adsorptive HPLC techniques.

## Applications

SEC analyses do not normally result in the denaturation of samples, making the technique a suitable choice for biological samples where activity must be retained. A wide range of biomolecules and organic polymers are separated by SEC. For samples of wide molecular weight distribution, it can be useful to use a mixed pore size phase or to couple columns of one or more pore sizes in series.

## Size Exclusion Chromatography Phases

| Phase / Series | Manufacturer | Base Material / Bonding | Mode | Pore Sizes (Å) | Typical Applications | Page |
|---|---|---|---|---|---|---|
| GPC PEPTIDE | Eprogen | Glycerol bonded silica | GFC | 50 | Small peptides | 84 |
| GPC100, 300, 500, 1000, 4000 | | | GFC | 100, 300, 500, 1000, 4000 | Proteins, carbohydrates, nucleic acids, water soluble polymers | 84 |
| GPC LINEAR | | | GFC | 100-1000 | Organic polymers, denatured proteins | 84 |
| CATSEC | | Silica with polymerised polyamine coating | GFC | 100, 300, 1000 | Cationic polymers | 84 |
| MCI GEL CQP | Mitsubishi Chemical Corp. | Polyhydroxymethacrylate | GFC | 120, 200, 600 | Proteins, peptides, enzymes and other biomolecules | 108 |
| PLgel | Agilent Technologies | Polystyrene-divinylbenzene | GPC | 50, 100, 500, 1000, 10,000, 100,000, MIXED | Oils, oligomers, high MW synthetic polymers, starches, polystyrenes, resins | - |
| PL aquagel-OH 30, 40, 50, 60, MIXED | | Polystyrene-divinylbenzene with polyhydroxyl functionality | GFC | - | Surfactants, polysaccharides, polyacrylamides, starches, gum | - |
| PolyHYDROXYETHYL A | PolyLC | Silica with hydroxyethylaspartamide coating | GFC | 60, 100, 200, 300, 500, 1000, 1500 | Peptides, proteins, carbohydrates, small molecules | 127, 130 |
| Asahipak GF | Showa Denko | Polyvinyl alcohol | GFC/ GPC | 400, 2000, 10000 | Hydrophilic and hydrophobic compounds | 142 |
| Shodex GPC | | Styrene-divinylbenzene | GPC | Various | Polymers, plastics | 142 |
| Shodex OHpak SB | | Polyhydroxymethacrylate | GFC | Various | Water soluble samples | 141 |
| Shodex PROTEIN KW | | Silica | GFC | 400, 1000, 1500 | Proteins | 141 |
| Acclaim SEC | Thermo Scientific | Hydrophilic polymethacrylate | GFC | 300, 1000 | Water soluble polymers | - |
| BioBasic SEC | | Silica | GFC | 60, 120, 300, 1000 | Peptides and proteins | 153 |
| MAbPac SEC-1 | | Silica | GFC | 300 | Monoclonal antibodies and aggregates | 153 |
| TSKgel SW | Tosoh Bioscience | Silica | GFC | 125, 250, 450 | Proteins, antibodies, enzymes, nucleic acids | 154 |
| TSKgel PW | | Polymethacrylate | GFC | <100, 125, <200, 200, 500, 1000, >1000 | Water soluble organic polymers, polysaccharides, DNA | 154 |
| TSKgel H | | Polystyrene-divinylbenzene | GPC | - | Oligomers, polymers and polymer additives | 154 |
| TSKgel Alpha and SuperAW | | Hydrophilic polyvinyl | GFC/ GPC | - | Organic and water soluble polymers | 154 |
| ZORBAX GF Series | Agilent Technologies | Zirconia-clad silica | GFC | 150, 300 | Proteins, peptides | - |

# AFFINITY CHROMATOGRAPHY PHASES



## Introduction

Affinity chromatography offers the highest specificity and selectivity in biomolecular separations and purifications. Purifications up to several orders of magnitude can be achieved in a single step. Affinity separations can often remove contaminants difficult to eliminate using conventional chromatographic procedures.

## Mechanism

The basis of affinity chromatography is a 'lock and key' type mechanism. An affinity ligand, specific for a binding site on the target molecule, is coupled to an inert chromatography matrix. Using suitable binding conditions, target molecules are bound to the affinity ligand according to its specificity. Unbound solutes are washed through the column. The adsorbed target molecules are then desorbed and eluted from the column. Purification of several thousand-fold may be obtained due to the high selectivity of the affinity interactions.

Group specific affinity resins (eg. Tosoh Bioscience) bind molecules sharing specific structural features. Alternatively, if greater specificity is required, ligands with precise specificity for the target molecule can be used. For example, the Protein A phase has specific affinity for the Fc region of immunoglobulins.

## Immunoaffinity Chromatography

Immunoaffinity chromatography is a specialised form of affinity chromatography, utilising an antibody or antibody fragment as the ligand immobilised on to a solid support in such a manner that its binding capacity is retained.

Figure 1 shows a schematic diagram of the stages involved in a typical immunoaffinity analysis: loading the sample, washing the column to remove matrix components and impurities and eluting the target compound.

## Applications

Affinity and immunoaffinity chromatography techniques are applicable in a variety of disciplines including biochemistry, immunochemistry, virology and molecular biology. Due to the increasing availability of a variety of antibodies, separations based on immunoaffinity techniques are being increasingly used in a wide range of applications involving the purification of complex biological samples.

The main application areas of immunoaffinity chromatography are proteins and enzymes. Method selectivity can be enhanced by combining the pre-concentration and pre-treatment of samples offered by immunoaffinity phases, with the separation capabilities of reversed-phase HPLC. The technique can also be used prior to MS analyses in proteomics.

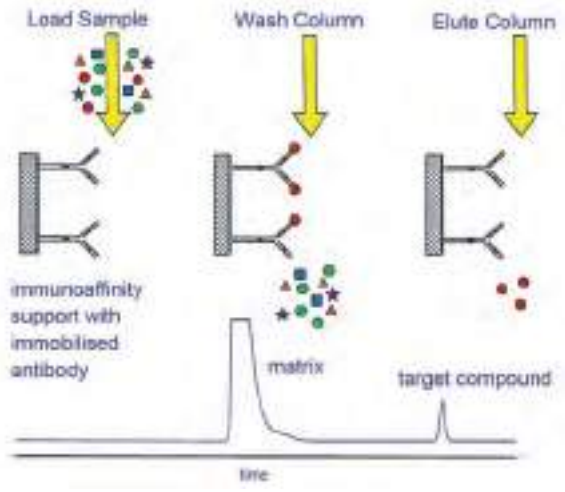



Figure 1. Typical Immunoaffinity Analysis

## Affinity and Immunoaffinity Phases

| Phase Range | Manufacturer | Base Material | Particle Size (µm) | Features | Page |
|---|---|---|---|---|---|
| AFpak | Shodex | Polyhydroxymethacrylate | 18 | 6 different ligands available | 142 |
| ProPac IMAC-10 | Thermo Scientific | Non-porous polystyrene-divinylbenzene | 10 | Used for separation of proteins and peptides by immobilised metal affinity chromatography | 153 |
| ProSwift ConA-1S | Thermo Scientific | Polymethacrylate monolith | - | Used for purification of Concanavalin A binding glycans, glycopeptides and glycoproteins | 153 |
| TSKgel | Tosoh Bioscience | Polymethacrylate | 10 | Group specific affinity phases for analysis of peptides, proteins and nucleic acids | 155 |
| TOYOPEARL | Tosoh Bioscience | Polymethacrylate | 40 – 90 (30 – 60 for Protein A) | Various ligands. Bulk media | 155 |

# CHIRAL PHASES



In many biological processes, the activity of one member of an enantiomeric pair can be contrasted with the inactivity or even harmful activity of the other. The successful development of chiral stationary phases (CSPs) for HPLC and SFC now allows us to monitor the optical purity of a bulk drug and its presence in formulations or biological fluids. Further applications can be found within the agrochemical and related industries. The main types of HPLC/SFC CSPs are discussed below, with examples listed on pages 52-53. Please contact us for information on GC chiral phases.

## Immobilised Polysaccharide CSPs

Coated polysaccharide CSPs are limited in the solvents that may be used in the eluent and as sample diluents. Newer immobilised CSPs allow the use of a more robust and expanded range of solvents and bring new selectivity and higher sample solubility relative to conventionally coated CSPs.

## Cellulose and Amylose Bound

Cellulose and amylose are linear polymers of optically active glucose units with molecular weights of 250,000 to 1,000,000. Cross-linked derivatives of these materials coated onto silica give unique chiral selectivity. Their chiral recognition properties depend on the 'steric fit' of guest enantiomers into the material's cavities. Choice of eluent is the key factor affecting chiral recognition.

## 'Brush-Type'

Although 'brush-type' (Pirkle) chiral selectors are relatively simple molecules, their well defined structure contains three types of functional groups capable of participating in charge transfer ($\pi$-$\pi$ bonding), hydrogen bonding ('dipole stacking' interactions) and steric effects. The monolayer of chiral selector covalently bound to the silica surface usually gives a column of relatively high capacity and efficiency but often with limited chiral discrimination ability. Since the synthesis of the popular D-3,5-dinitrobenzoylphenylglycine phase, significant numbers of these multiple interaction CSPs have been synthesised. Polyaromatic hydrocarbon derivative CSPs are the most recent additions to the range. All 'brush-type' phases are typically used with normal-phase eluents.

## Protein Bound

Proteins are high molecular weight polymers containing chiral sub-units. When bound to silica they act as very effective CSPs. The binding or complexation of small enantiomeric molecules is often stereospecific, especially for serum proteins such as $\alpha_1$-acid glycoprotein (AGP) or human serum albumin (HSA). The additional stability of the Ultron ES-OVM and ES-Pepsin columns enable them to be used with high organic content eluents. Immobilised enzymes can similarly be used. Protein immobilised CSPs are typically used in buffered aqueous eluents compatible with many biological samples. They offer good selectivity. Enantiomer retention and stereoselectivity can often be significantly altered by changes in eluent pH or modifier concentration. Their low capacity makes them unsuitable for preparative applications.

## Cyclodextrin Inclusion

Cyclodextrins are a class of oligosaccharides containing six to twelve optically active glucose units. They are covalently bound to silica to form the corresponding CSP. The physical shape of these molecules is that of a truncated cone, the internal diameter of which is proportional to the number of glucose units. The interior of the cavity is relatively hydrophobic. Secondary hydroxyl groups at the entrance to the cavity contribute to the separation process. The relative stability of the inclusion complexes formed by the enantiomers of the guest molecule at the edge of the cyclodextrin cavity dictates the degree of separation. $\beta$-Cyclodextrin and its derivatives are the most commonly used CSPs of this type. Cyclodextrin CSPs are used in reversed-phase and are suitable for preparative separations.

## Crown Ether

Chiral recognition with crown ether phases is achieved when a complex is formed between the crown ether and an ammonium ion from the analyte. These phases are used for solutes with a primary amino group at or near its chiral centre, such as amino acids and amino alcohols.

## Ligand Exchange

Ligand exchange chiral phases are characterised by the attachment of a chiral chelating ligand to the stationary support. In the presence of an appropriate transition metal cation such as copper (II), a molecular complex is formed with the chiral stationary phase ligand and the analyte. Compounds that are suitable for chiral ligand exchange are $\alpha$-amino acids, hydroxy acids and small peptides.

## Network Polymeric

In a network polymeric CSP the chiral selector is anchored into a network polymer by a cross-linking reaction which simultaneously bonds it to the silica. The aim is to combine in one CSP the efficiency and capacity of 'brush-type' structures with the chiral recognition power of those phases based on chiral polymers.

## Chiral Phases (continued)

### Chiral Phases

| Phase | Manufacturer | Chiral Type | Chiral Selector | Particle Size (µm) | Features | Page |
|---|---|---|---|---|---|---|
| CHIRA-chrom-1 | Hichrom | Brush | D-Phenylglycine | 5 | High efficiency and capacity. Low cost | 100 |
| | | | L-Phenylglycine | 5 | | 100 |
| | | | L-Leucine | 5 | | 100 |
| CHIRA-chrom-2 | | | Dinitrophenyltartramide | 5 | | 100 |
| ChiraDex | Merck | Cyclodextrin | β-Cyclodextrin | 5 | Forms inclusion complexes | 110 |
| CHIRALPAK AGP | Chiral Technologies[2] | Protein | $\alpha_1$-Acid glycoprotein | 5 | Widely used. pH variation a useful tool | 75 |
| CHIRALPAK CBH | | Enzyme | Cellobiohydrolase | 5 | | 75 |
| CHIRALPAK HSA | | Protein | Human serum albumin | 5 | | 75 |
| CHIRALPAK IA | | Amylose | Immobilised amylose derivative | 3, 5 | Broad application range | 74 |
| CHIRALPAK IB | | Cellulose | Immobilised cellulose derivative | 3, 5 | | 74 |
| CHIRALPAK IC | | Cellulose | | 3, 5 | | 74 |
| CHIRALPAK ID | | Amylose | Immobilised amylose derivative | 3, 5 | | 74 |
| CHIRALPAK IE | | Amylose | | 3, 5 | | 74 |
| CHIRALPAK IF | | Amylose | | 3, 5 | | 74 |
| CHIRALPAK AD | | Amylose | Amylose derivative | 3, 5, 10 | Unique separation applications. Very versatile | 74 |
| CHIRALPAK AS | | | | 3, 5, 10 | | 74 |
| CHIRALCEL OD | | Cellulose | Cellulose derivative | 3, 5, 10 | | 74 |
| CHIRALCEL OJ | | | | 3, 5, 10 | | 74 |
| CHIRALPAK QD-AX | | Anion-exchange | Quinidine derivative | 5 | Useful for chiral acids | 75 |
| CHIRALPAK QN-AX | | | Quinine derivative | 5 | | 75 |
| CROWNPAK | | Crown ether | 18-crown-6 type crown ether | 5 | Suitable for amino acids and primary amines | 75 |
| Chirobiotic R | Supelco[1] | Macrocyclic glycopeptide | Ristocetin A | 5 | Broad selectivity | - |
| Chirobiotic T | | | Teicoplanin | 5 | | - |
| Chirobiotic V | | | Vancomycin | 5 | | - |
| Cyclobond I | | Cyclodextrin | β-Cyclodextrin | 5 | Forms inclusion complexes | - |
| Cyclobond II | | | γ-Cyclodextrin | 5 | | - |
| ChiroSil | Regis/RStech | Crown ether | (18-crown-6)-tetracarboxylic acid | 5, 10 | Suitable for primary amines and amino acids | 137 |
| DACH-DNB | Regis | Brush | 3,5-Dinitrobenzoyl derivatives | 5 | π-electron acceptor/donor. Widely used | 136 |
| ULMO | | | | 5 | | 136 |
| Whelk-01/Whelk-02 | | | | 5, 10 | | 136 |
| α-Burke 2 | | | | 5 | π-electron acceptor | 136 |
| β-GEM 1 | | | | 5 | | 136 |
| Leucine | | | | 5 | | 136 |
| Phenylglycine | | | | 5 | | 136 |
| Pirkle-1J | | | β-Lactam derivative | 5 | | 136 |
| RegisCell | | Cellulose | Cellulose derivative | 5, 10 | Broad application range | 137 |
| RegisPack | | Amylose | Amylose derivative | 5, 10 | | 137 |
| RegisPack CLA-1 | | Amylose | Chlorinated amylose derivative | 10 | Complementary selectivity to RegisCell and RegisPack | 137 |
| Kromasil AmyCoat | Akzo Nobel | Amylose | Amylose derivative | 3, 5, 10 | Broad application range | - |
| Kromasil CelluCoat | | Cellulose | Cellulose derivative | | | - |
| Kromasil DMB | | Network polymer | Acylated N,N'-diallyl-L-tartardiamide | 5, 10 | High stability and capacity. Suitable for preparative applications | - |
| Kromasil TBB | | | | 5, 10 | | - |

[1] Please contact Hichrom for ordering information

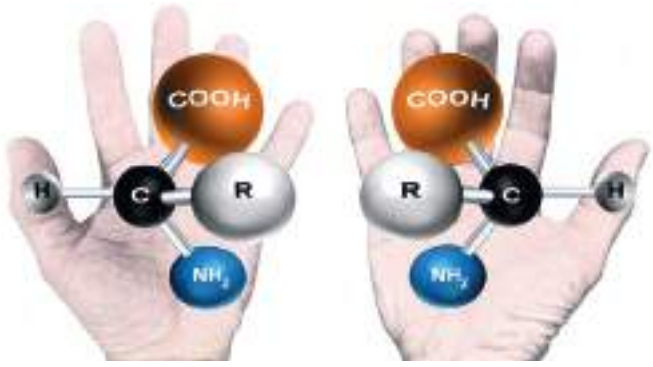
[2] CHIRALPAK ZWIX phases also available – see page 75

## Chiral Phases (continued)

### Chiral Phases (continued)

| Phase | Manufacturer | Chiral Type | Chiral Selector | Particle Size (µm) | Features | Page |
|---|---|---|---|---|---|---|
| NUCLEODEX β-OH | Macherey-Nagel | Cyclodextrin | β-Cyclodextrin | 5 | Reversed-phase applications | 102 |
| NUCLEODEX α-PM | | | Permethylated α-, β- and γ-cyclodextrins respectively | 5 | | 102 |
| NUCLEODEX β-PM | | | | 5 | | 102 |
| NUCLEODEX γ-PM | | | | 5 | | 102 |
| NUCLEOSIL CHIRAL-1 | | Ligand exchange | L-Hydroxyproline-$Cu^{2+}$ complex | 5 | α-Amino acid applications | 102 |
| RESOLVOSIL BSA-7 | | Protein | Bovine serum albumin | 7 | | 102 |
| NUCLEOCEL *DELTA* | | Cellulose | Cellulose derivative | 5 | Broad application range | 102 |
| ORpak CDA | Shodex | Cyclodextrin | α-Cyclodextrin | 6 | Polyhydroxymethacrylate base material | 142 |
| ORpak CDB | | | β-Cyclodextrin | 6 | | 142 |
| ORpak CDC | | | γ-Cyclodextrin | 6 | | 142 |
| ORpak CDBS | | | β-Cyclodextrin | 3 | Silica base | 142 |
| ORpak CRX | | Ligand exchange | L-Amino acid derivative | 6 | Suitable for underivatised amino acids | 142 |
| Ultron ES-OVM | Shinwa Chemical Industries | Protein | Ovomucoid | 5, 10 | USP L57 column | 158 |
| Ultron ES-Pepsin | | | Pepsin | 5 | Suitable for basic compounds | 158 |
| Ultron ES-CD | | Cyclodextrin | β-Cyclodextrin | 5 | Suitable for hydrophobic cyclic compounds | 158 |
| Ultron ES-PhCD | | | Phenylcarbamated β-cyclodextrin | 5 | | 158 |
| YMC Chiral CD BR | YMC[3] | Cyclodextrin | Bromide derivatives of cyclodextrin (α, β or γ) | 5 | Separates wide range of polar compounds | - |
| YMC Chiral NEA | | Brush | α-Naphthylethylamine | 5 | NP or RP applications | - |
| YMC Sumichiral OA series | | Various | Various | 5 | 17 different phases | - |
| ZirChrom Chiral LEU | ZirChrom | Brush | Leucine derivative | 3, 5 | Zirconia base material | 163 |
| ZirChrom Chiral NESA | | | Naphthylethylsuccinamic acid derivative | 3, 5 | | 163 |
| ZirChrom Chiral PG | | | Phenylglycine derivative | 3, 5 | | 163 |
| ZirChrom CelluloZe | | Cellulose | Cellulose derivative | 3, 5 | | 163 |

[3] YMC CHIRAL polysaccharide phases also available – please enquire



# HPLC COLUMN SELECTION BY USP SPECIFICATIONS



The following list of USP (United States Pharmacopoeia) column specifications (USP 35) includes a selection of recommended columns within each category. In most cases there are several columns available within a given category, but in a few indicated instances a packing very closely fitting the specification has been included. Please contact us for further advice and assistance on selecting a suitable column by USP specification. Please also contact us for advice on column selection by EP (European Pharmacopoeia) specification. The USP monographs allow chromatographers flexibility to make method adjustments within specified limits in order to meet system suitability requirements. Please see page 57 for further details.


| | |
|---|---|
| L1 | Octadecylsilane chemically bonded to porous or non-porous silica or ceramic microparticles, 1.5 to 10µm in diameter, or a monolithic rod<br>*Widely available* |
| L2 | Octadecylsilane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50µm in diameter<br>*Pellicular ODS* |
| L3 | Porous silica particles, 1.5 to 10µm in diameter, or a monolithic silica rod<br>*Widely available* |
| L4 | Silica gel of controlled surface porosity bonded to a solid spherical core, 30 to 50µm in diameter<br>*Pellicular silica* |
| L5 | Alumina of controlled surface porosity bonded to a solid spherical core, 30 to 50µm in diameter<br>*Please enquire* |
| L6 | Strong cation-exchange packing – sulphonated fluorocarbon polymer coated on a solid spherical core, 30 to 50µm in diameter<br>*Please enquire* |
| L7 | Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10µm in diameter, or a monolithic silica rod<br>*Widely available* |
| L8 | An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10µm in diameter<br>*Widely available* |
| L9 | Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10µm in diameter<br>*Partisil SCX  NUCLEOSIL SA* |
| L10 | Nitrile groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter<br>*Widely available* |
| L11 | Phenyl groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter<br>*Widely available* |
| L12 | A strong anion-exchange packing made by chemically bonding a quaternary amine to a solid silica spherical core, 30 to 50µm in diameter<br>*Please enquire* |
| L13 | Trimethylsilane chemically bonded to porous silica particles, 3 to 10µm in diameter<br>*YMC TMS  Exsil C1  Develosil TMS-UG* |
| L14 | Silica gel having a chemicallly bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10µm in diameter<br>*NUCLEOSIL SB  Exsil SAX  Partisil SAX* |
| L15 | Hexylsilane chemically bonded to totally porous silica particles, 3 to 10µm in diameter<br>*Spherisorb C6  Chromegabond C6* |
| L16 | Dimethylsilane chemically bonded to porous silica particles, 5 to 10µm in diameter<br>*NUCLEOSIL C2  Chromegabond C2* |
| L17 | Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12µm in diameter<br>*Hamilton HC-75 $H^+$  Shodex SUGAR SH1011  NUCLEOGEL Sugar 810H* |
| L18 | Amino and cyano groups chemically bonded to porous silica particles, 3 to 10µm in diameter<br>*Partisil PAC  Partisphere PAC* |
| L19 | Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form, about 9µm in diameter<br>*Hamilton HC-75 $Ca^{2+}$  NUCLEOGEL Sugar 810 Ca  Shodex SUGAR SC1011* |
| L20 | Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10µm in diameter<br>*LiChrospher Diol  NUCLEOSIL Diol  PrincetonSPHER Diol* |
| L21 | A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30µm in diameter<br>*Hamilton PRP-1  PLRP-S  Shodex RSpak RP18* |
| L22 | A cation-exchange resin made of porous polystyrene gel with sulphonic acid groups, about 10µm in size<br>*Hamilton PRP-X200  Shodex SUGAR SH1011* |
| L23 | An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, 7 to 12µm in size<br>*TSKgel BioAssist Q  COSMOGEL QA  Shodex IEC QA-825* |

## HPLC Column Selection by USP Specifications (continued)

| | |
|---|---|
| L24 | Polyvinyl alcohol chemically bonded to porous silica particles, 5µm in diameter<br>*YMC-Pack PVA-Sil* |
| L25 | Packing having the capacity to separate compounds with a molecular weight range from 100-5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contained some residual carboxyl functional groups) was found suitable<br>*TSKgel G2500PW$_{XL}$* *Shodex OHpak SB-802HQ* |
| L26 | Butylsilane chemically bonded to totally porous silica particles, 1.5 to 10µm in diameter<br>*Widely available* |
| L27 | Porous silica particles, 30 to 50µm in diameter<br>*YMC Silica* *LiChroprep Silica* *Develosil Silica* *NUCLEODUR Silica* |
| L28 | A multifunctional support, which consists of a high purity, 100Å, spherical silica substrate that has been bonded with anionic exchanger, amine functionality in addition to a conventional reversed-phase C8 functionality<br>*Alltech Mixed-Mode C8/Anion* |
| L29 | Gamma alumina, reversed-phase, low carbon percentage by weight, alumina-based polybutadiene spherical particles, 5µm in diameter with a pore volume of 80Å units<br>*GammaBond RP1* |
| L30 | Ethylsilane chemically bonded to totally porous silica particles, 3 to 10µm in diameter<br>*As for L16*[1] |
| L31 | A hydroxide-selective, strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 8.5µm macroporous particles having a pore size of 2000Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene<br>*IonPac AS11-HC* |
| L32 | A chiral ligand-exchange resin packing - L-proline copper complex covalently bonded to irregularly shaped silica particles, 5 to 10µm in diameter<br>*NUCLEOSIL Chiral-1* *CHIRALCEL WH* |
| L33 | Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability<br>*TSKgel G4000SW$_{XL}$* *Shodex PROTEIN KW-800 series* |
| L34 | Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 7 to 9µm in diameter<br>*Hamilton HC-75 Pb$^{2+}$* *Shodex SUGAR SP0810* |
| L35 | A zirconium-stabilised spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase having a pore size of 150Å<br>*ZORBAX GF-250* |
| L36 | A 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5µm aminopropyl silica<br>*Hichrom CHIRA-chrom-1* |
| L37 | Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 Da. It is a polymethacrylate gel<br>*TSKgel G3000PW$_{XL}$* *Shodex OHpak SB-803HQ* |
| L38 | A methacrylate-based size-exclusion packing for water-soluble samples<br>*TSKgel G1000-G6000PW$_{XL}$* *Shodex OHpak SB-800HQ Series* |
| L39 | A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin<br>*TSKgel G1000-G6000PW$_{XL}$* *Shodex OHpak SB-800HQ Series* |
| L40 | Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5µm to 20µm in diameter<br>*CHIRALCEL OD-H* *RegisCell* *NUCLEOCEL DELTA* |
| L41 | Immobilised $\alpha_1$-acid glycoprotein on spherical silica particles, 5µm in diameter<br>*CHIRALPAK AGP* |
| L42 | Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5µm in diameter<br>*Hichrom RPB* |
| L43 | Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10µm in diameter<br>*HALO PFP* *Hypersil GOLD PFP* *Partisphere TAC-1* |
| L44 | A multifunctional support, which consists of a high purity, 60Å, spherical silica substrate that has been bonded with a cationic exchanger, sulphonic acid functionality in addition to a conventional reversed phase $C_8$ functionality<br>*Chromegabond RP-SCX* |
| L45 | Beta cyclodextrin, R,S-hydroxypropyl ether derivative, bonded to porous silica particles, 5 to 10µm in diameter<br>*ChiraDex* *NUCLEODEX β-OH* *Ultron ES-CD* |
| L46 | Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalised latex beads, about 9µm to 11µm in diameter<br>*CarboPac PA1* |
| L47 | High capacity anion-exchange microporous substrate, fully functionalised with a trimethylamine group, 8µm in diameter<br>*CarboPac MA1* |

[1] Column represents the closest match to USP specifications

## HPLC Column Selection by USP Specifications (continued)

| | |
|---|---|
| L48 | Sulphonated, cross-linked polystyrene with an outer layer of sub-micron, porous, anion-exchange microbeads, 5 to 15µm in diameter<br>*IonPac AS5* |
| L49 | A reversed-phase packing made by coating a thin layer of polybutadiene on to spherical porous zirconia particles, 3 to 10µm in diameter<br>*ZirChrom PBD* |
| L50 | Multi-function resin with reversed-phase retention and strong anion-exchange functionalities. The resin consists of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15µm in diameter, and a surface area of not less than 350 $m^2/g$. Substrate is coated with quaternary ammonium functionalised latex particles consisting of styrene cross-linked with divinylbenzene<br>*OmniPac PAX-500* |
| L51 | Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 5 to 10µm in diameter<br>*CHIRALPAK AD* *RegisPack* |
| L52 | A strong cation-exchange resin made of porous silica with sulphopropyl groups, 5 to 10µm in diameter<br>*BioBasic SCX* *TSKgel SP-2SW* |
| L53 | Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15µm diameter. Substrate is surface grafted with carboxylic acid and/or phosphoric acid functionalised monomers. Capacity not less than 500 µEq/column<br>*IonPac CS14* |
| L54 | A size exclusion medium made of covalent bonding of dextran to highly cross-linked porous agarose beads, about 13µm in diameter<br>*Please enquire* |
| L55 | A strong cation-exchange resin made of porous silica coated with polybutadiene-maleic acid copolymer, about 5µm in diameter<br>*Universal Cation* |
| L56 | Propyl silane chemically bonded to totally porous silica particles, 3 to 10µm in diameter<br>*Zorbax SB-C3* |
| L57 | A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5µm in diameter, with a pore size of 120Å<br>*Ultron ES-OVM* |
| L58 | Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30µm in diameter<br>*PL Hi-Plex Na* *Shodex SUGAR KS series* |
| L59 | Packing for the size exclusion separations of proteins (separation by molecular weight) over the range of 5 to 7000 kDa. It is spherical (1.5 to 10µm), silica or hybrid packing with a hydrophilic coating<br>*TSKgel G3000$SW_{XL}$* *Shodex PROTEIN KW-803* |
| L60 | Spherical, porous silica gel, 10µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped<br>*HALO RP-Amide* *Discovery RP-Amide* |
| L61 | A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 13µm microporous particles having a pore size less than 10Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 85nm diameter microbeads bonded with alkanol quaternary ammonium ions (6%)<br>*IonPac AS11* |
| L62 | C30 silane bonded phase on a fully porous spherical silica, 3 to 15µm in diameter.<br>*PrincetonSPHER C30* *Develosil C30* *Cogent C30* |
| L63 | Glycopeptide teicoplanin linked through multiple covalent bonds to a 100Å units spherical silica<br>*CHIROBIOTIC T* |
| L64 | Strongly basic anion-exchange resin consisting of 8% crosslinked styrene-divinylbenzene copolymer with a quaternary ammonium group in the chloride form, 45 to 180µm in diameter<br>*AG 1-X8* |
| L65 | Strongly acidic cation-exchange resin consisting of 8% sulphonated crosslinked styrene-divinylbenzene copolymer with a sulphonic acid group in the hydrogen form, 63 to 250µm in diameter<br>*AG 50W-X2* |
| L66 | A crown ether coated on a 5µm particle size silica gel substrate. The active site is (S)-18-crown-6-ether<br>*CROWNPAK CR(+)* |
| L67 | Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10µm in diameter<br>*Asahipak ODP-50* *apHera C18* |
| L68 | Spherical, porous silica, 10µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped<br>*Suplex pKb-100* |
| L69 | Ethylvinylbenzene/divinylbenzene substrate agglomerated with quaternary amine functionalised 130nm latex beads, about 6.5µm in diameter<br>*CarboPac PA20* |
| L70 | Cellulose tris(phenyl carbamate) coated on 5µm silica<br>*CHIRALCEL OC-H* |
| L71 | A rigid, spherical polymethacrylate, 4 to 6µm in diameter<br>*Shodex RSpak DE* |

## HPLC Column Selection by USP Specifications (continued)

| | |
|---|---|
| L72 | (S)-phenylglycine and 3,5-dinitroaniline urea linkage covalently bonded to silica<br>*Sumichiral OA-3300 S* |
| L73 | A rigid, spherical polydivinylbenzene particle, 5 to 10µm in diameter<br>*Jordi-Gel DVB* |
| L74 | A strong anion-exchange resin consisting of a highly cross-linked core of 7µm macroporous particles having a 100Å average pore size and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and an anion-exchange layer grafted to the surface, which is functionalised with alkyl quaternary ammonium ions<br>*IonPac AS14A* |
| L75 | A chiral-recognition protein, bovine serum albumin (BSA), chemically bonded to silica particles, about 7µm in diameter, with a pore size of 300Å<br>*RESOLVOSIL BSA* |
| L76 | Silica based weak cation-exchange material, 5µm in diameter. Substrate is surface polymerised polybutadiene-maleic acid to provide carboxylic acid functionalities. Capacity not less than 29 µEq/column<br>*IonPac SCS-1* |
| L77 | Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 6 to 9µm diameter. Substrate is surface grafted with carboxylic acid functionalised groups. Capacity not less than 500 µEq/column (4mm x 25cm)<br>*IonPac CS17* |
| L78 | A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and anion-exchange (primary, secondary, tertiary or quaternary amino) functional groups chemically bonded to porous, non-porous or ceramic microparticles, 1.0 to 50µm in diameter or a monolithic rod<br>*Acclaim Mixed-Mode WAX-1* |
| L79 | A chiral-recognition protein, human serum albumin (HSA), chemically bonded to silica particles, about 5µm in diameter<br>*CHIRALPAK HSA* |
| L80 | Cellulose tris(4-methylbenzoate)-coated, porous spherical silica particles, 5µm in diameter<br>*CHIRALCEL OJ-H* |
| L81 | A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 9µm porous particles having a pore size of 2000Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 70nm diameter microbeads (6% cross-linked) bonded with alkanol quaternary ammonium ions<br>*IonPac AS11-HC* |
| L82 | Polyamine chemically bonded to cross-linked polyvinyl alcohol polymer, 4-5µm in diameter<br>*Asahipak NH2P-40  Asahipak NH2P-50* |
| L83 | A hydroxide-selective, strong anion-exchange resin, quaternary amine bonded on latex particles attached to a core of 10.5µm microporous particles having a pore size of 10Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene<br>*IonPac AS17-C* |
| L84 | Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 5µm diameter. Substrate is surface grafted with carboxylic acid functionalised groups. Capacity not less than 8400 µEq/column (5mm x 25cm)<br>*IonPac CS16* |

## USP and EP Allowable Adjustments

Allowable adjustments that can be made to a USP or EP (European Pharmacopoeia) method, without the method requiring revalidation, are summarised in the table below. Please contact us for further advice and assistance.

| Parameter | USP[1] Allowable Adjustment | EP[2] Allowable Adjustment |
|---|---|---|
| Column Length | ±70% | ±70% |
| Column i.d. | ±25% | ±25% |
| Particle Size | –50% | –50% |
| Column Temperature | ±10°C | ±10°C |
| Flow Rate | ±50% | ±50% |
| Eluent pH | ±0.2 units | ±0.2 units |
| Concentration of Buffer Salts | ±10% | ±10% |
| Solvent A:B Ratio | Minor ±30% relative, but ≤±10% absolute | Minor ±30% relative or ±2% absolute, ≤ ±10% absolute for other |
| Injection Volume | Any reduction | Any reduction |
| Change of UV Detector Wavelength | 0, but ±3nm between detectors | No change |

[1] United States Pharmacopoeia 34 (2011) Section 621  [2] European Pharmacopoeia 6.0 (2010) Section 2.2.4.6

# ACE®



- ACE HPLC and ACE Excel® UHPLC options
- Ultra high purity base deactivated silica
- 2, 3, 5, 10 and 15µm particle sizes
- Unique selectivity phases
- Exceptional reproducibility
- Capillary to preparative dimensions

The ACE® range of ultra pure silica phases is manufactured by Advanced Chromatography Technologies Ltd (ACT). A wide range of particle sizes, pore sizes, bonded chemistries and column dimensions are available. Excellent performance and reproducible chromatography with basic, acidic and neutral molecules are ensured by the most stringent of validation protocols. ACE Excel® columns (see page 65), with increased pressure rating, are available for UHPLC applications in 2, 3 and 5µm particle sizes.

## ACE Phases

| Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | USP Listing |
|---|---|---|---|---|---|---|---|
| SuperC18 | Octadecyl encapsulated | Yes | 2, 3, 5, 10 | 90 | 400 | 14.8 | L1 |
| C18-AR | Octadecyl with integral Phenyl | Yes | 2, 3, 5, 10 | 100 | 300 | 15.5 | L1 |
| C18-PFP | Octadecyl with integral PFP | Yes | 2, 3, 5, 10 | 100 | 300 | 14.3 | L1 |
| C18-Amide | Octadecyl with integral amide polar group | Yes | 2, 3, 5, 10 | 100 | 300 | 16.4 | L1/L60 |
| C18-HL | Octadecyl | Yes | 3, 5, 10, 15 | 90 | 400 | 20.0 | L1 |
| C18 | Octadecyl | Yes | 2, 3, 5, 10 | 100 | 300 | 15.5 | L1 |
| C8 | Octyl | Yes | 2, 3, 5, 10 | 100 | 300 | 9.0 | L7 |
| C4 | Butyl | Yes | 2, 3, 5, 10 | 100 | 300 | 5.5 | L26 |
| CN-ES | Cyano with proprietary extended alkyl spacer | Yes | 2, 3, 5, 10 | 100 | 300 | 12.6 | L10 |
| CN | Cyano | Yes | 2, 3, 5, 10 | 100 | 300 | 5.5 | L10 |
| Ph | Phenyl | Yes | 2, 3, 5, 10 | 100 | 300 | 9.5 | L11 |
| AQ | Proprietary | Yes | 2, 3, 5, 10 | 100 | 300 | 14.0 | L1 |
| SIL | Unbonded | - | 2, 3, 5, 10 | 100 | 300 | - | L3 |
| C18-300 | Octadecyl | Yes | 3, 5, 10 | 300 | 100 | 9.0 | L1 |
| C8-300 | Octyl | Yes | 3, 5, 10 | 300 | 100 | 5.0 | L7 |
| C4-300 | Butyl | Yes | 3, 5, 10 | 300 | 100 | 2.6 | L26 |
| CN-300 | Cyano | Yes | 3, 5, 10 | 300 | 100 | 2.6 | L10 |
| Ph-300 | Phenyl | Yes | 3, 5, 10 | 300 | 100 | 5.3 | L11 |

## Wide Range of Stationary Phase Selectivities

Selectivity is an important consideration for method development. Although changing organic modifier, buffer pH, gradient time etc. will all change retention times and therefore compound resolution, the column stationary phase is the feature which affects selectivity the greatest. In order to successfully separate compounds with wide variations in hydrophobicity and polarity, a number of phases with significantly different selectivity and which separate according to very different interaction mechanisms, are required.

The ACE range of phases, which now includes several novel chemistry phases, was developed in order to offer the chromatographer a wider choice of interaction mechanisms for method development. For instance, the unique ACE C18-AR and ACE C18-PFP phases offer C18, combined with Phenyl or PFP selectivities respectively, but exhibit the ultra low bleed and excellent temperature and pH stability characteristics of C18 phases.

Figure 1 demonstrates the power of selectivity in developing a method for the separation of a mixture of 7 components. In this case, using the combination of hydrophobic, π-π, dipole-dipole and shape selectivity interactions of the ACE C18-PFP column, all 7 components were fully resolved. In addition, comparing the results obtained with columns of significantly different selectivity for an unknown sample, can give confidence that all components which may be present in the mixture are detected.

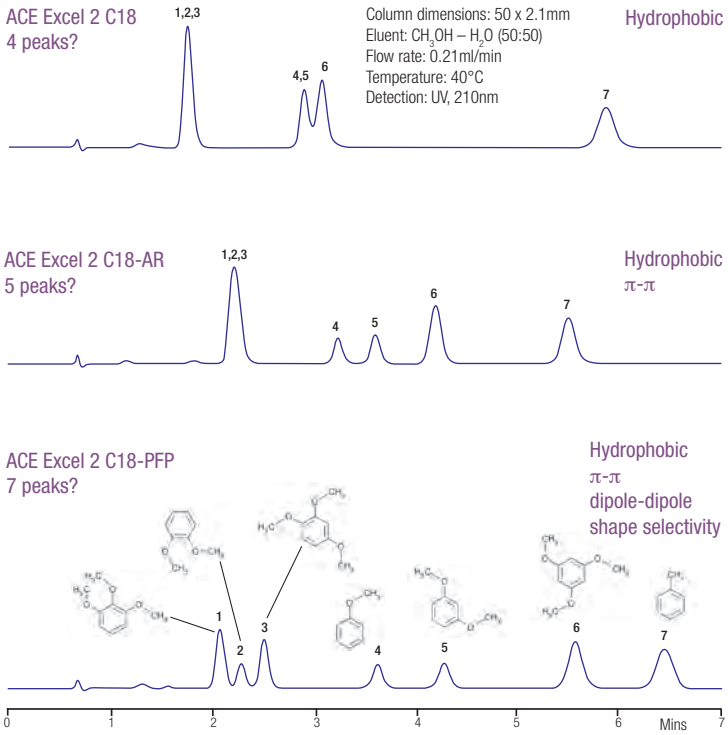
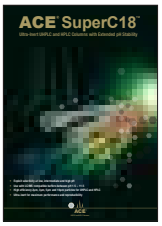



Figure 1. The power of stationary phase selectivity

## ACE® SuperC18™

- Exploit selectivity at low, intermediate and high pH
- Use with LC-MS compatible buffers between pH 1.5 and 11.5
- Ultra low bleed for LC-MS compatibility
- Encapsulated Bonding Technology (EBT™) for improved stability
- Ideal for method development and screening
- Useful for preparative separations at high pH

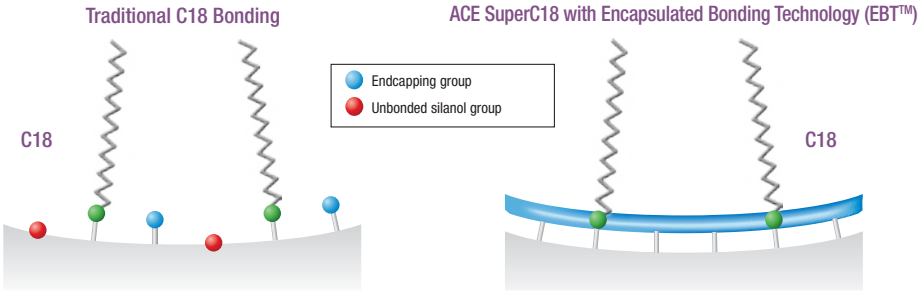



**Please request your copy of the ACE SuperC18 brochure**

ACE® SuperC18™ is a new highly inert stationary phase developed by Advanced Chromatography Technologies Ltd. It is ideal for investigating selectivity at low, intermediate and high pH due to its excellent robustness and extended pH range. This phase can be used in both methanol and acetonitrile containing eluents, offers rapid re-equilibration and is ideal for method development and screening systems. ACE SuperC18 is also an ideal phase for purification and isolation work at high eluent pH. All ACE SuperC18 analytical dimension columns are supplied in the dual compatible UHPLC/HPLC Excel hardware (see page 65).

### Encapsulated Bonding Technology

The unique Encapsulated Bonding Technology (EBT) developed for ACE SuperC18 columns considerably increases ligand coverage of the silica surface and effectively eliminates the effect of unbonded silanol groups from separations. This higher ligand coverage results in improved inertness, chromatographic performance and stability.




Under aggressive acidic conditions ACE SuperC18 is highly resistant to ligand cleavage, due to a combination of the Encapsulated Bonding Technology and ultra-inert ACE silica. Many C18 bonded columns however, will exhibit ligand cleavage under such acidic conditions, resulting in retention shifts and/or peak tailing.

Under basic conditions with LC-MS compatible eluents, the Encapsulated Bonding Technology shields the ACE SuperC18 from dissolution, whilst maintaining excellent chromatographic performance. Traditional C18 bonded silica columns however, are prone to silica dissolution under such aggressive conditions, which can result in premature column deterioration.

### Exploiting Selectivity by Adjusting pH

Figure 2 illustrates the differences in selectivity that can be obtained for a mixture of compounds when using the ACE SuperC18 at low pH (3.0) and high pH (10.7). Changes in retention and elution order were observed. In addition to offering a powerful tool for method development, analysis at high and low pH on the same column allows the chromatographer to ensure that all components in a mixture are observed, with no coelution occurring.

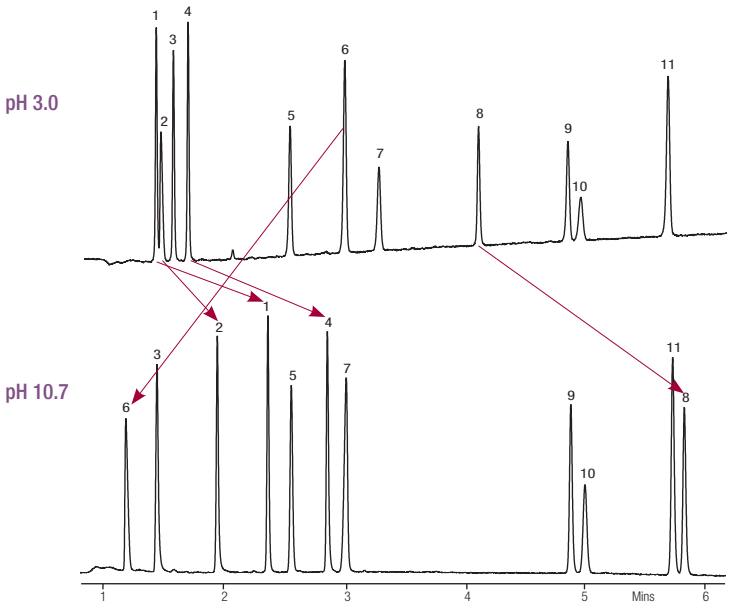



Figure 2. Selectivity at low and high pH

Column: ACE Excel 3 SuperC18 (3µm, 50 x 2.1mm)
Eluent: **Low pH conditions:**
A: 10mM ammonium formate, pH 3.0 in $H_2O$
B: 10mM ammonium formate, pH 3.0 in $CH_3CN$-$H_2O$ (9:1)
**High pH conditions:**
A: 0.1% $NH_3$ (18mM), pH 10.7 in $H_2O$
B: 0.1% $NH_3$ (18mM), pH 10.7 in $CH_3CN$-$H_2O$ (9:1)
Gradient: 3 to 100% B in 7mins
Flow rate: 0.42ml/min
Temperature: 40°C
Detection: UV, 254nm

1. Nizatidine
2. Salbutamol
3. Amiloride
4. N-Acetylprocainamide
5. Quinoxaline
6. Methyl paraben
7. p-Cresol
8. Reserpine
9. Piperine
10. Toluene
11. Felodipine

Please see pages 71-72 for ordering information for ACE SuperC18 columns.

## ACE® C18-AR

- C18 bonded phase with integral phenyl (AR) selectivity
- Provides alternative selectivity to standard C18 columns
- Compatible with highly aqueous eluents
- Ultra low bleed phase for UV and LC-MS compatibility
- Exceptional bonded phase stability for elevated temperature applications
- USP L1 designation


**Please request your copy of the ACE C18-AR brochure**

### Alternative Selectivity

C18 columns manufactured from high purity silicas show near identical selectivity. Therefore, simply changing from one leading C18 brand to another will not significantly improve a problematic separation. ACE® C18-AR, however, is a C18 phase that has been specifically developed to provide alternative selectivity to other C18 columns.

The ACE C18-AR phase utilises a specially developed ligand combining a C18 chain with integral phenyl functionality, thus combining the benefits of both C18 and phenyl characteristics into a single phase. ACE C18-AR can be used for 'standard' C18 column separations, but is additionally recommended for separations that involve compounds containing aromatic functionality.

In addition to strong hydrophobic interactions, ACE C18-AR exhibits strong π-π and moderate dipole-dipole interactions, with a moderate degree of shape selectivity. This makes the phase particularly suitable for the analysis of analytes containing π-bonding, different dipole moments or electron delocalization and electron withdrawing groups, such as halogens, nitro groups, ketones, esters and acids.

Figure 3 compares the selectivity offered by ACE 3 C18 (typical of conventional C18 phases) and ACE 3 C18-AR for a mixture containing aromatic nitrobenzene compounds. Hydrophobic retention of a neutral molecule marker is the same on both columns. However, selectivity towards the aromatic compounds is significantly enhanced, with increased retention and a complete reversal of elution order.

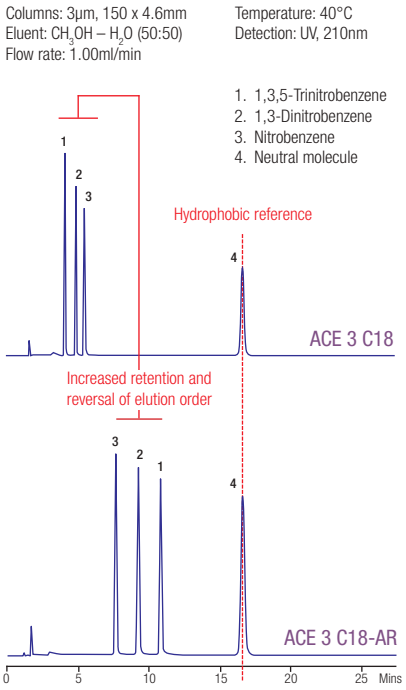



Figure 3. Aromatic nitrobenzenes

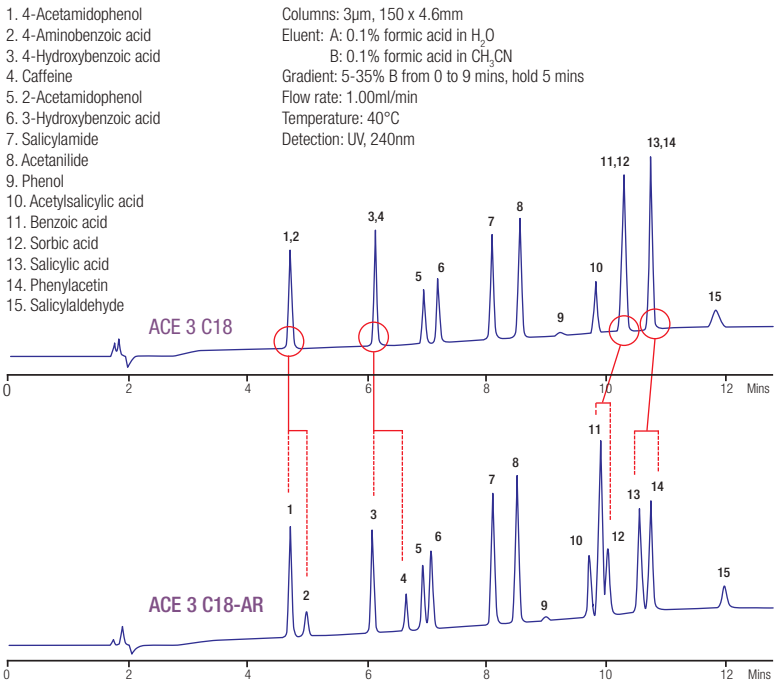



Figure 4. Analgesics separation

Figure 4 compares the separation of a mixture of 15 analgesics on ACE 3 C18 and ACE 3 C18-AR columns, under the same analytical conditions. Whereas on the ACE C18 column, 4 pairs of co-eluting peaks were observed, with ACE C18-AR all 15 components were resolved. This demonstrates the alternative selectivity offered by the ACE C18-AR phase, due to the presence of strong π-π and moderate dipole-dipole interactions, in addition to the hydrophobic interactions due to the C18 ligand.

### Compatibility with High Aqueous Conditions

The integral phenyl functionality of the ACE C18-AR phase protects against de-wetting and subsequent retention loss, which may occur with conventional C18 phases when separating very polar, water-soluble compounds under highly aqueous (>95%) eluent conditions. As a result, ACE C18-AR produces highly reproducible chromatography even under highly aqueous conditions.

### Very Low Bleed for UV and LC-MS Compatibility

Whereas most high purity C18 bonded phases show low bleed characteristics, alternative bonded phases such as phenyl and polar embedded phases, can exhibit significantly higher bleed, which is particularly evident at low UV wavelengths or by LC-MS. In contrast, ACE C18-AR shows very low column bleed, comparable to that observed for C18 phases.

Please see pages 71-72 for ordering information for ACE C18-AR columns.

# ACE® C18-PFP

- Combines C18 and PFP separation mechanisms
- Improved retention of polar basic compounds
- Compatible with highly aqueous eluents
- Ultra low bleed phase for UV and LC-MS compatibility
- Exceptional bonded phase stability for elevated temperature applications
- USP L1 designation


**Please request your copy of the ACE C18-PFP brochure**

ACE® C18-PFP is the second of the unique selectivity phases developed by ACT as an alternative to conventional C18 phases. In recent years the use of PFP bonded phases (see page 41) has grown significantly due to the alternative selectivity offered. However, compared to C18 phases, PFP phases have traditionally been compromised with reduced hydrophobicity, reduced stability and significant column bleed. The ACE C18-PFP phase utilises a specially developed ligand combining a C18 chain with integral PFP functionality. This results in a phase that maintains the hydrophobic, stability and low bleed characteristics of leading C18 phases, yet provides the multiple retention mechanisms of a PFP phase.

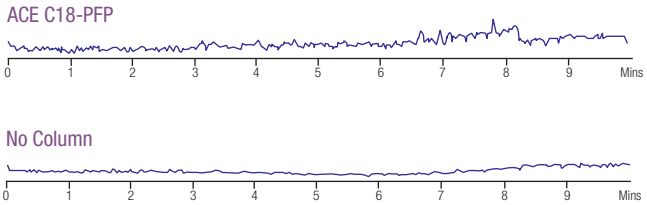
## ACE C18-PFP Separation Mechanisms

The ACE C18-PFP phase exhibits multiple retention mechanisms including hydrophobic, π-π interactions, dipole-dipole interactions, hydrogen bonding and shape selectivity. Whilst approximations of relative strengths are shown in the table adjacent, the predominance of each retention mechanism is dictated by the solute's physico-chemical properties, its structure and the chromatographic conditions employed.

| Separation Mechanism | C18 | PFP | ACE C18-PFP |
|---|---|---|---|
| Hydrophobicity | ++++ | +/++ | ++++ |
| π-π Interaction | - | +++ | +++ |
| Dipole-Dipole Interaction | - | ++++ | ++++ |
| Hydrogen Bonding | - | +++ | +++ |
| Shape Selectivity | ++ | +++ | ++++ |

## Very Low Bleed for UV and LC-MS Compatibility

Whereas most high purity C18 bonded phases show low bleed characteristics, alternative bonded phases including PFP exhibit significantly higher bleed. This is particularly evident at low UV wavelengths or by LC-MS. ACE C18-PFP combines a low bleed level (typical of leading C18 brands) with an alternative selectivity, thus providing a valuable method development tool. Figure 5 shows the TIC trace obtained for ACE C18-PFP compared with a blank run, demonstrating that the ACE C18-PFP column has very low bleed and is therefore highly suitable for LC-MS analyses.

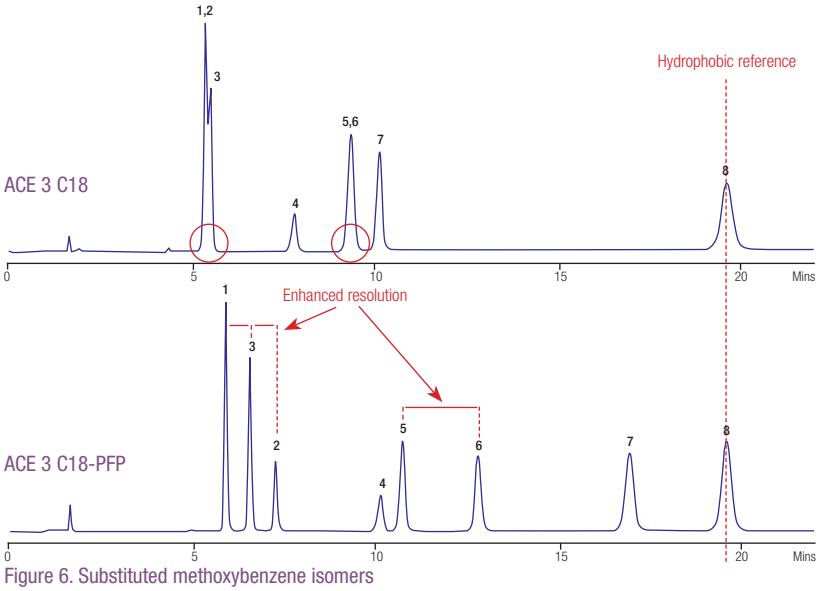



Column dimensions: 50 x 3.0mm
Eluent: A: 0.1% formic acid in $H_2O$
B: 0.1% formic acid in $CH_3CN$
Flow rate: 0.43ml/min
Gradient: T(mins) 0 0.1 5 10 10.5
%B 5 5 95 95 5
Temperature: 60°C
Detection: +ve ESI fast scan mode, full scan range 50-1000m/z

Figure 5. Low column bleed for LC-MS

## Applications of ACE C18-PFP

Due to their similar hydrophobic characteristics, ACE C18-PFP columns may be used for applications in which 'standard' C18 columns would normally be considered. However, due to its integral pentafluorophenyl functionality, ACE C18-PFP is additionally recommended for separations that involve halogenated aromatic compounds, regioisomers and those analytes with differing shape constraints. Figure 6 demonstrates excellent resolution of all components in a mixture of methoxybenzene isomers when using an ACE C18-PFP column. Analysed on an ACE C18 column (typical of conventional C18 phases), two pairs of components coeluted, but on an ACE C18-PFP column all peaks were resolved. The neutral molecule marker had the same retention time on both columns due to hydrophobic interactions.




Columns: 3µm, 150 x 4.6mm
Eluent: $CH_3OH$ - $H_2O$ (50:50)
Flow rate: 1.00ml/min
Temperature: 40°C
Detection: UV, 254nm

1. 1,2,3-Trimethoxybenzene
2. 1,2,4-Trimethoxybenzene
3. 1,2-Dimethoxybenzene
4. 1,4-Dimethoxybenzene
5. Methoxybenzene
6. 1,3-Dimethoxybenzene
7. 1,3,5-Trimethoxybenzene
8. Neutral molecule

Figure 6. Substituted methoxybenzene isomers

Please see pages 71-72 for ordering information for ACE C18-PFP columns.

## ACE® C18-Amide

- Offers combined C18 and Amide separation mechanisms
- Alternative selectivity to C18 for method development
- Improved separations of polar, acidic, basic and phenolic compounds
- Compatible with 100% aqueous eluents
- Low bleed for UV and LC-MS compatibility


**Please request your copy of the ACE C18-Amide brochure**

ACE® C18-Amide combines a C18 with a polar amide group on a single ligand. The extended spacer technology leads to increased column lifetime and stability. The ACE C18-Amide phase offers complementary selectivity to C18 columns due to multiple modes of interaction from the unique C18-Amide ligand, leading to increased polar retention. Figure 7 illustrates the difference between two ACE bonded phases, C18-Amide and C18. Although both phases offer the possibility of strong hydrophobic interaction with their respective C18 chains, the embedded amide group of the C18-Amide phase introduces additional modes of interaction, which results in increased retention for polar compounds and alternative selectivity.

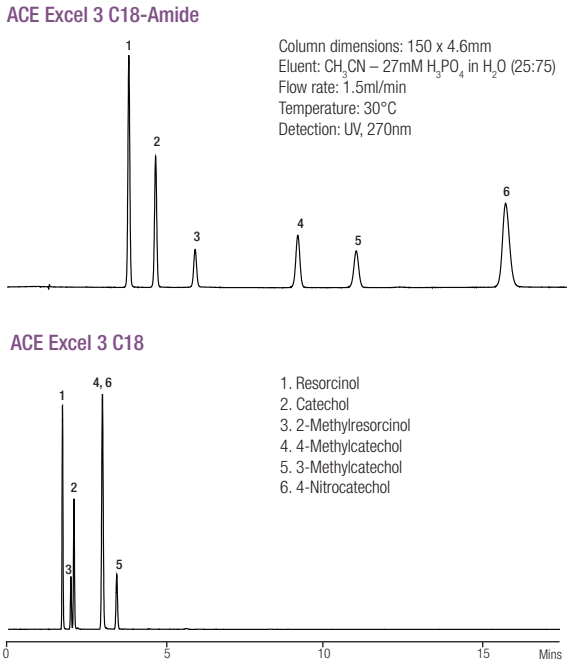



Figure 7. Enhanced selectivity with ACE C18-Amide

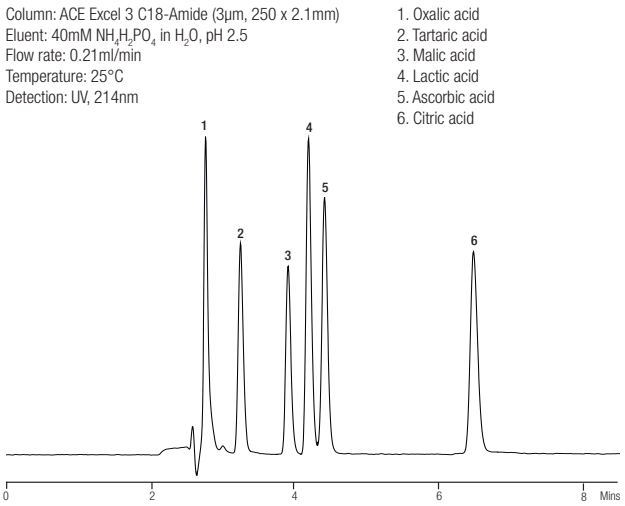



Figure 8. Separation of organic wine acids

Figure 8 demonstrates the separation of polar organic wine acids on ACE C18-Amide using a 100% aqueous eluent.
Figure 9 shows an example of the alternative selectivity obtained for vanillins using ACE C18-Amide compared with ACE C18.

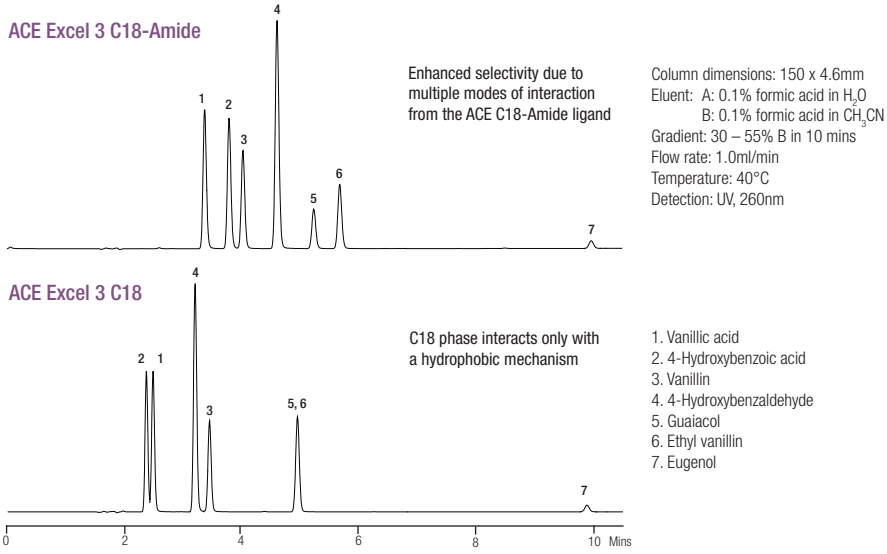


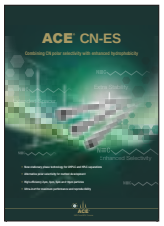

Figure 9. Separation of vanillins

All ACE C18-Amide analytical dimension columns are supplied in the dual compatible UHPLC/HPLC Excel hardware (see page 65).

Please see pages 71-72 for ordering information for ACE C18-Amide columns.

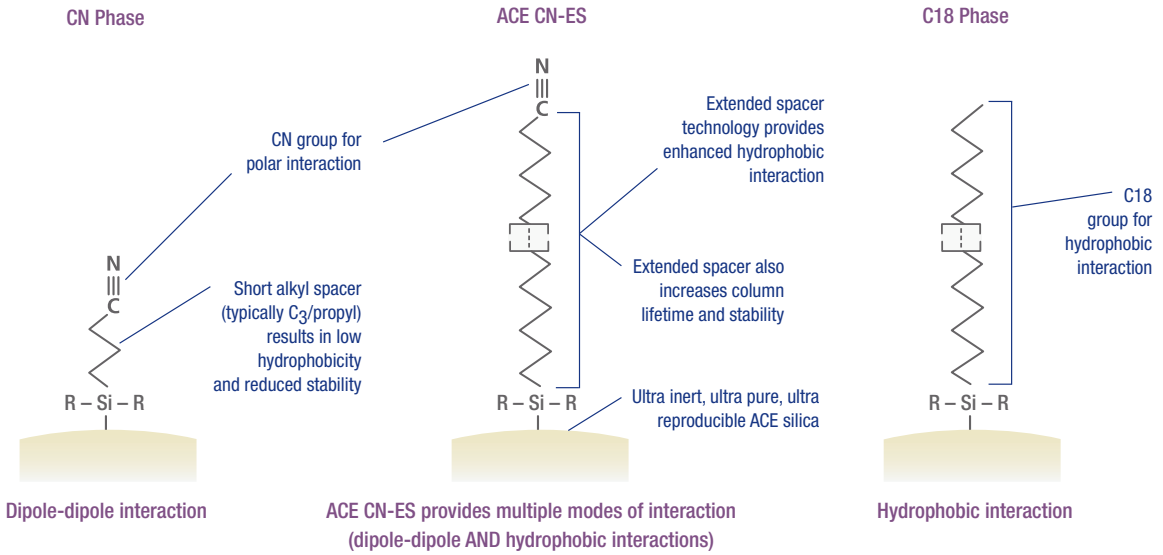
## ACE® CN-ES

- Combines CN polar selectivity with enhanced hydrophobicity
- Alternative polar selectivity for method development
- Separations due to hydrophobic and dipole-dipole interactions
- Suitable for RP and NP separations
- Compatible with 100% aqueous eluents




**Please request your copy of the ACE CN-ES brochure**

ACE® CN-ES combines a polar CN group with an extended spacer that provides increased hydrophobic retention. Separations are achieved by a combination of strong dipole-dipole interactions and hydrophobic binding interactions. The extended spacer technology additionally provides increased column lifetime and stability, compared to traditional CN bonded phases.




ACE CN-ES is recommended for separations where traditional CN bonded phases show insufficient stability or column lifetime or for applications where a typical C18 column does not provide adequate separation. ACE CN-ES also shows good selectivity for the separation of analytes with double and triple bonds and for mixtures of polar and non-polar analytes. Figure 10 shows how alternative selectivity can be exploited for the UHPLC separation of steroids. Whereas both the ACE Excel 2 C18 and the ACE Excel 2 CN columns fail to separate all nine components of this mixture, the ACE Excel 2 CN-ES column shows complete resolution of all compounds.

All ACE CN-ES analytical dimension columns are supplied in the dual compatible UHPLC/HPLC Excel hardware (see page 65).

Please see pages 71-72 for ordering information for ACE CN-ES columns.

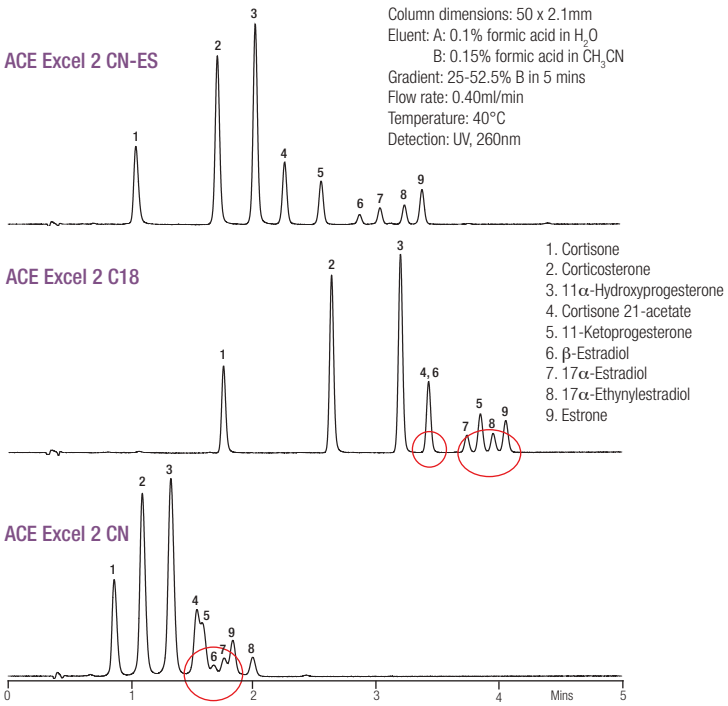



Figure 10. Selectivity comparison for UHPLC of Steroids

## ACE® AQ

- Retains polar compounds in 100% aqueous eluent
- C18 bonded phase with integral polar functionality
- Resistant to retention loss in 100% aqueous eluent
- Rapid gradient re-equilibration
- Excellent peak shape with acidic, basic and neutral molecules
- Ideal for LC-MS applications

ACE® AQ is an ultra-inert phase designed for the reversed-phase separation of very polar compounds with up to 100% aqueous eluent. ACE AQ is ideal for use with fast gradients due to its rapid re-equilibration properties and resistance to retention loss.

### Maximum Reproducibility in High Aqueous Eluents

When separating very polar, water-soluble compounds, highly aqueous (>95%) eluents are often required to achieve sufficient retention. However, operating a C18 column under such conditions can lead to dewetting or 'phase collapse' which can result in poor chromatographic reproducibility. Over time peaks will elute with shorter and shorter retention times and resolution between peaks will deteriorate. ACE AQ columns introduce an integral polar functionality, which prevents this retention loss when using highly aqueous eluents.

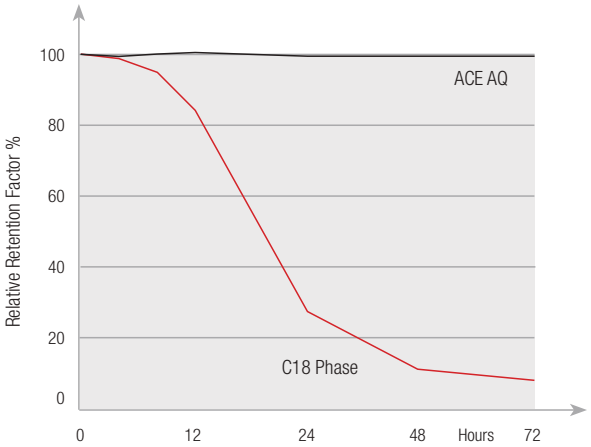



Figure 11. Change in retention with 100% aqueous eluent.*

*The comparative data presented here may not be representative for all applications.

## ACE C18-HL (Hi-Load)

- High surface area, high carbon load phase
- Increased loading and retention
- Optimised for preparative and process scale applications

The increased retention characteristics of ACE C18-HL make it an ideal selection for LC-MS applications. Retention can be maintained whilst reducing the aqueous content of the eluent, thus increasing sensitivity. For preparative applications, the higher surface area leads to increased loading capacity. Available in 3, 5, 10 and 15µm particle sizes and a wide range of column dimensions, ACE C18-HL columns show reproducible scale-up from LC-MS to preparative scale dimensions.

## ACE Guard Cartridges

- Protection of columns from 1.0 to 50mm i.d.
- No loss in column performance or selectivity
- Significantly extends column lifetime
- Available for all phases and dimensions

ACE guard cartridges are available for every ACE HPLC phase and column dimension to ensure maximum column protection for all applications. ACE analytical columns (2.1 to 4.6mm i.d.) are available with an integral zero dead volume guard holder, which also allows simple cartridge replacement. Compared to conventional 'stand alone' designs, this zero dead volume holder provides improved system efficiency, especially with 2.1mm i.d. columns.

Please note that these integral holders cannot be used with ACE Excel UHPLC columns. For protection of ACE Excel UHPLC columns, ACE UHPLC pre-column filters (see page 66) are recommended.

ACE integral zero dead volume guard holder (H0005) attached to a 4.6mm i.d. column


**Please contact Hichrom to request a copy of the ACE Application Guide containing over 100 applications**

## ACE® Excel® UHPLC Columns


**Please request your copy of the ACE Excel UHPLC columns brochure**

- 2, 3 and 5µm particle size columns available
- Unique phases for rapid method development
- Compatible with UHPLC and HPLC systems
- HSC™ (High Stability Columns): ultra robust up to 1,000 bar (15,000psi)
- Optimised UHPLC hardware

ACE® Excel® 2µm particle size columns offer all the advantages of ACE HPLC columns but in a format suitable for UHPLC. Selectivity is unchanged from existing 3, 5 and 10µm ACE HPLC phases, enabling excellent scale-up of methods. Excellent peak shape, great column-to-column reproducibility and exceptional column ruggedness is now available for UPLC® and UHPLC users. All ACE Excel UHPLC columns are manufactured using a proprietary HSC™ (High Stability Column) manufacturing process that results in ultra robust UHPLC columns. ACE Excel columns utilize specially developed low dispersion hardware which enables high efficiency UHPLC separations up to a maximum pressure limit of 1,000 bar (15,000psi).

### Compatible with all UPLC and UHPLC Systems

ACE Excel UHPLC columns are designed to be fully compatible with all commercial UPLC and UHPLC instruments and have been engineered to benefit from the high flow rate, ultra-high pressure and low dispersion of these systems.

Owing to the optimal 2µm particle size and a rigorous classification protocol, column back pressure is significantly lower than for traditional UPLC and UHPLC columns packed with <2µm particles. Therefore ACE Excel 2µm columns may also be used on HPLC systems to provide a performance boost compared to standard HPLC columns, subject to HPLC system pressure limitations.

Figure 12 illustrates the excellent peak shapes and resolution obtained during rapid UHPLC screening of 16 pharmaceuticals and related compounds on an ACE Excel 2µm C18 column in less than 5 minutes.

Reusable UHPLC column connectors EXL-CC (see page 66) are recommended for UHPLC connections and offer ultra low dead volume connections.

1. N-Acetylprocainamide
2. 3-Hydroxybenzoic acid
3. Pindolol
4. Methyl phenyl sulphoxide
5. Benzyl alcohol
6. Quinoxaline
7. 1,4-Dinitrobenzene
8. Phenacetin
9. 1,2-Dimethoxybenzene
10. Furosemide
11. Anisole
12. Methyl benzoate
13. Remacemide
14. Nimesulide
15. Ethyl benzoate
16. Diflunisal

Column: ACE Excel 2 C18 (2µm, 50 x 2.1mm)
Eluent: A: 20mM $KH_2PO_4$, pH 2.7
B: 20mM $KH_2PO_4$, pH 2.7 in $CH_3OH$ – $H_2O$ (65:35)
Gradient: 3 to 100% B in 5 mins
Flow rate: 0.6ml/min
Temperature: 60°C
Detection: UV, 214nm

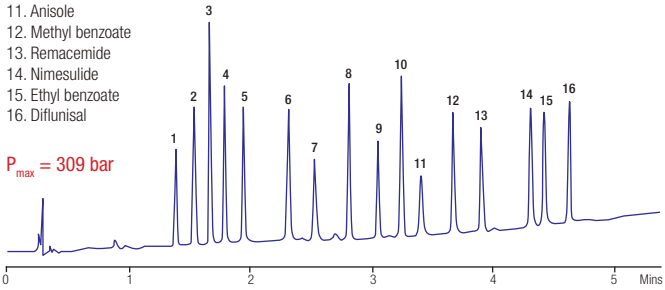
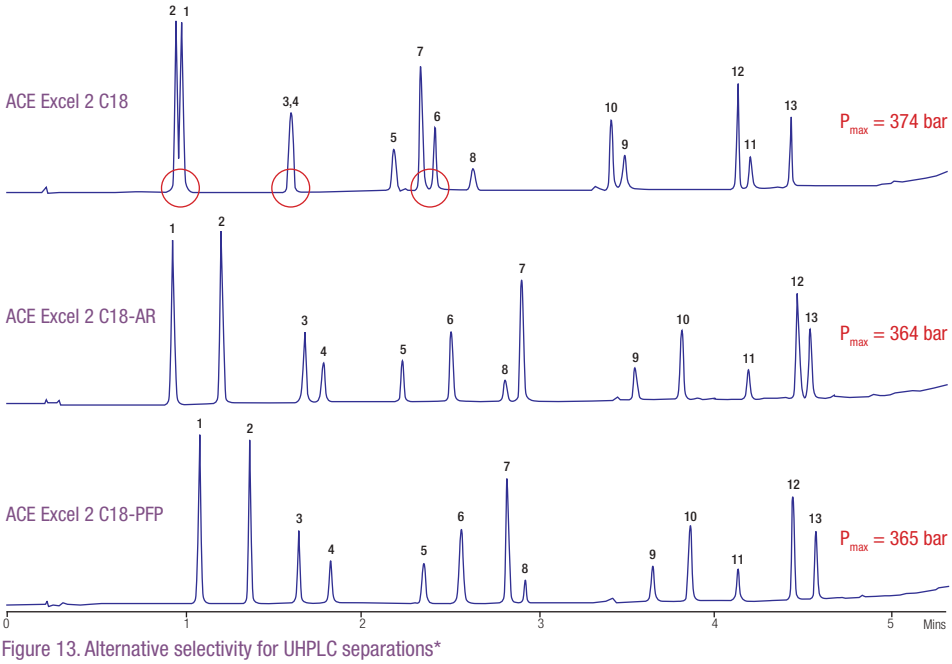



Figure 12. Rapid UHPLC screening

### Unique Selectivity Phases

In addition to the wide range of standard ACE phases (C18, C8, C4, CN, Phenyl, AQ and SIL), the benefits of the extra selectivity offered by the unique phases ACE C18-AR, ACE C18-PFP, ACE C18-Amide, ACE CN-ES and ACE SuperC18 (see pages 59-63), are now also available for UHPLC applications. These alternative selectivity 2µm phases complement the standard ACE Excel phases, providing chromatographers with increased choice for method development. Figure 13 shows the different selectivity obtained using three ACE Excel columns (C18, C18-AR and C18-PFP) for a mixture of pharmaceuticals and related compounds. Whereas the ACE Excel 2µm C18 column (typical of other leading UHPLC C18 columns) fails to resolve all the components under these conditions, the unique selectivity of both the ACE Excel C18-AR and C18-PFP phases enable improved separations. In total ACE Excel columns are now available in 12 phases.




Column dimensions: 50 x 2.1mm
Eluent: A: 5mM formic acid in $H_2O$
B: 5mM formic acid in $CH_3OH$
Gradient: 3 to 100% B in 5 mins
Flow rate: 0.6ml/min
Temperature: 40°C
Detection: UV, 254nm

1. Paracetamol
2. Hydrochlorothiazide
3. Methyl phenyl sulphoxide
4. Methyl phenyl sulphone
5. Aspirin
6. Phenacetin
7. 1,3-Dinitrobenzene
8. 1,2,4-Trimethoxybenzene
9. Ethyl benzoate
10. Nimesulide
11. Ibuprofen
12. Indomethacin
13. Mefenamic acid

Figure 13. Alternative selectivity for UHPLC separations*

*The comparative separations presented here may not be representative for all applications.

Please see pages 71-72 for ordering information for ACE Excel columns.

## ACE® Excel® UHPLC Columns (continued)

### Column-to-Column Reproducibility

Like all ACE columns, ACE® Excel UHPLC columns are subjected to extensive controls at each stage of the manufacturing process. In addition to the HSC™ packing technology used to manufacture ACE Excel® UHPLC columns, they incorporate an inlet frit design that reduces the risk of plugging. It is still recommended that you filter samples and eluents as you would with any UHPLC column, but ACE Excel columns provide the same levels of durability and lifetime that you would expect from HPLC columns.

Figure 14 illustrates the excellent lifetime and reproducibility provided by an ACE Excel C18 column under searching fast gradient UHPLC conditions. After over 2,000 gradient cycles incorporating a $P_{max}$ of 1,000 bar (15,000psi), the column efficiency, peak shape and retention times were essentially unchanged.

### Easy scalability from UHPLC to HPLC to preparative HPLC

All ACE Excel columns are available in larger particle size equivalents and larger non-analytical dimensions. The consistent selectivity inherent in all ACE stationary phases, regardless of particle size, permits easier transfer of methods from UHPLC to HPLC or HPLC to UHPLC. It also makes scale-up from analytical to preparative applications easier.

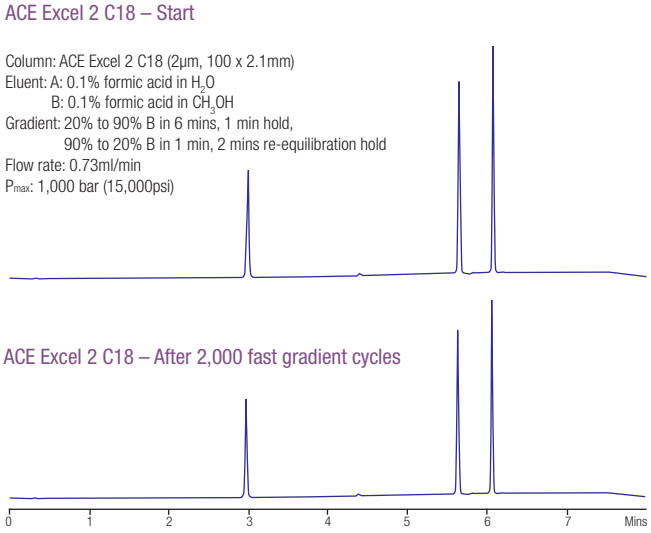
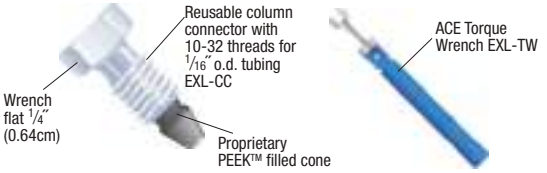



Figure 14. Exceptional durability of ACE Excel UHPLC columns

## ACE UHPLC Reusable Column Connector

- Pressure rating >1700 bar (>25,000psi)
- Compatible with all UHPLC systems
- Compatible with all UHPLC column brands
- Eliminates poor connections
- Unique reusable design

ACE UHPLC column connectors are reusable and enable UHPLC columns to be correctly installed every time. Their unique design ensures that they maintain pressure rating with repeated use, yet do not permanently swage onto the inlet tubing. This has the advantage of avoiding the introduction of extra column volume to the system and minimising leaks at the inlet fitting connection. To maximize the lifetime of the fitting, the use of an ACE Torque Wrench (EXL-TW) is required. Standard ACE HPLC PEEK fingertight connectors (ACE-CC, pressure rated to 350 bar/5,000psi) may be used at the outlet end of the UHPLC column, where pressure demands are lower but a correct connection remains important.

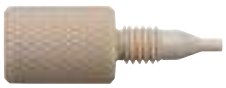



**Please contact Hichrom for your copy of the 'Making Good Connections' flyer**

## ACE Pre-column Filters

Pre-column filters may be used to protect the column inlet frit from blockage and extend column lifetime. Due to their ultra-low dispersion design, column performance and retention remain unaffected.


ACE UHPLC Pre-column filter


ACE Microbore HPLC Pre-column filter


ACE Analytical HPLC Pre-column filter

### ACE UHPLC Pre-column Filters

ACE UHPLC Pre-column filters are designed for use in UHPLC separations requiring high eluent velocities and ultra-high pressure. ACE UHPLC Pre-column filters (p/n EXL-PCF10, 10/pk) can be installed on any UHPLC column in seconds and are leak tight to 1000 bar. Simply finger tighten initially, then wrench tighten a further ¼ turn. For connection to a Waters Acquity system, the use of a pre-column filter incorporating the Waters Acquity column port profile is alternatively recommended (p/n EXL-PCF10/ACQ, 10/pk).

### ACE HPLC Pre-column Filters

ACE HPLC Pre-column filters utilise a PEEK fingertight design that connects directly into any 1/16″ 10-32 internal thread column inlet, and are thus compatible with all HPLC brands.

For 2.1mm i.d. HPLC columns, the use of ACE Microbore HPLC Pre-column filters is recommended. For 3.0 - 4.6mm i.d. HPLC columns, the use of ACE Analytical HPLC Pre-column filters is recommended.

ACE HPLC Pre-column filters are simply hand tightened into the column inlet to achieve a pressure rating of 350 bar (5000psi). If higher pressure rating is required, ACE UHPLC Pre-column filters are alternatively recommended.

Please see page 71 for ordering information for ACE Excel columns and ACE UHPLC column connectors, and page 73 for ACE Pre-column filters

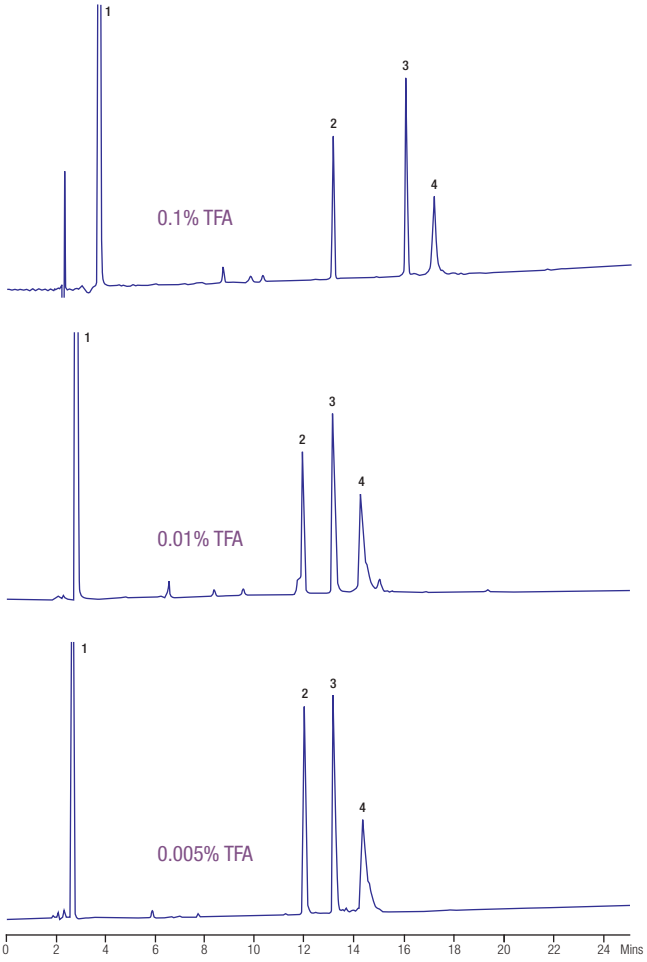
## ACE® 300Å Columns for Biotechnology

- 300Å ultra high purity silica
- Ultimate protein and peptide application column
- C18, C8, C4, CN and Phenyl chemistries
- 3, 5 and 10µm particle sizes
- Exceptional chemical stability

ACE® 300Å pore size columns produce high efficiency, reproducible separations for a wide range of peptides, proteins and other high molecular weight biomolecules. They are available in an extensive range of dimensions and particle sizes for use in micro-scale separations, LC-MS analyses and analytical and preparative scale analyses.

### Increased Sensitivity

TFA is typically used as an eluent additive to improve both peak shape and resolution of complex mixtures of peptides and proteins. However, although TFA complexation with polypeptides can enhance selectivity, mass spectral sensitivity is decreased. ACE 300Å phases maintain good separation characteristics even when using reduced levels of TFA, leading to increased sensitivity of detection. Figure 15 demonstrates that for the separation of a peptide mixture, with an ACE 5 C18-300 column, performance is maintained when TFA concentration is decreased down to 0.005%.




Column: ACE 5 C18-300, 250 x 4.6mm
Eluent: A: TFA in $H_2O$
B: TFA in $CH_3CN$
10 to 55% B in 37.5 mins
Flow rate: 1.5ml/min
Detection: UV, 200nm

1. Gly-Tyr
2. Oxytocin
3. Angiotensin II
4. Neurotensin

Figure 15. Separation of peptide mix

### Optimising Selectivity

The ability of TFA and other eluent additives to complex with peptides and proteins can be used to adjust selectivity and improve resolution. As shown in Figure 16, lowering TFA concentration from 0.1% to 0.01% enabled the resolution of angiotensin II and III on an ACE 5 C18-300 column.

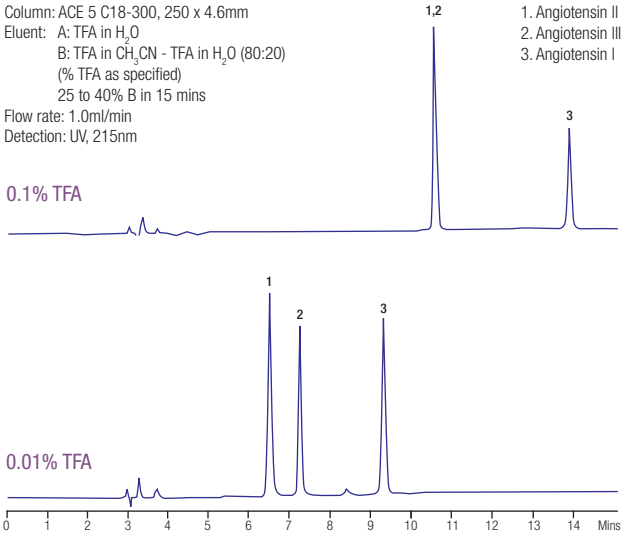



Figure 16. Selectivity as a function of TFA concentration

Figure 17 shows the separation of whey proteins from milk on an ACE C4-300 column.

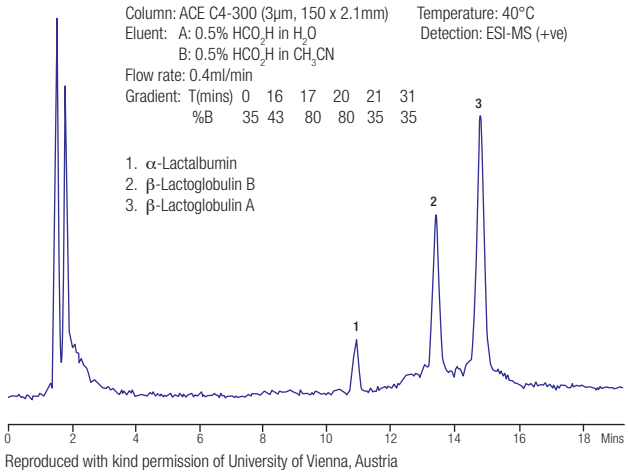



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Figure 17. Whey proteins from whole milk

Part numbers for ACE 300Å columns are listed on pages 72-73.

## ACE® LC-MS Columns

- High performance – excellent peak shape for higher sensitivity
- Wide range of bonded phases available
- Ultra-inert silica enables MS compatible buffers to be used
- 20, 30, 35 and 50mm column lengths

The use of truly ultra-inert base deactivated HPLC columns is imperative for high-throughput analysis or LC-MS. Even subtle differences in silanol activity between columns can markedly affect the chromatography in the very short, fast columns typically used. Any peak tailing due to silanol activity can have a profound effect on detection limits in high sensitivity assays. ACE® ultra-inert base deactivated HPLC columns virtually eliminate the negative effects of silanols in HPLC separations and are ideal for LC-MS applications.

In Figure 18, the chromatograms show the LC-MS signal intensity obtained for equivalent injections on an ultra-inert ACE 30 x 4.6mm i.d. C8 column and a C8 column with less inert silica. The expanded chromatograms show that there is almost perfect symmetry (virtually no baseline tailing) with the ACE column.

### Increased Column Robustness

ACE LC-MS and rapid analysis columns are manufactured by a unique process which results in increased column bed stability, thus increasing column lifetime.

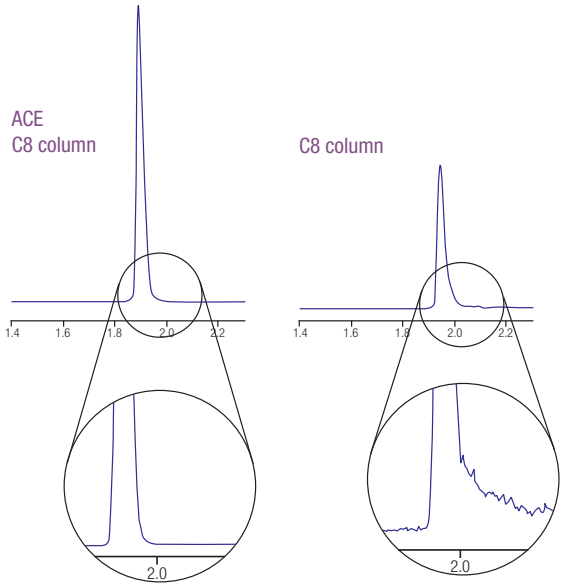
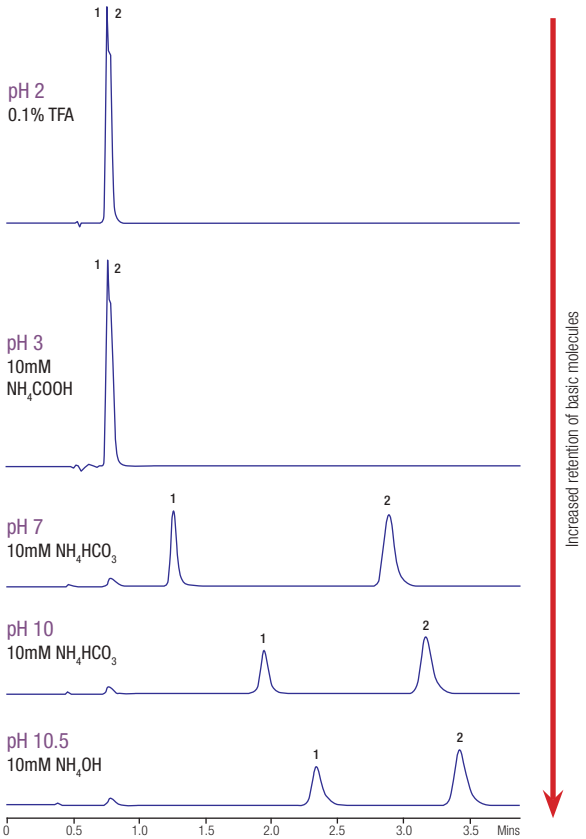



Figure 18. Effect of peak tailing on signal strength in LC-MS*

*The comparative separations presented here may not be representative for all applications.

### LC-MS Buffer Compatibility

ACE columns, by virtue of their high bonding density and ultra-inert characteristics, can be used with eluent conditions that are optimal for LC-MS applications. ACE LC-MS columns have an extremely low requirement for buffer salts or modifiers in order to achieve good peak shape. ACE columns have been demonstrated to be extremely robust at high and low pH. For maximum LC-MS compatibility under acidic conditions, organic acids such as formic and acetic are recommended. Under basic conditions ammonium bicarbonate, ammonium acetate and ammonium hydroxide buffers are recommended. Figure 19 demonstrates the resolution obtained with 2 basic compounds at different eluent pHs on an ACE 3 C18 column. For extra high and low pH conditions ACE SuperC18 columns are recommended (see page 59).




Column: ACE 3 C18, 50 x 2.1mm
Eluent: $CH_3OH$ - Buffer (80:20)
Flow rate: 0.2ml/min
Temperature: 22ºC

1. Nortriptyline
2. Amitriptyline

Figure 19. pH investigation with LC-MS buffers

Please contact Hichrom for a copy of the LC-MS Buffer Guide

## ACE® Capillary and Nano Columns

- Capillary (500 and 300µm) and nano (100 and 75µm) dimensions
- Wide range of bonded phases available
- 100Å and 300Å pore sizes
- High efficiency, long lifetime and guaranteed reproducibility
- LC-MS and LC-MS/MS applications

In addition to the extensive range of analytical (1.0 – 4.6mm i.d.) through to preparative (21.2 – 50mm i.d.) columns, ACE® columns are available in capillary (500 and 300µm) and nano (100 and 75µm) dimensions. ACE capillary and nano columns are available with all ACE bonded phase chemistries in 90Å, 100Å and 300Å pore sizes.

### Improved Mass Limit of Detection

Capillary and nano HPLC are advantageous for applications where limited sample amounts lead to problems in detection sensitivity. This is relevant in the areas of pharmacokinetics, trace analysis and in particular the expanding fields of bioanalytical and proteomic analysis. ACE capillary and nano columns are ideal for use with detectors requiring very low flow rates, such as electrospray LC-MS.

ACE capillary and nano HPLC columns offer high sensitivity due to their low dispersion characteristics. Table 1 shows the theoretical sensitivity increase of each i.d. column compared with a 4.6mm i.d. analytical column and 1.0mm i.d. microbore column. This high sensitivity can be important for accurate quantitation of sample limited applications.

For maximum performance, these columns should be used with fully optimised HPLC systems (e.g. minimise system dead volume by using short lengths of <75µm i.d. connection tubing).

**Table 1. Sensitivity Increase**

| Column i.d. (mm) | Typical Flow Rate (µl/min) | Theoretical Sensitivity Increase[1] |
|---|---|---|
| 4.6 | 1000 | 1 |
| 1.0 | 40 | 21 |
| 0.5 | 10 | 85 |
| 0.3 | 3 | 235 |
| 0.1 | 0.5 | 2100 |
| 0.075 | 0.3 | 3760 |

[1] For same sample mass

### ACE Trace Enrichment/Guard Columns

Capillary HPLC guard columns (5mm x 300µm or 500µm i.d.) prolong the lifetime of the capillary column. They are also suitable for trace enrichment and column switching applications, particularly for concentration of low abundance analytes or desalting of biological samples. These short columns can be used to separate analyte from matrix prior to analysis with detectors such as ESI-MS, where baseline resolution is not required. Figure 20 shows the connection of an ACE capillary column to a guard cartridge using a capillary column coupler.

Figure 20. ACE capillary column, guard and connector (C0003)

### ACE Capillary and Nano Column Availability

ACE capillary and nano columns are available with all bonded phase chemistries, 90Å, 100Å or 300Å pore sizes and 3, 5 or 10µm particle sizes.

Please see pages 71 and 72 for ordering information.

## ACE® Preparative HPLC Columns

- Ultra high purity base deactivated silica
- 5, 10 and 15µm particle sizes available
- Fully validated columns
- Exceptional reproducibility
- Excellent efficiencies
- High sample recovery
- Excellent column lifetime
- 90Å, 100Å and 300Å pore sizes

### Achieve Reproducible High Performance Preparative Separations

For preparative HPLC, resolution and loadability are of the utmost importance. The greater the resolution, the higher the sample load and the faster pure compound is obtained. The ability to optimise resolution at the preparative scale means starting with high performance separations at the analytical scale. The same features that make ACE® ultra-inert base deactivated analytical columns the choice of method development chemists, also make them the ideal choice for scale-up and process methods.

ACE preparative HPLC columns offer the following benefits:

**1) Loadability –** high surface area and carbon load for maximum sample capacity
**2) Selectivity –** available in unbonded and 12 bonded phases, including ACE SuperC18 and all ACE 'extra selectivity' phases, to optimise resolution and maximise sample capacity
**3) Rugged –** reliable, long-term performance
**4) Guaranteed reproducibility –** complete column/batch validation as for ACE analytical columns
**5) Guard cartridges –** available for maximum column protection

### Get High Purity Product Fast

ACE preparative HPLC columns are available in a wide range of column dimensions and particle sizes. For maximum loadability, choose 30mm i.d. columns. Use a 50mm length column with a 5µm particle size to optimise the speed of your separation. To maximise resolution, choose a 250mm length column with a 5µm particle size.

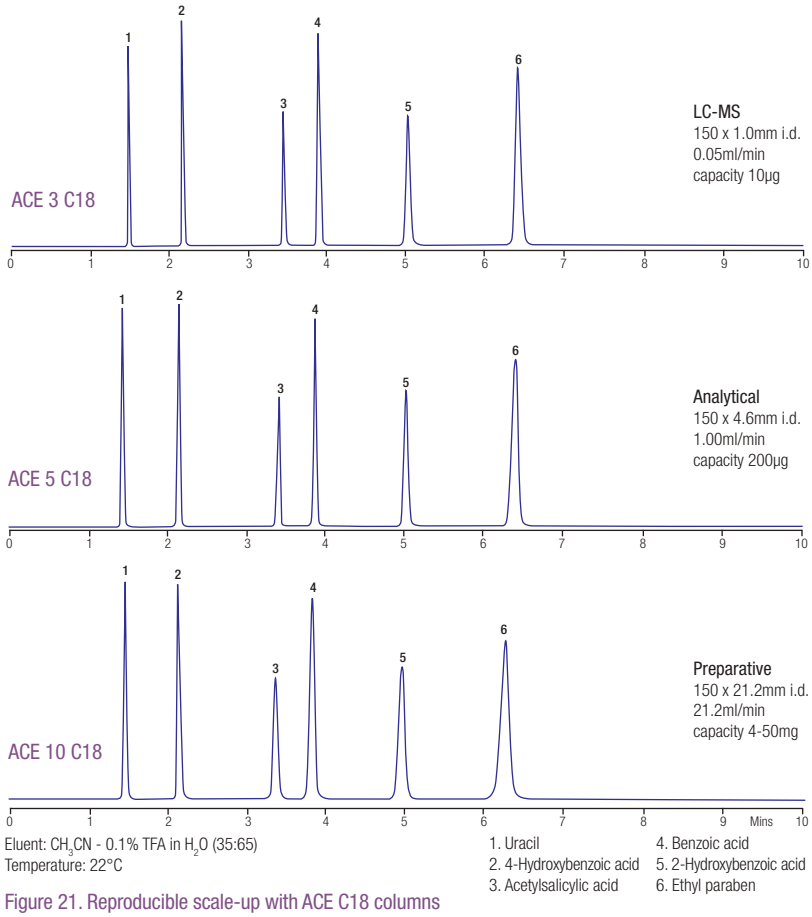



Figure 21. Reproducible scale-up with ACE C18 columns

Part numbers for ACE preparative columns are listed on pages 71-73.

## Ordering Information – ACE® Columns

### ACE Excel 2µm UHPLC Columns - when ordering please replace '**X**' with the appropriate material code.

ACE Excel UHPLC columns also available with 3 and 5µm particle size – please enquire for further details

| ACE Excel 2µm Phase | C18 | C8 | C4 | CN | Phenyl | AQ |
|---|---|---|---|---|---|---|
| **X** | EXL-101 | EXL-102 | EXL-103 | EXL-104 | EXL-105 | EXL-106 |

| ACE Excel 2µm Phase | SIL | C18-AR | C18-PFP | SuperC18 | C18-Amide | CN-ES |
|---|---|---|---|---|---|---|
| **X** | EXL-107 | EXL-109 | EXL-1010 | EXL-1011 | EXL-1012 | EXL-1013 |

Example: For a 50 x 2.1mm i.d. ACE Excel 2µm C18 UHPLC column – Catalogue No. = EXL-101-0502U

| Column Diameter | Column Length (mm) | | | | | | | | UHPLC Reusable Connector[1, 2, 3] |
|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | |
| 2.1mm | **X** -0202U | **X** -0302U | **X** -3502U | **X** -0502U | **X** -7502U | **X** -1002U | **X** -1202U | **X** -1502U | EXL-CC |
| 3.0mm | **X** -0203U | **X** -0303U | **X** -3503U | **X** -0503U | **X** -7503U | **X** -1003U | **X** -1203U | **X** -1503U | EXL-CC |
| 4.6mm | **X** -0246U | **X** -0346U | **X** -3546U | **X** -0546U | **X** -7546U | **X** -1046U | **X** -1246U | **X** -1546U | EXL-CC |

[1] Also available as 10/pk - EXL-CC10 [2] Torque Wrench (EXL-TW) is recommended [3] Starter kit available (EXL-CCSK) containing 4x EXL-CC plus 1x EXL-TW

### ACE 3µm 100Å HPLC Columns - when ordering, please replace '**X**' with the appropriate material code.

ACE Excel 3µm UHPLC columns also available – please enquire for details

| ACE 3µm Phase | C18 | C8 | C4 | CN | Phenyl | AQ | SIL |
|---|---|---|---|---|---|---|---|
| **X** | ACE-111 | ACE-112 | ACE-113 | ACE-114 | ACE-115 | ACE-116 | ACE-117 |

| ACE 3µm Phase | C18-AR | C18-PFP | SuperC18 | C18-Amide | CN-ES | C18-HL | - |
|---|---|---|---|---|---|---|---|
| **X** | ACE-119 | ACE-1110 | ACE-1111 | ACE-1112 | ACE-1113 | ACE-311 | - |

Example: For a 150 x 4.6mm i.d. ACE 3 C18 HPLC column – Catalogue No. = ACE-111-1546

| Column Diameter | Column Length (mm) | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | 250 | |
| 75µm | - | - | - | - | - | **X** -1000075 | - | **X** -1500075 | - | - |
| 100µm | - | - | - | - | - | **X** -10001 | - | **X** -15001 | - | - |
| 300µm | - | **X** -03003 | **X** -35003 | **X** -05003 | **X** -75003 | **X** -10003 | **X** -12003 | **X** -15003 | **X** -25003 | **X** -005003GD[1] |
| 500µm | - | **X** -03005 | **X** -35005 | **X** -05005 | **X** -75005 | **X** -10005 | **X** -12005 | **X** -15005 | **X** -25005 | **X** -005005GD[1] |
| 1.0mm | - | **X** -0301 | **X** -3501 | **X** -0501 | **X** -7501 | **X** -1001 | **X** -1201 | **X** -1501 | **X** -2501[a] | **X** -0101GD[2] |
| 2.1mm | **X** -0202[2] | **X** -0302 | **X** -3502 | **X** -0502 | **X** -7502 | **X** -1002 | **X** -1202 | **X** -1502 | **X** -2502[a] | **X** -0102GD[3] |
| 3.0mm | **X** -0203[2] | **X** -0303 | **X** -3503 | **X** -0503 | **X** -7503 | **X** -1003 | **X** -1203 | **X** -1503 | **X** -2503[a] | **X** -0103GD[4] |
| 4.0mm | - | - | **X** -3504 | **X** -0504 | **X** -7504 | **X** -1004 | **X** -1204 | **X** -1504 | **X** -2504[a] | **X** -0103GD[4] |
| 4.6mm | **X** -0246[2] | **X** -0346 | **X** -3546 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | **X** -2546[a] | **X** -0103GD[4] |

[1] 1/pk – use with capillary column coupler C0003 – no holder required
[2] 5/pk – guards for 20mm length and 1.0mm i.d. columns require cartridge holder H0001 and coupler C0001
[3] 5/pk – use with integral microbore cartridge holder H0004
[4] 5/pk – use with integral analytical cartridge holder H0005
[a] Available to special order - consider operating pressure limitations for maximum column lifetime

### ACE 5µm 100Å HPLC Columns - when ordering please replace '**X**' with the appropriate material code.

ACE Excel 5µm UHPLC columns also available – please enquire for details

| ACE 5µm Phase | C18 | C8 | C4 | CN | Phenyl | AQ | SIL |
|---|---|---|---|---|---|---|---|
| **X** | ACE-121 | ACE-122 | ACE-123 | ACE-124 | ACE-125 | ACE-126 | ACE-127 |

| ACE 5µm Phase | C18-AR | C18-PFP | SuperC18 | C18-Amide | CN-ES | C18-HL | - |
|---|---|---|---|---|---|---|---|
| **X** | ACE-129 | ACE-1210 | ACE-1211 | ACE-1212 | ACE-1213 | ACE-321 | - |

| Column Diameter | Column Length (mm) | | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | 250 | 300 | |
| 75µm | - | - | - | - | - | **X** -1000075 | - | **X** -1500075 | **X** -2500075 | - | - |
| 100µm | - | - | - | - | - | **X** -10001 | - | **X** -15001 | **X** -25001 | - | - |
| 300µm | - | **X** -03003 | **X** -35003 | **X** -05003 | **X** -75003 | **X** -10003 | **X** -12003 | **X** -15003 | **X** -25003 | **X** -30003 | **X** -005003GD[1] |
| 500µm | - | **X** -03005 | **X** -35005 | **X** -05005 | **X** -75005 | **X** -10005 | **X** -12005 | **X** -15005 | **X** -25005 | **X** -30005 | **X** -005005GD[1] |
| 1.0mm | - | **X** -0301 | **X** -3501 | **X** -0501 | **X** -7501 | **X** -1001 | **X** -1201 | **X** -1501 | **X** -2501 | - | **X** -0101GD[2] |
| 2.1mm | **X** -0202[2] | **X** -0302 | **X** -3502 | **X** -0502 | **X** -7502 | **X** -1002 | **X** -1202 | **X** -1502 | **X** -2502 | **X** -3002 | **X** -0102GD[3] |
| 3.0mm | **X** -0203[2] | **X** -0303 | **X** -3503 | **X** -0503 | **X** -7503 | **X** -1003 | **X** -1203 | **X** -1503 | **X** -2503 | **X** -3003 | **X** -0103GD[4] |
| 4.0mm | - | - | **X** -3504 | **X** -0504 | **X** -7504 | **X** -1004 | **X** -1204 | **X** -1504 | **X** -2504 | **X** -3004 | **X** -0103GD[4] |
| 4.6mm | **X** -0246[2] | **X** -0346 | **X** -3546 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | **X** -2546 | **X** -3046 | **X** -0103GD[4] |
| 7.75mm | - | - | - | **X** -0508 | **X** -7508 | **X** -1008 | **X** -1208 | **X** -1508 | **X** -2508 | **X** -3008 | **X** -0110GD[5] |
| 10.0mm | - | - | - | **X** -0510 | **X** -7510 | **X** -1010 | **X** -1210 | **X** -1510 | **X** -2510 | **X** -3010 | **X** -0110GD[5] |
| 21.2mm | - | - | - | **X** -0520 | **X** -7520 | **X** -1020 | **X** -1220 | **X** -1520 | **X** -2520 | **X** -3020 | **X** -0110GD[5] |
| 30.0mm | - | - | - | **X** -0530 | **X** -7530 | **X** -1030 | - | **X** -1530 | **X** -2530 | **X** -3030 | **X** -0220GD[6] |

[1] 1/pk – use with capillary column coupler C0003 – no holder required
[2] 5/pk – guards for 20mm length and 1.0mm i.d. columns require cartridge holder H0001 and coupler C0001
[3] 5/pk – use with integral microbore cartridge holder H0004
[4] 5/pk – use with integral analytical cartridge holder H0005
[5] 3/pk – use with semi-prep cartridge holder H0002 and column coupler C0001
[6] 1/pk – use with prep cartridge holder H0006 and column coupler C0002

## Ordering Information – ACE® Columns (continued)

### ACE 10µm 100Å HPLC Columns - when ordering please replace '**X**' with the appropriate material code.

| ACE 10µm Phase | C18 | C8 | C4 | CN | Phenyl | AQ | SIL |
|---|---|---|---|---|---|---|---|
| **X** | ACE-131 | ACE-132 | ACE-133 | ACE-134 | ACE-135 | ACE-136 | ACE-137 |

| ACE 10µm Phase | C18-AR | C18-PFP | SuperC18 | C18-Amide | CN-ES | C18-HL | - |
|---|---|---|---|---|---|---|---|
| **X** | ACE-139 | ACE-1310 | ACE-1311 | ACE-1312 | ACE-1313 | ACE-331 | - |

| Column Diameter | Column Length (mm) | | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 50 | 75 | 100 | 125 | 150 | 250 | 300 | 500 | |
| 4.6mm | **X** -0246[2] | **X** -0346 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | **X** -2546 | **X** -3046 | **X** -5046 | **X** -0103GD[4] |
| 7.75mm | - | - | **X** -0508 | **X** -7508 | **X** -1008 | **X** -1208 | **X** -1508 | **X** -2508 | **X** -3008 | **X** -5008 | **X** -0110GD[5] |
| 10.0mm | - | - | **X** -0510 | **X** -7510 | **X** -1010 | **X** -1210 | **X** -1510 | **X** -2510 | **X** -3010 | **X** -5010 | **X** -0110GD[5] |
| 21.2mm | - | - | **X** -0520 | **X** -7520 | **X** -1020 | **X** -1220 | **X** -1520 | **X** -2520 | **X** -3020 | **X** -5020 | **X** -0110GD[5] |
| 30.0mm | - | - | **X** -0530 | **X** -7530 | **X** -1030 | - | **X** -1530 | **X** -2530 | **X** -3030 | **X** -5030 | **X** -0220GD[6] |
| 50.0mm | - | - | - | **X** -7550 | **X** -1050 | - | **X** -1550 | **X** -2550 | - | - | **X** -0220GD[6] |

[2] 5/pk – Guards for 20mm length and 1.0mm i.d. columns require cartridge holder H0001 and coupler C0001
[4] 5/pk – Use with integral analytical cartridge holder H0005
[5] 3/pk – Use with semi-prep cartridge holder H0002 and column coupler C0001
[6] 1/pk – Use with prep cartridge holder H0006 and column coupler C0002

### ACE 3µm 300Å HPLC Columns - when ordering please replace '**X**' with the appropriate material code.

| Phase Required | ACE 3 C18-300 | ACE 3 C8-300 | ACE 3 C4-300 | ACE 3 CN-300 | ACE 3 Phenyl-300 |
|---|---|---|---|---|---|
| **X** | ACE-211 | ACE-212 | ACE-213 | ACE-214 | ACE-215 |

| Column Diameter | Column Length (mm) | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | 250 | |
| 75µm | - | - | - | - | - | **X** -1000075 | - | **X** -1500075 | - | - |
| 100µm | - | - | - | - | - | **X** -10001 | - | **X** -15001 | - | - |
| 300µm | - | **X** -03003 | **X** -35003 | **X** -05003 | **X** -75003 | **X** -10003 | **X** -12003 | **X** -15003 | **X** -25003 | **X** -005003GD[1] |
| 500µm | - | **X** -03005 | **X** -35005 | **X** -05005 | **X** -75005 | **X** -10005 | **X** -12005 | **X** -15005 | **X** -25005 | **X** -005005GD[1] |
| 1.0mm | - | **X** -0301 | **X** -3501 | **X** -0501 | **X** -7501 | **X** -1001 | **X** -1201 | **X** -1501 | - | **X** -0101GD[2] |
| 2.1mm | **X** -0202[2] | **X** -0302 | **X** -3502 | **X** -0502 | **X** -7502 | **X** -1002 | **X** -1202 | **X** -1502 | - | **X** -0102GD[3] |
| 3.0mm | **X** -0203[2] | **X** -0303 | **X** -3503 | **X** -0503 | **X** -7503 | **X** -1003 | **X** -1203 | **X** -1503 | - | **X** -0103GD[4] |
| 4.0mm | - | - | **X** -3504 | **X** -0504 | **X** -7504 | **X** -1004 | **X** -1204 | **X** -1504 | - | **X** -0103GD[4] |
| 4.6mm | **X** -0246[2] | **X** -0346 | **X** -3546 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | - | **X** -0103GD[4] |

[1] 1/pk – Use with capillary column coupler C0003 – no holder required
[2] 5/pk – Guards for 20mm length and 1.0mm i.d. columns require cartridge holder H0001 and coupler C0001
[3] 5/pk – Use with integral microbore cartridge holder H0004
[4] 5/pk – Use with integral analytical cartridge holder H0005

### ACE 5µm 300Å HPLC Columns - when ordering please replace '**X**' with the appropriate material code.

| Phase Required | ACE 5 C18-300 | ACE 5 C8-300 | ACE 5 C4-300 | ACE 5 CN-300 | ACE 5 Phenyl-300 |
|---|---|---|---|---|---|
| **X** | ACE-221 | ACE-222 | ACE-223 | ACE-224 | ACE-225 |

| Column Diameter | Column Length (mm) | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | 250 | |
| 75µm | - | - | - | - | - | **X** -1000075 | - | **X** -1500075 | **X** -2500075 | - |
| 100µm | - | - | - | - | - | **X** -10001 | - | **X** -15001 | **X** -25001 | - |
| 300µm | - | **X** -03003 | **X** -35003 | **X** -05003 | **X** -75003 | **X** -10003 | **X** -12003 | **X** -15003 | **X** -25003 | **X** -005003GD[1] |
| 500µm | - | **X** -03005 | **X** -35005 | **X** -05005 | **X** -75005 | **X** -10005 | **X** -12005 | **X** -15005 | **X** -25005 | **X** -005005GD[1] |
| 1.0mm | - | **X** -0301 | **X** -3501 | **X** -0501 | **X** -7501 | **X** -1001 | **X** -1201 | **X** -1501 | **X** -2501 | **X** -0101GD[2] |
| 2.1mm | **X** -0202[2] | **X** -0302 | **X** -3502 | **X** -0502 | **X** -7502 | **X** -1002 | **X** -1202 | **X** -1502 | **X** -2502 | **X** -0102GD[3] |
| 3.0mm | **X** -0203[2] | **X** -0303 | **X** -3503 | **X** -0503 | **X** -7503 | **X** -1003 | **X** -1203 | **X** -1503 | **X** -2503 | **X** -0103GD[4] |
| 4.0mm | - | - | **X** -3504 | **X** -0504 | **X** -7504 | **X** -1004 | **X** -1204 | **X** -1504 | **X** -2504 | **X** -0103GD[4] |
| 4.6mm | **X** -0246[2] | **X** -0346 | **X** -3546 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | **X** -2546 | **X** -0103GD[4] |
| 7.75mm | - | - | - | **X** -0508 | **X** -7508 | **X** -1008 | **X** -1208 | **X** -1508 | **X** -2508 | **X** -0110GD[5] |
| 10.0mm | - | - | - | **X** -0510 | **X** -7510 | **X** -1010 | **X** -1210 | **X** -1510 | **X** -2510 | **X** -0110GD[5] |
| 21.2mm | - | - | - | **X** -0520 | **X** -7520 | **X** -1020 | **X** -1220 | **X** -1520 | **X** -2520 | **X** -0110GD[5] |
| 30.0mm | - | - | - | **X** -0530 | **X** -7530 | **X** -1030 | - | **X** -1530 | **X** -2530 | **X** -0220GD[6] |

[1] 1/pk – Use with capillary column coupler C0003 – no holder required
[2] 5/pk – Guards for 20mm length and 1.0mm i.d. columns require cartridge holder H0001 and coupler C0001
[3] 5/pk – Use with integral microbore cartridge holder H0004
[4] 5/pk – Use with integral analytical cartridge holder H0005
[5] 3/pk – Use with semi-prep cartridge holder H0002 and column coupler C0001
[6] 1/pk – Use with prep cartridge holder H0006 and column coupler C0002

## Ordering Information – ACE® Columns (continued)

### ACE 10µm 300Å HPLC Columns - when ordering please replace '**X**' with the appropriate material code.

| Phase Required | ACE 10 C18-300 | ACE 10 C8-300 | ACE 10 C4-300 | ACE 10 CN-300 | ACE 10 Phenyl-300 |
|---|---|---|---|---|---|
| **X** | ACE-231 | ACE-232 | ACE-233 | ACE-234 | ACE-235 |

| Column Diameter | Column Length (mm) | | | | | | | | | Guard Cartridges |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 35 | 50 | 75 | 100 | 125 | 150 | 250 | |
| **4.6mm** | **X** -0246[2] | **X** -0346 | **X** -3546 | **X** -0546 | **X** -7546 | **X** -1046 | **X** -1246 | **X** -1546 | **X** -2546 | **X** -0103GD[4] |
| **7.75mm** | - | - | - | **X** -0508 | **X** -7508 | **X** -1008 | **X** -1208 | **X** -1508 | **X** -2508 | **X** -0110GD[5] |
| **10.0mm** | - | - | - | **X** -0510 | **X** -7510 | **X** -1010 | **X** -1210 | **X** -1510 | **X** -2510 | **X** -0110GD[5] |
| **21.2mm** | - | - | - | **X** -0520 | **X** -7520 | **X** -1020 | **X** -1220 | **X** -1520 | **X** -2520 | **X** -0110GD[5] |
| **30.0mm** | - | - | - | **X** -0530 | **X** -7530 | **X** -1030 | - | **X** -1530 | **X** -2530 | **X** -0220GD[6] |

[2] Guards for 20mm columns require cartridge holder H0001 and column coupler C0001
[4] 5/pk – Use with integral analytical cartridge holder H0005
[5] 3/pk – Use with semi-prep cartridge holder H0002 and column coupler C0001
[6] 1/pk – Use with prep cartridge holder H0006 and column coupler C0002

### ACE UHPLC and HPLC Pre-column Filters

| Description | Maximum Pressure | Pack Size | Part No. |
|---|---|---|---|
| ACE UHPLC Pre-column filter | 1000 bar | 1 | EXL-PCF01 |
| | | 10 | EXL-PCF10 |
| ACE Waters compatible UHPLC Pre-column filter | 1000 bar | 1 | EXL-PCF01/ACQ |
| | | 10 | EXL-PCF10/ACQ |
| ACE Microbore HPLC Pre-column filter for 2.1mm i.d. columns | 350 bar | 5 | ACE-HP205 |
| | | 10 | ACE-HP210 |
| ACE Analytical HPLC Pre-column filter for more typical standard bore (3.0 to 4.6mm i.d.) columns | 350 bar | 5 | ACE-CS205 |
| | | 10 | ACE-CS210 |

## ACE UHPLC and HPLC Method Development Kits

Method development kits enable the optimum bonded phase for an application to be identified. ACE columns are available with a unique range of highly selective phases in 2, 3, 5 and 10µm particle sizes, specially developed for challenging UHPLC and HPLC applications. The availability of a range of phases offering complementary selectivity enables resolution to be maximised, improves the chances of impurity detection and increases the speed at which methods can be systematically developed.

Please enquire for further details.

**Please contact Hichrom for a free copy of any ACE technical guide**

# CHIRAL TECHNOLOGIES

Daicel chiral HPLC columns are the most widely referenced chiral columns. Chiral Technologies, a subsidiary of Daicel Corporation, offers the complete range of Daicel chiral columns and has the largest portfolio of chiral stationary phases for the separation of racemic mixtures. This includes the CHIRALPAK®, CHIRALCEL® and CROWNPAK® trademarks. The wide range of polysaccharide chiral stationary phases (CSPs) can be divided into two distinct groups - modern immobilized phases and traditional, coated phases.

## CHIRALPAK® Immobilized Phases

CHIRALPAK IA, IB, IC, ID, IE and IF HPLC and SFC columns are a newer generation of CSPs, in which the polysaccharide chiral selector has been immobilized on to a wide pore silica matrix. This confers universal solvent compatibility on these highly selective chiral phases, without compromising phase stability. In addition, the phases show increased robustness compared with traditional coated polysaccharide phases. Analytical and preparative dimension columns are available for each of these phases.

### Chiral Selectors

CHIRALPAK IA - based on amylose *tris*(3,5-dimethylphenyl)carbamate (as in CHIRALPAK AD)
CHIRALPAK IB - based on cellulose *tris*(3,5-dimethylphenyl)carbamate (as in CHIRALCEL OD)
CHIRALPAK IC - based on cellulose *tris*(3,5-dichlorophenyl)carbamate
CHIRALPAK ID - based on amylose *tris*(3-chlorophenyl)carbamate
CHIRALPAK IE – based on amylose *tris*(3,5-dichlorophenyl)carbamate
CHIRALPAK IF – based on amylose *tris*(3-chloro, 4-methylphenyl)carbamate

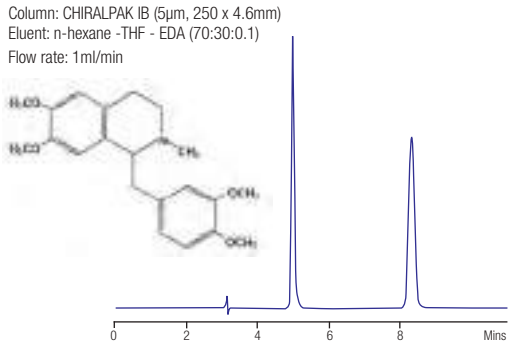



Figure 1. Analysis of laudanosine on CHIRALPAK IB

## Features of Immobilized Phases

**1) Wide Solvent Compatibility –** The immobilization of the selector confers a wide solvent compatibility to these highly selective chiral stationary phases, without compromising phase stability. This is in contrast to traditional, coated polysaccharide phases which have restricted solvent compatibility due to solubility of the polymer coating in certain solvents, including chloroform, methylene chloride, ethyl acetate, acetone, THF and DMF.

**2) Column Regeneration –** CHIRALPAK immobilized columns are more robust than their coated analogues. If columns have been used with additives or with multiple solvent changes, a regeneration procedure may be implemented to eliminate any change in chiral recognition.

**3) Screening Strategies –** Column screening is simpler, faster and more successful using the four main Daicel immobilized phases (IA, IB, IC and ID). When used in alkane/alcohol solvents, the immobilized columns can separate a significant number of small molecules, combined with the advantage of speed and ease of injecting in any suitable solvent.

## Polysaccharide Coated Phases

The original Daicel CSPs were formed by coating the chiral polymer from the derivatization of the amylose and cellulose on to 10µm silica. Subsequently, these CHIRALPAK AD and AS and CHIRALCEL OD and OJ phases were superseded by products made with higher efficiency 5µm particles. These CSPs are designated CHIRALPAK AD-H and AS-H and CHIRALCEL OD-H and OJ-H.

Reversed-phase versions of these 5µm normal-phase are also available. These include CHIRALPAK AD-RH and AS-RH and CHIRALCEL OD-RH and OJ-RH. These were specifically developed for use with aqueous-organic eluents. They are suited for applications where the sample is in aqueous media (eg. biological samples) or for samples that require flexibility in terms of pH range. More recently, 3µm particle size NP and RP phases have been introduced for higher resolution, fast analyses (CHIRALPAK AD-3, AD-3R, AS-3, AS-3R and CHIRALCEL OD-3, OD-3R, OJ-3, OJ-3R). These phases enable enhanced chromatographic separations to be achieved using conventional HPLC systems. Figure 2 demonstrates that enantiomer separation can be achieved in less than 30 seconds.

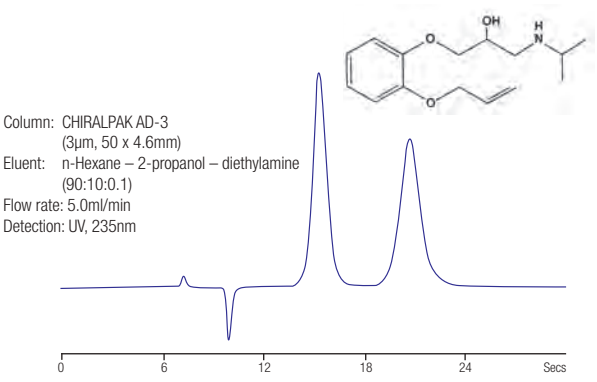



Figure 2. Fast analysis of oxprenolol on CHIRALPAK AD-3

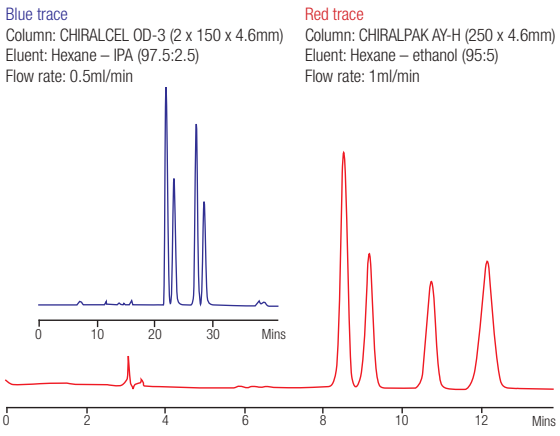



Figure 3. Separation of metolachlor diastereomers

Please contact us for ordering information on all Chiral Technologies products.

## Newer Polysaccharide Coated Phases

CHIRALPAK® AY-H, AZ-H and CHIRALCEL® OZ-H, OX-H 5µm and CHIRALPAK AY-3, AZ-3 and CHIRALCEL OZ-3, OX-3 3µm HPLC and SFC columns contain significantly different chiral selectors to the immobilized and coated polysaccharide phases discussed on the previous page. They show new recognition profiles, allowing effective method development for compounds not fully resolved on other Daicel columns. Figure 3 (page 74) shows a comparison of the separation of the four diastereomers of the herbicide metolachlor using CHIRALPAK AY-H and CHIRALCEL OD-3.

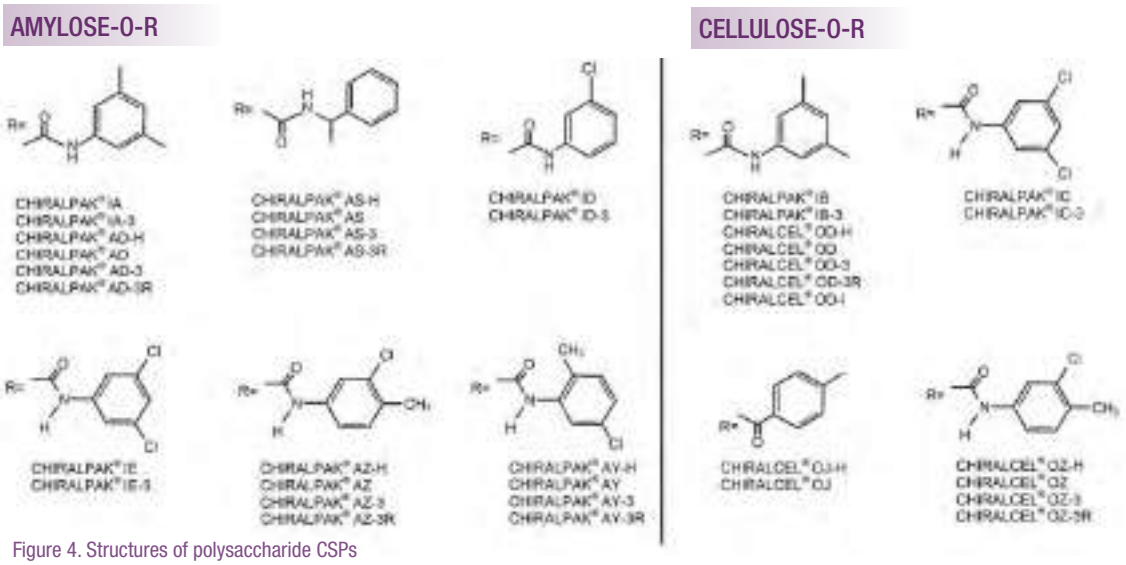



Figure 4. Structures of polysaccharide CSPs

## Daicel Columns for SFC

Daicel polysaccharide columns are well established for use in SFC separations. In addition to the benefits of speed of separation, speed of method development and improved column efficiency, green SFC technology reduces the use of organic solvents. Columns packed specifically for SFC are available in both the immobilized and coated polysaccharide product lines.

## CHIRALPAK Ion-Exchange Columns

### A. CHIRALPAK QN-AX and QD-AX Anion-Exchange Columns

CHIRALPAK QN-AX and QD-AX are enantioselective weak anion-exchange (AX) columns. They are based on complementary stereoisomeric quinine (QN) and quinidine (QD) derivatives. Due to their pseudo enantiomeric character they usually reveal reversed elution order for opposite enantiomers. These phases are designed specifically for enantioselective HPLC of chiral acids and show exceptional separation capabilities for acidic chiral compounds containing carboxylic, phosphonic, phosphinic, phosphoric or sulphonic acid groups.

### B. CHIRALPAK ZWIX™

The zwitterionic CHIRALPAK ZWIX(+) and ZWIX(-) stationary phases are quinine- and quinidine-derived chiral supports respectively that incorporate both anion- and cation-exchange functional groups. These novel selectors exhibit enantioseparation capabilities toward zwitterionic molecules such as underivatized amino acids and peptides.

## CHIRALPAK Protein Phase Columns

Protein stationary phases were originally developed and manufactured by ChromTech Ltd, but are now manufactured by Chiral Technologies Europe. The range consists of three protein-based columns – CHIRALPAK AGP, CHIRALPAK CBH and CHIRALPAK HSA (previously called CHIRAL-AGP, CHIRAL-CBH and CHIRAL-HSA), where the protein is immobilized on 5µm porous spherical silica particles. These columns are used with reversed-phase solvents, using buffers with low organic content and at moderate pH.

**CHIRALPAK AGP** has the broadest applicability of the three chiral phases, separating a wide range of compound types.

**CHIRALPAK CBH** has a narrower applicability, preferentially separating compounds containing one or more nitrogen atoms together with one or more hydrogen accepting or donating groups, and is particularly suited for the analysis of very hydrophilic amines.

**CHIRALPAK HSA** is also more suitable for specific applications, particularly very hydrophilic acids.

## Crown Ether Chiral Columns

The CROWNPAK® CR(+) and CR(-) phases contain a chiral crown ether as chiral selector, which is coated on to a 5µm silica support. They can be used for the enantiomeric separation of amino acids and other small molecules containing a primary amino group near the chiral centre. The CR(-) column gives the reversed elution order compared to the CR(+) column. Acidic eluents such as perchloric acid at pH 1 to 2 are used as standard with these columns.

## Ligand Exchange Chiral Columns

The ligand exchange phases, CHIRALPAK MA(+) and CHIRALPAK WH consist of amino acids and their derivatives coated on to silica supports. They are used in conjunction with an aqueous $CuSO_4$ eluent (0.1 to 2mM) and are useful for the separation of amino acids and their derivatives.

Please contact us for ordering information on all Chiral Technologies products.

# COSMOSIL®

- Spherical porous silica
- Monomeric and polymeric bonded C18
- Unique speciality bonded phases

Nacalai Tesque of Kyoto, Japan manufactures the COSMOSIL® range of columns. Their products include four C18 bonded phases and a number of unique speciality phases.

## COSMOSIL Phases

| Phase[2] | Functional Group | Bonding | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapping |
|---|---|---|---|---|---|---|---|
| SL-II | - | - | 3, 5 | 120 | 300 | - | - |
| C18-MS-II | Octadecyl | Monomeric | 2.5[1], 3, 5 | 120 | 300 | 16 | Yes |
| C18-AR-II | Octadecyl | Polymeric | 3, 5, 15 | 120 | 300 | 17 | Yes |
| C18-PAQ | Octadecyl | Polymeric | 5 | 120 | 300 | 11 | Yes |
| Protein-R | Octadecyl | Polymeric | 5 | 300 | 150 | Proprietary | Yes |
| PYE | 2-(1-Pyrenyl)ethyl | - | 5 | 120 | 300 | 18 | Yes |
| NPE | Nitrophenylether | - | 5 | 120 | 300 | 9 | Yes |
| PBB | Pentabromobenzyl | - | 5 | 120 | 300 | 8 | Yes |
| HIC | Diol | - | 5 | 300 | 150 | 7 | Proprietary |
| Sugar-D | Proprietary | - | 5 | Proprietary | Proprietary | Proprietary | Proprietary |
| Cholester | Cholesteryl | Monomeric | 2.5[1], 5 | 120 | 300 | 20 | Yes |
| HILIC | Triazole | - | 2.5[1], 5 | 120 | 300 | Proprietary | - |
| πNAP | Naphthylethyl | Monomeric | 2.5[1], 5 | 120 | 300 | 11 | Yes |

[1] Pore size 130Å, surface area $330m^2/g$ [2] Additional bonded phases available – see below and also page p.79

## COSMOSIL C18 Phases

The availability of a range of C18 phases allows the chromatographer to select the most appropriate phase for a specific application.

**1) COSMOSIL C18-MS-II** is a monomerically bonded C18 phase. A new endcapping treatment with polar groups has extended the pH range of the material (2-10) and improved peak shape for basic compounds. This phase is recommended for the separation of low molecular weight organic compounds.

**2) COSMOSIL C18-AR-II** is a polymerically bonded C18 phase exhibiting strong acid resistance (down to pH 1.5). It is particularly effective for the separation of chelating compounds as well as both acidic and basic compounds, including biopolymers. Compared with the monomeric COSMOSIL C18-MS-II, the polymeric C18-AR-II shows superior molecular shape selectivity.

**3) COSMOSIL C18-PAQ** maintains stable retention time even in 100% aqueous eluents, due to partial coverage of the silica with C18 chains. A new mode of polymeric linkage gives the phase a strong acidic resistance and provides good separation for hydrophilic compounds.

**4) COSMOSIL Protein-R** is a polymerically bonded wide pore phase, designed specifically for the separation of large peptides and proteins. High recovery is observed for a variety of proteins.

Figures 1 and 2 show the application of COSMOSIL 5C18-AR-II and 5C18-MS-II phases for the analysis of isoflavones and antipyretics respectively.

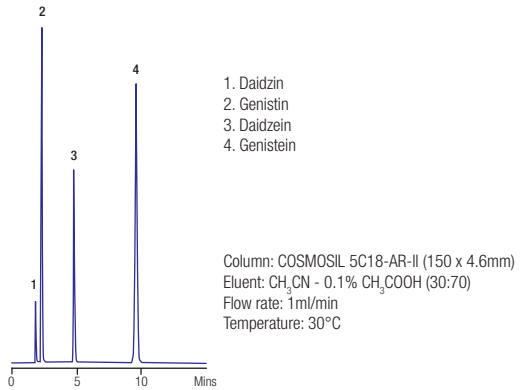



Figure 1. Separation of isoflavones on COSMOSIL 5C18-AR-II

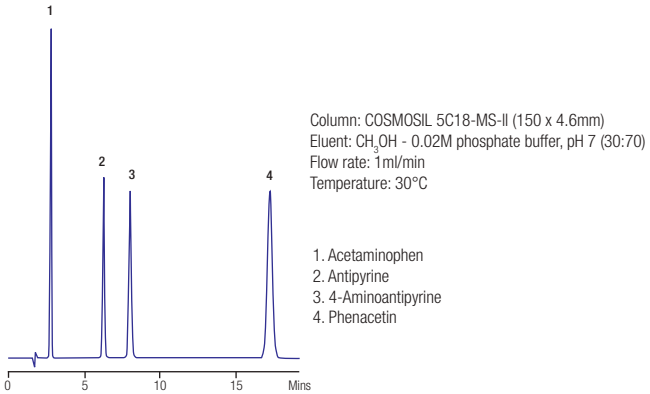


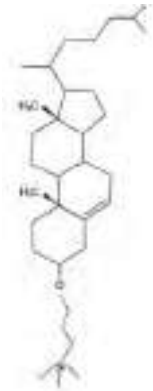
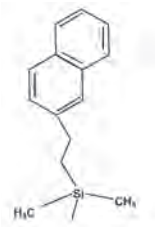
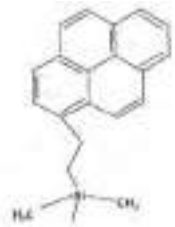
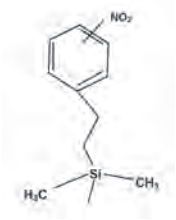

Figure 2. Separation of antipyretics on COSMOSIL 5C18-MS-II

## Additional Bonded Phases

Alternative alkyl chain length columns, COSMOSIL C22-AR-II, 5C8-MS, 5C4-MS, and 5TMS-MS, and COSMOSIL 5NH2-MS, 5CN-MS and 5Diol columns are also available. Please contact Hichrom for further details.

## Speciality Phases

### Bonding and Main Interactions

| Cholester | πNAP | PYE | NPE | PBB-R |
|---|---|---|---|---|
|  |  |  |  |  |
| **Cholesteryl group** | **Naphthylethyl group** | **Pyrenylethyl group** | **Nitrophenylethyl group** | **Pentabromobenzyl group** |
| Hydrophobic Interaction<br>Molecular Shape Selectivity | Hydrophobic Interaction<br>π-π Interaction | Hydrophobic Interaction<br>π-π Interaction<br>Charge-transfer Interaction<br>Dispersion Force | Hydrophobic Interaction<br>π-π Interaction<br>Dipole-dipole Interaction | Hydrophobic Interaction<br>Dispersion Force |

**COSMOSIL® Cholester** is a novel 3-[(cholesteryl)oxy]propylsilyl bonded silica phase. It shows the same hydrophobicity as C18 bonded phases, so can be used under similar reversed-phase conditions. However, a slightly different selectivity may be obtained. In particular, the rigid structure of the cholesterol imparts greater separation capabilities for isomeric, double-bonded and polyaromatic compounds. Figure 3 shows a comparison of stereoselectivity of Cholester and C18 phases for o-terphenyl and triphenylene.

**COSMOSIL πNAP** is a naphthylethyl bonded silica phase. The presence of two aromatic rings leads to stronger π-π interactions than with phenyl phases. COSMOSIL πNAP columns offer improved separation of positional isomers that are difficult to separate with alkyl bonded materials.

**COSMOSIL PYE** is a reversed-phase column with 2-(1-pyrenyl)ethyl groups bonded to the silica material. This column utilises π-π interactions originating from the planar pyrene ring structure to separate structural isomers (see Figure 4). Symmetrical isomers are retained more strongly. COSMOSIL PYE shows the strongest π-π interactions of all the speciality phases.

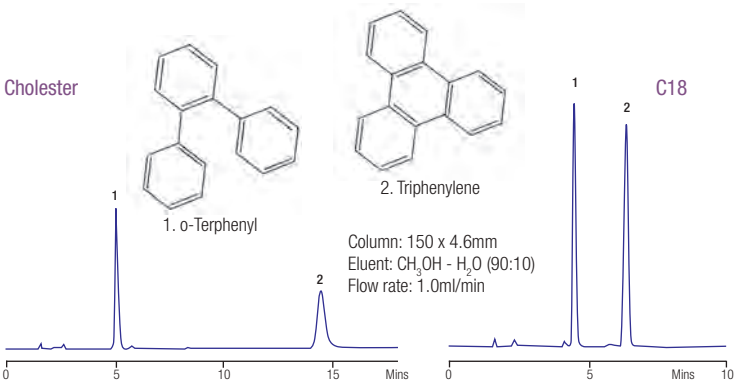
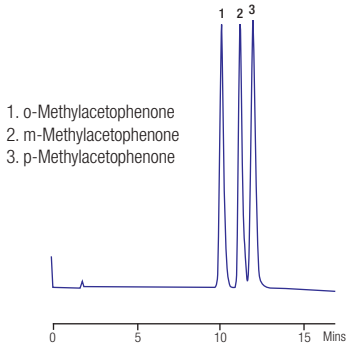



Figure 3. Comparison of stereoselectivity



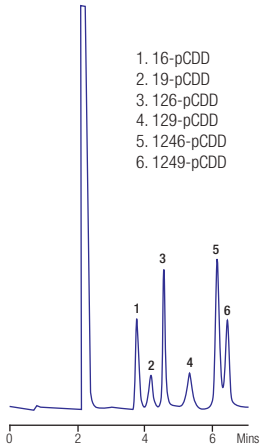

Column: COSMOSIL 5PYE (150 x 4.6mm)
Eluent: $CH_3OH$ - $H_2O$ (60:40)
Flow rate: 1.0ml/min

Figure 4. Separation of di-substituted benzenes

**COSMOSIL NPE** provides unique retention characteristics, utilising both dipole-dipole and π-π interactions. This is illustrated (Figure 5 on page 78) by the separation of a mixture of polychlorodibenzo-p-dioxins (pCDDs). Isomers with a strong dipole moment exhibit greater retention.

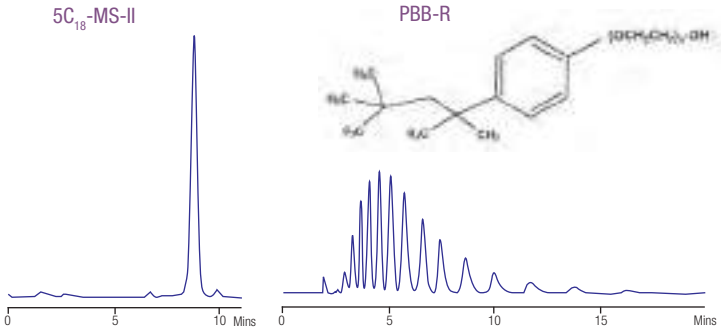
**COSMOSIL PBB-R** provides unique selectivity for structurally similar compounds utilising dispersion force interactions, and can separate structural isomers which differ by only a double bond. Figure 6 on page 78 shows the separation by dispersion force of Triton X-100 components on COSMOSIL PBB-R. Using a C18 column no separation was achieved, due to the poor hydrophobicity of the (-$OCH_2CH_2$-) group.

## Speciality Phases (continued)




Column: COSMOSIL 5NPE (150 x 4.6mm)
Eluent: $CH_3OH$ - $H_2O$ (80:20)
Flow rate: 1.0ml/min

Figure 5. Polychlorodibenzo-p-dioxins (pCDDs)




Column: 150 x 4.6mm
Eluent: COSMOSIL 5C18-MS-II $CH_3OH$ – $H_2O$ (80:20) COSMOSIL PBB-R $CH_3OH$ (100%)
Flow rate: 1.0ml/min Temperature: 30°C Detection: UV, 254nm

Figure 6. Analysis of Triton X-100 on COSMOSIL PBB-R

### COSMOSIL® HILIC

- Novel triazole bonded phase
- Strong retention for acidic compounds
- Enhanced sensitivity in LC-MS
- Good retention of polar compounds

COSMOSIL® HILIC is a triazole bonded silica phase. It has a higher polarity than non-bonded silica commonly used for HILIC, resulting in stronger hydrophilic interactions. The positively charged triazole stationary phase also shows an anion-exchange mechanism, enabling acidic compounds to be strongly retained. The two separation modes – HILIC and ionic interaction – can be controlled by varying key eluent parameters such as pH, concentration of organic solvent and buffer ionic strength.

Figure 7 shows the advantage of using a triazole-bonded silica over non-bonded silica for the HILIC separation of trimethylene glycol and ethylene glycol.

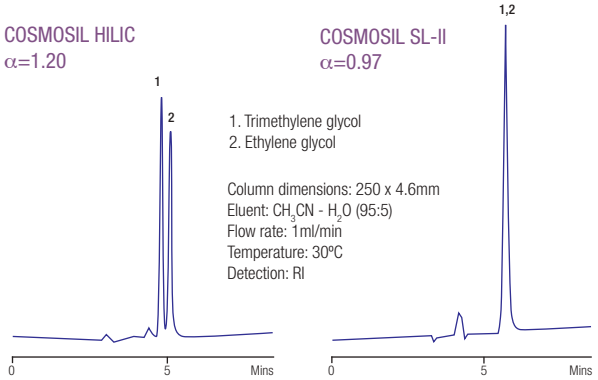


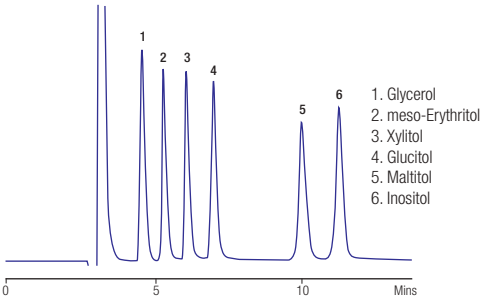

Figure 7. Hydrophilic interactions

**COSMOSIL HILIC Applications Guide available on request**

### COSMOSIL Sugar-D

- Novel amino column for saccharides
- Improved column lifetime
- Useful for hydrophilic compounds

COSMOSIL Sugar-D is a novel phase designed for the analysis of saccharides. According to Nacalai Tesque it shows improved column durability compared to standard aminopropyl bonded phases, due to a new 'defence shield' bonding technology. This protection also minimises undesirable adsorption of certain saccharides. Figure 8 shows the separation of polyols on COSMOSIL Sugar-D. In addition, COSMOSIL Sugar-D is useful for separating highly hydrophilic compounds which are not retained on conventional C18 phases.




Column: COSMOSIL Sugar-D (250 x 4.6mm)
Eluent: $CH_3CN$ - $H_2O$ (75:25) Flow rate:1ml/min
Temperature: 30°C Detection: RI

Figure 8. Analysis of polyols on COSMOSIL Sugar-D phase

## Other Phases

### COSMOSIL® Wide Pore Phases

The COSMOSIL® AR-300 series of packed columns are designed for the analysis of proteins and peptides and show good reproducibility and stability when used with TFA. Four phases are available: 5C18-AR-300, 5C8-AR-300, 5C4-AR-300 and 5Ph-AR-300.

### COSMOSIL HIC

COSMOSIL 5HIC is a wide pore diol-bonded column for hydrophobic interaction chromatography. It is designed for the one-step desalting and separation of proteins, without denaturation. It has a higher loading capacity than ODS columns.

### Special Columns for Fullerenes

The COSMOSIL range offers a variety of columns designed for the preparative scale separation of fullerenes and metallofullerenes. These include COSMOSIL Buckyprep and COSMOSIL PBB.

### COSMOGEL IEX Series

The COSMOGEL IEX Series consists of anion-exchange, cation-exchange and amphoteric ion-exchange phase types (see table below). These are useful for the separation of biopolymers including proteins and nucleic acids.

| Ion Exchange Mode | Anion-exchange Type | | Cation-exchange Type | | Amphoteric Ion-exchange Type | |
|---|---|---|---|---|---|---|
| **Packing Material** | Type Q | Type Q-N | Type S | Type S-N | Type M | Type M-N |
| **Gel / Average Particle Size** | Hydrophilic Polymer / 5µm | | Hydrophilic Polymer / 5µm | | Hydrophilic Polymer / 5µm | |
| **Average Pore Size** | 1000Å | Non-porous | 1000Å | Non-porous | 1000Å | Non-porous |
| **Functional Group** | $-CH_3N^+(CH_3)_3$ | | $-(CH_2)_3SO_3^-$ | | $-CH_3N^+(CH_3)_3$ + $-(CH_2)_3SO_3^-$ | |
| **Target Sample** | Acidic proteins and DNA | | Basic proteins | | All proteins | |

### COSMOSIL SFC Columns

Two bonded phases have been developed to enhance the capability of SFC separations - COSMOSIL 3-Hydroxyphenyl and COSMOSIL Quinoline. COSMOSIL Quinoline shows improved separations of structural isomers of polar lipids, such as cholesterol, used as potential biomarkers.
Please contact Hichrom for further information.

### Ordering Information

| 5µm COSMOSIL Phase | Column Dimensions[1] (mm) | | | | | | Guard Column (10 x 4.6mm, 1/pk) |
|---|---|---|---|---|---|---|---|
| | 150 x 2.0 | 250 x 2.0 | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| SL-II | - | - | 37999-81 | 38000-01 | 38001-91 | 38002-81 | 37997-01 |
| C18-AR-II | 37992-51 | 05272-71 | 38142-51 | 38143-41 | 38144-31 | 38145-21 | 38141-61[2] |
| C18-MS-II | 38025-91 | 05761-61 | 38017-01 | 38018-91 | 38019-81 | 38020-41 | 38014-31[2] |
| C18-PAQ | 34449-71 | 05795-31 | 34451-21 | 05799-91 | 02486-71 | 02485-81 | 02484-91 |
| Protein-R | 06514-71 | - | 06525-31 | - | 06526-21 | 06527-11 | 06518-31 |
| PYE | 38042-61 | 34450-31 | 38043-51 | - | 37837-91 | 37989-11 | 37903-11 |
| NPE | 34328-51 | 34379-91 | - | - | 37902-21 | 37990-71 | 37904-01 |
| PBB-R | 05900-31 | - | - | - | 05697-21 | 05698-11 | 05704-11 |
| HIC | - | - | 04263-21 | - | - | - | - |
| Sugar-D | 05688-41 | 05689-31 | 07975-11 | - | 05395-71 | 05397-51 | 05394-81 |
| Cholester | 05971-11 | 05972-01 | - | 06591-61 | 05976-61 | 05977-51 | 05975-71 |
| HILIC | 07054-71 | - | - | - | 07056-51 | 07057-41 | 07055-61 |
| πNAP | 08078-41 | 08079-31 | 08083-61 | 08084-51 | 08085-41 | 08086-31 | 08082-71 |

[1] Other dimensions available, including preparative columns
[2] Guard cartridges (3/pk) also available – please enquire

Please contact Hichrom for further details and ordering information on COSMOSIL and COSMOGEL phases not listed above.

# DEVELOSIL®

- Spherical porous silica
- Range of C18 phases for different selectivity
- Range of C30 phases for high aqueous eluents and polar selectivity

Develosil® columns are manufactured by Nomura Chemical Co. Ltd, Japan. Six different C18 phases are offered: ODS-UG, ODS-HG, ODS-MG, ODS-SR, XG-C18 and the speciality PAHS. Each phase offers slightly different selectivity, enabling the chromatographer to fine-tune difficult separations. A series of C30 bonded columns provide added retention for polar compounds using 100% aqueous eluents. Develosil wide pore (300Å) C4-HG, C8-HG and ODS-HG can also be supplied.

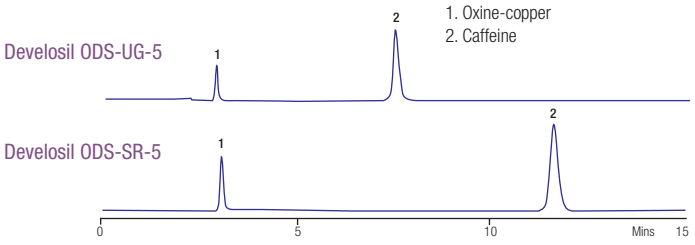
## Develosil Phases[3]

| Develosil Phase[1] | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Bonding Type |
|---|---|---|---|---|---|---|---|
| TMS-UG | C1 | Yes | 3, 5 | 140 | 300 | 4.5 | Monomeric, monofunctional |
| C8-UG | C8 | Yes | 3, 5 | 140 | 300 | 11 | Monomeric, monofunctional |
| ODS-UG | C18 | Yes | 3, 5 | 140 | 300 | 18 | Monomeric, monofunctional |
| Phenyl-UG | Phenyl | Yes | 3, 5 | 140 | 300 | 8 | Monomeric, monofunctional |
| CN-UG | CN | Yes | 5 | 140 | 300 | 7 | Monomeric, monofunctional |
| RPAQUEOUS (C30-UG)[2] | C30 | Yes | 3, 5 | 140 | 300 | 18 | Monomeric, monofunctional |
| RPAQUEOUS-AR | C30 | Yes | 3, 5 | 140 | 300 | 18 | Monomeric, trifunctional |
| ODS-MG | C18 | Yes | 3, 5 | 100 | 450 | 15 | Monomeric, difunctional |
| ODS-HG | C18 | Yes | 3, 5 | 140 | 300 | 18 | Monomeric, trifunctional |
| ODS-SR | C18 | Yes | 3, 5 | 80 | - | 18 | Monomeric, difunctional |
| PAHS | C18 | No | 3, 5 | 120 | 350 | 23 | Polymeric, trifunctional |
| XG-C18 | C18 | Yes | 3, 5 | 140 | 300 | 19 | Monomeric, monofunctional |
| XG-C30 | C30 | Yes | 3, 5 | 140 | 300 | 19.5 | Monomeric, monofunctional |
| XG-CN | CN | Yes | 3, 5 | 140 | 300 | 7.5 | Monomeric, monofunctional |

[1] Develosil wide pore (300Å) phases C4-HG, C8-HG and ODS-HG also available
[2] The Combi-RP phase (see p. 81) is identical to the RPAQUEOUS (C30-UG) phase
[3] Please enquire for phases not listed

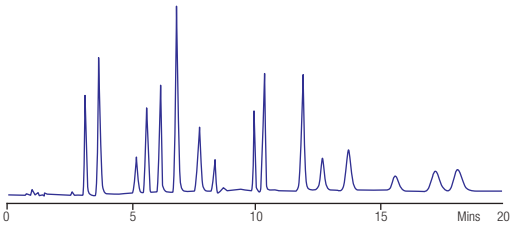
## Comparison of ODS (C18) Columns

**Develosil ODS-UG** is the most stable Develosil phase under alkaline conditions and can be used at pH 1 – 10.
**Develosil ODS-HG** is the most stable under acidic conditions (pH 1 – 9) and can be used with up to 0.5% TFA.
**Develosil ODS-MG** equilibrates quickly and shows higher retention of polar compounds.
**Develosil ODS-SR** (Super Retentive) is the most retentive of the C18 phases and is ideal for LC-MS, as the higher organic solvent content required leads to higher sensitivity. Figure 1 shows a comparison of retention times for oxine-copper and caffeine on ODS-SR-5 and ODS-UG-5.
**Develosil PAHS** is an ODS bonded non-endcapped phase with polymeric bonding and shows the greatest steric selectivity. Figure 2 shows the separation of the 16 priority pollutant polyaromatic hydrocarbons.
**Develosil XG-C18** is the newest addition to the C18 range and is based on high purity silica. The recommended pH range is 1.5 – 8.




Column: 150 x 4.6mm
Eluent: $CH_3CN$ - 20mM $H_3PO_4$ (10:90)
Flow rate: 1ml/min
Temperature: 30°C

Figure 1. Comparison of retention times for ODS-SR-5 and ODS-UG-5




Column: Develosil PAHS-5 (150 x 4.6mm)
Eluent: $H_2O$ - $CH_3OH$ (gradient of 80% to 100% $CH_3OH$)
Flow rate: 1.5ml/min
Temperature: 30°C

Figure 2. Analysis of 16 PAHs on Develosil PAHS-5

## Bulk Preparative Develosil

Bulk preparative scale Develosil material can be supplied. Please contact Hichrom for further details of Develosil bulk material.

## RPAQUEOUS*

Develosil® RPAQUEOUS is a C30 bonded phase that can be used under 100% aqueous conditions. In Figure 3 it is shown to be water stable for >150 hours, whereas the ODS-UG phase showed considerable loss of retention. It is believed that the C30 ligands remain in the solid state at a typical HPLC operating temperature of 30°C under these eluent conditions.

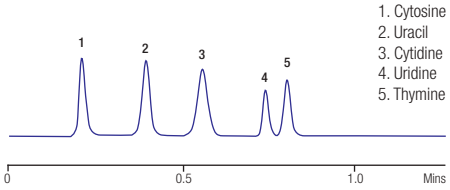
## Combi-RP*

The Combi-RP phase is the same C30 material as the RPAQUEOUS phase. However, whereas RPAQUEOUS columns are intended for general purpose and LC-MS applications, Combi-RP is specially aimed at high throughput applications requiring 100% aqueous eluents (pH 2-8). Figure 4 shows the separation of five nucleic acid bases in under a minute.

## RPAQUEOUS-AR

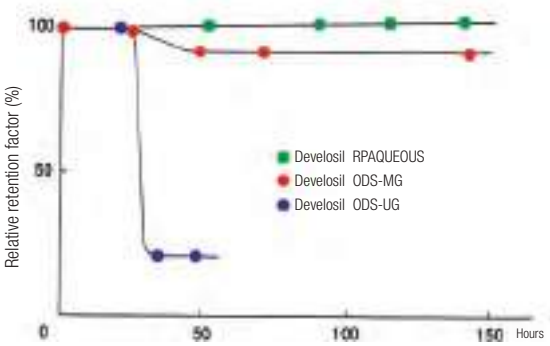
RPAQUEOUS-AR is an alternative C30 phase which shows increased stability under acidic conditions due to the nature of the trifunctional bonding. It is suitable for polar compounds such as sugars and nucleotides and also fat soluble compounds such as tocopherols and carotenoids.

* RPAQUEOUS, C30-UG and Combi-RP are the same material




Column: Develosil Combi-RP (5µm, 50 x 4.6mm)
Eluent: 100% 10mM Na phosphate, pH 7.0 to 100% $CH_3CN$ in 5 mins
Flow rate: 3ml/min

Figure 4. Separation of nucleic acid bases on Combi-RP




Column: Develosil RPAQUEOUS, Develosil ODS-MG-5, Develosil ODS-UG-5 (250 x 4.6 mm)
Sample: Thymine Eluent: $H_2O$ Flow rate: 1.0ml/min Temperature: 40°C

Figure 3. Effect of aqueous eluent on Develosil RPAQUEOUS, ODS-MG and ODS-UG phases

## Ordering Information

| Develosil 3µm Phase | Column Dimensions[1] (mm) | | |
|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 |
| ODS-UG-3 | UG11346050W | UG11346100W | UG11346150W |
| ODS-MG-3 | MG11346050W | MG11346100W | MG11346150W |
| ODS-HG-3 | HG11346050W | HG11346100W | HG11346150W |
| ODS-SR-3 | SR11346050W | SR11346100W | SR11346150W |
| PAHS-3 | PAHS346050W | PAHS346100W | PAHS346150W |
| RPAQUEOUS-3 | RPAQ346050W | RPAQ346100W | RPAQ346150W |
| RPAQUEOUS-AR-3 | RPAR346050W | RPAR346100W | RPAR346150W |

[1] Other dimensions available

| Develosil 5µm Phase | Column Dimensions[1] (mm) | | | Guard Cartridges[2] (1/pk, 10 x 4.0mm) |
|---|---|---|---|---|
| | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| ODS-UG-5 | UG11546100W | UG11546150W | UG11546250W | UG11540010W |
| ODS-MG-5 | MG11546100W | MG11546150W | MG11546250W | MG11540010W |
| ODS-HG-5 | HG11546100W | HG11546150W | HG11546250W | HG11540010W |
| ODS-SR-5 | SR11546100W | SR11546150W | SR11546250W | SR11540010W |
| PAHS-5 | PAHS546100W | PAHS546150W | PAHS546250W | - |
| TMS-UG-5 | UG14546100W | UG14546150W | UG14546250W | UG14540010W |
| C8-UG-5 | UG12546100W | UG12546150W | UG12546250W | UG12540010W |
| Phenyl-UG-5 | UG15546100W | UG15546150W | UG15546250W | UG15540010W |
| CN-UG-5 | UG16546100W | UG16546150W | UG16546250W | UG16540010W |
| RPAQUEOUS-5 | RPAQ546100W | RPAQ546150W | RPAQ546250W | UG17540010W |
| RPAQUEOUS-AR-5 | RPAR546100W | RPAR546150W | RPAR546250W | RPAR540010W |

[1] Other dimensions available [2] Holder included

| Develosil Phase | Particle Size (µm) | Column Dimensions[1] (mm) | | | | |
|---|---|---|---|---|---|---|
| | | 35 x 2.0 | 50 x 2.0 | 35 x 4.6 | 50 x 4.6 | 50 x 20.0 |
| Combi-RP-3 | 3 | COMB320035W | COMB320050W | COMB346035W | COMB346050W | - |
| Combi-RP-5 | 5 | COMB520035W | COMB520050W | COMB546035W | COMB546050W | COMB5P2050W |

[1] Other dimensions available

Please contact Hichrom for phases and column dimensions not listed.

# DIKMA® TECHNOLOGIES

Dikma® Technologies Inc. manufactures several ranges of HPLC and UHPLC columns, based on high purity metal-free silica.

## Phase Specifications

| Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | pH Range |
|---|---|---|---|---|---|---|
| Endeavorsil C18 | 1.8 | 120 | 300 | 20 | Yes | 1.5 - 10 |
| Leapsil C18 | 2.7 | 100 | 440 | 27 | Yes | 1.5 - 10 |
| Spursil C18 | 3, 5, 10 | 100 | 440 | 25 | Yes | 1.5 - 10 |
| Spursil C18-EP | 3, 5, 10 | 100 | 440 | 24 | Yes | 1.5 - 10 |
| Inspire C18 | 3, 5, 10 | 100 | 440 | 27 | Yes | 1 - 11 |
| Inspire C8 | 3, 5, 10 | 100 | 440 | 17 | Yes | 1 - 11 |
| Bio-Bond C18 | 3, 5, 10 | 300 | 100 | 8 | Yes | 2 - 8 |
| Bio-Bond C8 | 3, 5, 10 | 300 | 100 | 5 | Yes | 2 - 8 |
| Bio-Bond C4 | 3, 5, 10 | 300 | 100 | 3 | Yes | 2 - 8 |

## Endeavorsil™ C18

- 1.8µm particle size
- Combines speed, resolution and sensitivity

Endeavorsil C18 is a 1.8µm silica phase designed for ultra-fast separations with high efficiency, sensitivity and resolution. Columns show excellent separation characteristics over a wide pH range.

## Leapsil™ C18

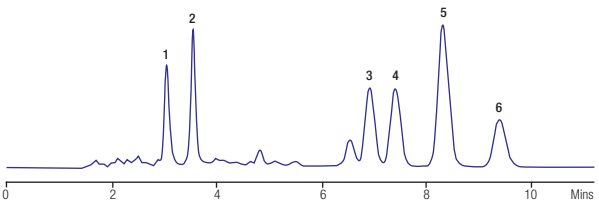
- 2.7µm particle size
- Compatible with all HPLC and UHPLC instruments
- Wide pH stability

Leapsil C18 is a 2.7µm particle size phase designed for ultra fast separations using HPLC or UHPLC instrumentation. Higher flow rates can be used, without compromising resolution or column back pressure.

## Spursil™

- Unique selectivity and enhanced resolution
- Enhanced retention for polar compounds
- Stable retention in highly aqueous eluents

Spursil phases combine the benefits of high purity silica with unique polar modification technology. These columns maximise polar retention and selectivity, whilst virtually eliminating silanol activity. Figure 1 shows the separation of a group of cephalosporins on a Spursil C18 column.




Column: Spursil C18 (5µm, 150 x 4.6mm)
Eluent: $CH_3OH$ – 0.1% TFA in $H_2O$ (30:70)
Flow rate: 1.0ml/min
Temperature: Ambient Detection: UV, 230nm

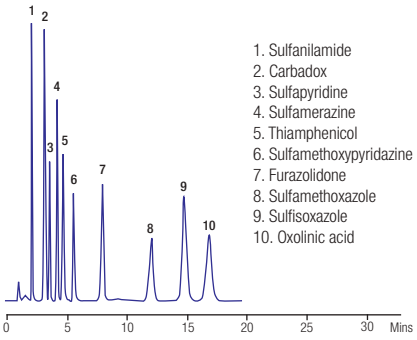
1. Ceftazidime
2. Cefadroxil
3. Cefuroxime
4. Cefazoline
5. Cefaclor
6. Cephalexin

Figure 1. Separation of cephalosporins on Spursil C18

## Inspire™

- High efficiency and selectivity
- Stable from pH 1 to 11
- Excellent batch-to-batch reproducibility

Inspire C18 and C8 columns are manufactured from high purity silica, using proprietary bonding techniques and tightly controlled manufacturing processes. Figure 2 shows the separation of antibacterials on an Inspire C18 column.




1. Sulfanilamide
2. Carbadox
3. Sulfapyridine
4. Sulfamerazine
5. Thiamphenicol
6. Sulfamethoxypyridazine
7. Furazolidone
8. Sulfamethoxazole
9. Sulfisoxazole
10. Oxolinic acid

Column: Inspire C18 (5µm, 150 x 4.6mm)
Eluent: 0.1% HCOOH in $CH_3CN$ – 0.1% HCOOH in $H_2O$ (20:80)
Flow rate: 1.0ml/min
Temperature: Ambient Detection: UV, 254nm

Figure 2. Separation of antibacterials on Inspire C18

## Bio-Bond™

- C18, C8 and C4 wide-pore phases
- Proteins, peptides and biomolecule analyses

Bio-Bond phases are designed for the analysis and purification of proteins, peptides and other biomolecules. These are excellent columns for LC-MS due to low phase bleed. Dikma offers direct scale-up to preparative dimension columns and bulk materials.

## Ordering Information

### Endeavorsil™

| 1.8µm Phase | Column Dimensions (mm) | | |
|---|---|---|---|
| | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 |
| Endeavorsil C18 | 87001 | 87002 | 87003 |

### Leapsil™

| 2.7µm Phase | Column Dimensions[1] (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 |
| Leapsil C18 | 86004 | 86005 | 86006 | 86001 | 86002 | 86003 |

[1] 3.0mm i.d. columns also available

### Spursil™

| 3µm Phase | Column Dimensions[1] (mm) | | | | |
|---|---|---|---|---|---|
| | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 |
| Spursil C18 | 82030 | 82004 | 82012 | 82013 | 82015 |
| Spursil C18-EP | 82130 | 82104 | 82112 | 82113 | 82115 |
| | **30 x 4.6** | **50 x 4.6** | **100 x 4.6** | **150 x 4.6** | **250 x 4.6** |
| Spursil C18 | 82031 | 82016 | 82017 | 82018 | 82020 |
| Spursil C18-EP | 82131 | 82116 | 82117 | 82118 | 82120 |

| 5µm Phase | Column Dimensions[1] (mm) | | | | |
|---|---|---|---|---|---|
| | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 |
| Spursil C18 | 82033 | 82003 | 82007 | 82002 | 82009 |
| Spursil C18-EP | 82133 | 82103 | 82107 | 82102 | 82109 |
| | **30 x 4.6** | **50 x 4.6** | **100 x 4.6** | **150 x 4.6** | **250 x 4.6** |
| Spursil C18 | 82034 | 82010 | 82011 | 82001 | 82006 |
| Spursil C18-EP | 82134 | 82110 | 82111 | 82101 | 82106 |

[1] 3.0mm i.d. columns also available

### Inspire™

| 3µm Phase | Column Dimensions[1] (mm) | | | | | Guard Cartridges[3], 2/pk |
|---|---|---|---|---|---|---|
| | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | 10 x 2.1 |
| Inspire C18 | 81030 | 81004 | 81012 | 81013 | 81015 | 6501 |
| Inspire C8 | 81130 | 81104 | 81112 | 81113 | 81115 | 6502 |
| | **30 x 4.6** | **50 x 4.6** | **100 x 4.6** | **150 x 4.6** | **250 x 4.6** | **10 x 4.0** |
| Inspire C18 | 81031 | 81016 | 81017 | 81018 | 81020 | 6601 |
| Inspire C8 | 81131 | 81116 | 81117 | 81118 | 81120 | 6602 |

| 5µm Phase | Column Dimensions[1, 2] (mm) | | | | | Guard Cartridges[3], 2/pk |
|---|---|---|---|---|---|---|
| | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | 10 x 2.1 |
| Inspire C18 | 81033 | 81003 | 81007 | 81002 | 81009 | 6503 |
| Inspire C8 | 81133 | 81103 | 81107 | 81102 | 81109 | 6504 |
| | **30 x 4.6** | **50 x 4.6** | **100 x 4.6** | **150 x 4.6** | **250 x 4.6** | **10 x 4.0** |
| Inspire C18 | 81034 | 81010 | 81011 | 81001 | 81006 | 6603 |
| Inspire C8 | 81134 | 81110 | 81111 | 81101 | 81106 | 6604 |

[1] 3.0mm i.d. columns also available [2] 5 and 10µm semi-preparative columns also available [3] Use with EasyGuard holder 6220

### Bio-Bond™

| 3µm Phase | Column Dimensions[1] (mm) | | | | | Guard Cartridges[2], 2/pk | |
|---|---|---|---|---|---|---|---|
| | 50 x 2.1 | 150 x 2.1 | 50 x 4.6 | 150 x 4.6 | 250 x 4.6 | 10 x 2.1 | 10 x 4.0 |
| Bio-Bond C18 | 84004 | 84013 | 84016 | 84018 | 84020 | 6901 | 6951 |
| Bio-Bond C8 | 84104 | 84113 | 84116 | 84118 | 84120 | 6902 | 6952 |
| Bio-Bond C4 | 84404 | 84413 | 84416 | 84418 | 84420 | 6905 | 6955 |
| **5µm Phase** | **50 x 2.1** | **150 x 2.1** | **50 x 4.6** | **150 x 4.6** | **250 x 4.6** | **10 x 2.1** | **10 x 4.0** |
| Bio-Bond C18 | 84003 | 84002 | 84010 | 84001 | 84006 | 6903 | 6953 |
| Bio-Bond C8 | 84103 | 84102 | 84110 | 84101 | 84106 | 6904 | 6954 |
| Bio-Bond C4 | 84403 | 84402 | 84410 | 84401 | 84406 | 6906 | 6956 |

[1] Semi-preparative columns also available [2] Use with EasyGuard holder 6220

Please contact Hichrom for further information on Dikma columns.

# EPROGEN

- Reversed-phase and ion-exchange silica phases
- SynChropak bonding chemistry
- Well suited for preparative formats

Eprogen Inc. manufactures a range of phases for reversed-phase, ion-exchange and size exclusion chromatography. The products listed below are based on the well-established SynChropak® bonding chemistry. The range is well suited to scaling up to preparative formats.

## Eprogen Phases

| Phase | Functional Group | Particle Size (µm) | Pore Size (Å) | Applications |
|---|---|---|---|---|
| **Reversed-phase** | | | | |
| RP8 | C8 (monomeric) | 5 | 300 | Peptides, proteins |
| SCD | Short alkyl chain | 5 | 100 | Pharmaceuticals |
| **Ion-exchange** | | | | |
| AX300 | Polyethyleneimine | 6 | 300 | Peptides, proteins |
| Q300 | Quaternary amine | 6 | 300 | Proteins |
| CM300 | Carboxymethyl | 6 | 300 | Proteins, haemoglobin |
| S300 | Sulphonic acid | 6 | 300 | Basic peptides, proteins |

Column: Eprogen Q300 (250 x 4.6mm)
Eluent: A: 0.02M Tris, pH 7.0
B: 0.02M Tris, 0.5M Na acetate
0 to 100% B in 30 mins
Flow rate: 1ml/min
Detection: post column reaction

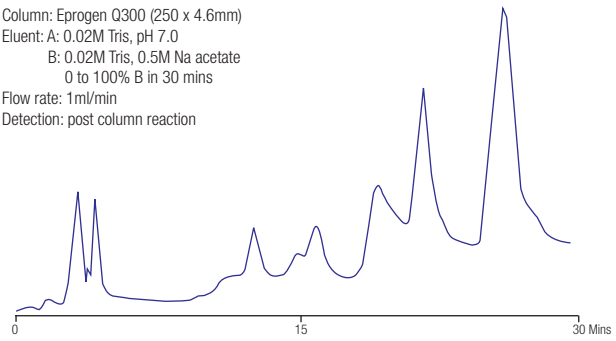



Figure 1. Analysis of lactate dehydrogenase isoenzymes

Column: Eprogen CM300 (250 x 4.6mm)
Eluent: A: 0.02M Tris-acetate, 1mM EDTA, 1.0mM DTT,
0.02% $NaN_3$, pH 6.0
B: A + 0.5M Na acetate, pH 6.0
10% B for 10mins, then 10-40% B in 15mins
Flow rate: 1ml/min

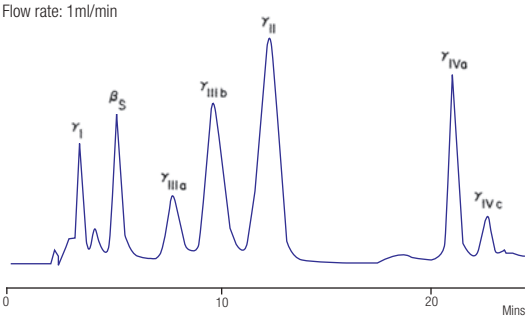



Figure 2. Analysis of calf lens crystallins

## Ordering Information – Hichrom Manufactured Columns

| Eprogen Phase | Column Dimensions[1] (mm) | | Guard Cartridges[2,3] (For 4.6mm i.d. Columns) |
|---|---|---|---|
| | 150 x 4.6 | 250 x 4.6 | |
| RP8 | C8R103-150A | C8R103-250A | C8R103-10C5 |
| SCD | SCD100-150A | SCD100-250A | SCD100-10C5 |
| AX300 | A103-150A | A103-250A | A103-10C5 |
| Q300 | Q103-150A | Q103-250A | Q103-10C5 |
| CM300 | CM103-150A | CM103-250A | CM103-10C5 |
| S300 | S103-150A | S103-250A | S103-10C5 |

[1] Other column dimensions available – please enquire
[2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20
[3] 5/pk – Starter kits also available – see p. 21

In addition to the Hichrom manufactured columns listed above, Eprogen packed columns can be provided, including:

- Non-porous silica (1.5µm) phases based on MICRA® NPS® technology
- Eprogen IEX columns – strong and weak anionic and cationic ion exchangers on polymer coated silica supports (AX300, Q300, CM300, S300 and S1000)
- Eprogen GPC and CATSEC columns – size exclusion technology for polymer and biomolecule separations (based on original SynChropak® bonding technology)

Please contact Hichrom for further details of Eprogen packed columns.

# ES INDUSTRIES



ES Industries, based in the north of the USA, have been manufacturing HPLC columns for over 30 years and more recently also manufacture columns specifically for SFC. An outline of some of their more popular product ranges is given below.

## Epic®

The Epic range is based on high density bonded monomeric phases, produced through a proprietary bonding process. Epic columns are compatible with a wide range of organic modifiers and buffers and stable over a wide pH range. All phases have a pore size of 120Å and are available with particle sizes of 1.8, 3, 5 and 10µm and with microbore to preparative dimensions.

**Available phases:** C18, C18-MS, C18-SD, C8, C4-SD, Polar, PFP, HILIC, Phenyl-Hexyl, SCX

## AquaSep™

AquaSep was developed for the separation of polar compounds using high aqueous eluents. The 100Å phase contains an ether linkage near the point of attachment to the silica base. This allows water to penetrate the surface and prevents 'pore dewetting'. AquaSep can retain highly water soluble compounds such as small organic acids (see Figure 1).

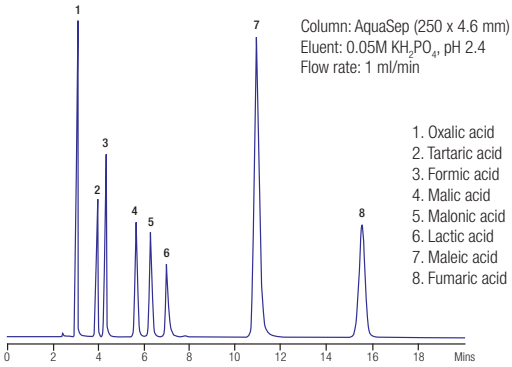



Figure 1. Separation of organic acids

## FluoroSep™-RP

FluoroSep-RP is a family of reversed-phase fluorinated ligands bonded to high efficiency silica. These phases are stable from pH 2 to 8 and may be heated to 80°C.

Three phases are available:
**FluoroSep-RP Phenyl (FSP)** – Pentafluorophenyl functional group, 60Å pore size
**FluoroSep-RP Octyl (FO)** – Perfluorooctyl functional group, 60Å pore size
**FluoroSep-RP Propyl (FP)** – Perfluoropropyl functional group, 300Å pore size

## GammaBond™ Alumina

GammaBond Alumina is a family of exceptionally stable spherical alumina-based HPLC columns designed to provide high efficiency and unique selectivity for extreme pH applications (pH 1.3 to 12).

Two phases are available:
**GammaBond Alumina** – manufactured by bonding a polymer to 5µm porous spherical alumina particles, with a pore size of 130Å.
**GammaBond RP-1** – a low load polybutadiene coated alumina, 80Å pore size with both reversed-phase and ion-exchange characteristics. This phase is classified as USP L29.

## Chromegabond®

Chromegabond columns manufactured by ES Industries constitute a wide range of bonded phases, some being exclusive to ES Industries. Spherical silica phases in 3, 5 or 10µm particle sizes are available with pore diameters ranging from 60 to 1000Å.

**Phases include:** C18, C8, C4, Dimethyl, Cyclohexyl, Alkyl Phenyl, Diol, Amino, Diamine, Triamine, C22, Fluorinated C18, Monoalcohol (NPl), Nitro, Mixed amino/cyano, columns for petroleum product applications (RingSep, DNAP, Silver/Sl) and others.

## GreenSep™ Columns for SFC

ES Industries offer the GreenSep range of phases specifically designed for SFC separations. These 120Å pore size phases have proved to show superior separation, selectivity, peak shape and loading capacity compared to conventional normal-phase HPLC materials adapted for SFC (eg. amino, cyano, diol). All GreenSep phases are available as analytical, semi-preparative (10mm and 15mm i.d.) and preparative (20mm, 30mm and 50mm i.d.) columns.

**Phases include:**
Ethylpyridine – good for strongly basic compounds
Nitro – useful for geometrical isomers and compounds containing aromatic groups, polarisable electrons and conjugate systems
Basic – based on imidazole chemistry, good retention of amine-containing basic compounds
Pyridyl Amide – good for compounds with both amine and acidic functional groups
Additional phases include Amino Phenyl, DEAP (Diethylaminopropyl), PFP and Silica

Please contact us for ordering information on all ES Industries products.

# EXSIL™

- Spherical porous silica
- 80 and 100Å pore sizes
- Competitively priced
- Hichrom high efficiency

Exsil™, initially developed by Exmere and now manufactured by Grace Materials Technologies / Discovery Sciences, is firmly established as a useful alternative to the classical spherical HPLC silicas. Exsil 100, the standard material, is supplied in a range of particle sizes and bonded phases to cover a wide range of applications. Exsil 80 is a higher surface area, complementary range of materials. Please enquire for further details.

## Exsil Phases

| Exsil Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Silica | - | - | 5, 10 | 100 | 200 | - |
| C1 | Methyl | No | 3, 5 | 100 | 200 | 3 |
| C8 | Octyl | Yes | 3, 5 | 100 | 200 | 6 |
| ODS | Octadecyl | Yes | 3, 5, 10 | 100 | 200 | 11 |
| ODS1 | Octadecyl | No | 3, 5 | 100 | 200 | 7 |
| ODSB | Octadecyl | Yes | 3, 5 | 100 | 200 | 12 |
| CN | Cyano | No | 3, 5 | 100 | 200 | 3.5 |
| NH2 | Amino | No | 3, 5 | 100 | 200 | 2 |
| SAX | Tetramethyl ammonium | No | 5 | 100 | 200 | 3 |
| SCX | Sulphonic acid | No | 5 | 100 | 200 | 3 |

## Ordering Information – Hichrom Manufactured Columns

### Microbore (1.0 – 2.1mm i.d.) and Medium Bore (3.2mm i.d.) Columns

Please contact Hichrom for further details of 1.0, 2.1 and 3.2mm i.d. Exsil columns

### Analytical (4.6mm i.d.) Columns

| Exsil Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **3µm** | | | | | |
| C1 | EXC1-3-50A | EXC1-3-100A | EXC1-3-150A | - | EXC1-3-10C5 |
| C8 | EXC8-3-50A | EXC8-3-100A | EXC8-3-150A | - | EXC8-3-10C5 |
| ODS | EXODS-3-50A | EXODS-3-100A | EXODS-3-150A | - | EXODS-3-10C5 |
| ODS1 | EXODS1-3-50A | EXODS1-3-100A | EXODS1-3-150A | - | EXODS1-3-10C5 |
| CN | EXCN-3-50A | EXCN-3-100A | EXCN-3-150A | EXCN-3-250A | EXCN-3-10C5 |
| NH2 | EXNH-3-50A | EXNH-3-100A | EXNH-3-150A | EXNH-3-250A | EXNH-3-10C5 |
| ODSB | EXODSB-3-50A | EXODSB-3-100A | EXODSB-3-150A | - | EXODSB-3-10C5 |
| **5µm** | | | | | |
| Silica | EX-5-50A | EX-5-100A | EX-5-150A | EX-5-250A | EX-5-10C5 |
| C1 | EXC1-5-50A | EXC1-5-100A | EXC1-5-150A | EXC1-5-250A | EXC1-5-10C5 |
| C8 | EXC8-5-50A | EXC8-5-100A | EXC8-5-150A | EXC8-5-250A | EXC8-5-10C5 |
| ODS | EXODS-5-50A | EXODS-5-100A | EXODS-5-150A | EXODS-5-250A | EXODS-5-10C5 |
| ODS1 | EXODS1-5-50A | EXODS1-5-100A | EXODS1-5-150A | EXODS1-5-250A | EXODS1-5-10C5 |
| CN | EXCN-5-50A | EXCN-5-100A | EXCN-5-150A | EXCN-5-250A | EXCN-5-10C5 |
| NH2 | EXNH-5-50A | EXNH-5-100A | EXNH-5-150A | EXNH-5-250A | EXNH-5-10C5 |
| ODSB | EXODSB-5-50A | EXODSB-5-100A | EXODSB-5-150A | EXODSB-5-250A | EXODSB-5-10C5 |
| SAX | EXSAX-5-50A | EXSAX-5-100A | EXSAX-5-150A | EXSAX-5-250A | EXSAX-5-10C5 |
| SCX | EXSCX-5-50A | EXSCX-5-100A | EXSCX-5-150A | EXSCX-5-250A | EXSCX-5-10C5 |
| **10µm** | | | | | |
| Silica | - | EX-10-100A | EX-10-150A | EX-10-250A | EX-10-10C5 |
| ODS | - | EXODS-10-100A | EXODS-10-150A | EXODS-10-250A | EXODS-10-10C5 |

[1] Other column dimensions available – please enquire [2] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] Starter kits also available – see p. 21

# GL SCIENCES



GL Sciences of Tokyo, Japan manufacture the well established Inertsil® series of HPLC columns. InertSustain® is their latest series of high purity silica bonded phases, with the C18 phase showing increased durability over a wide pH range. The Inertsil 2, 3 and 4 Series are successive generations of silica bonded phases. GL Sciences also manufacture Titansphere®, which consists of porous spherical particles of titanium dioxide, that displays amphoteric ion-exchange properties. These products are specifically designed for biological applications involving phosphopeptide extraction.

## InertSustain®

InertSustain phases are based on a new type of silica, the surface of which is uniquely modified, enabling precise control of the silica properties. The InertSustain C18 phase combines the advantages of all previous Inertsil columns (eg. inertness, low back pressure, high efficiency) with durability over a wide pH range. Further bonded phases in the InertSustain range include C8, Phenyl and NH2.

## Inertsil® 4 Series

The Inertsil 4 series is based on enhanced deactivation techniques which produce highly inert silica, enabling successful analysis of trace levels of the most demanding basic and acidic compounds. Strictly classified particle size, with narrow distribution width and an ideal carbon loading, enables high efficiency columns to be guaranteed. Available phases – ODS-4, ODS-4V (validated) and C8-4.

## Inertsil 3 Series

Compared with Inertsil 2 Series, Inertsil 3 Series phases are based on a purer, higher surface area silica which is specially manufactured to provide maximum phase coverage. The result is a series of columns which provide excellent peak shapes using simple eluents while operating at low pressure.

### Inertsil 3 Series Phases

| Phase | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Recommended pH Range* |
|---|---|---|---|---|---|---|
| SIL | - | 3, 5 | 100 | 450 | - | 2 - 7.5 |
| ODS-3 | Yes | 2, 3, 4, 5, 10 | 100 | 450 | 15 | 2 - 7.5 |
| ODS-3V | Yes | 3, 5 | 100 | 450 | 15 | 2 - 7.5 |
| ODS-P | No | 3, 5 | 100 | 450 | 29 | 2 - 7.5 |
| ODS-EP | No | 5 | 100 | 450 | 9 | 2 - 7.5 |
| Peptide C18 | Yes | 5 | 100 | 450 | 15 | 2 - 7.5 |
| ODS-Sprint | Yes | 3, 5 | 100 | 450 | 8.5 | 2 - 7.5 |
| Sulfa C18 | Yes | 3, 5 | 100 | 450 | 15 | 2 - 7.5 |
| C8-3 | Yes | 2, 3, 5 | 100 | 450 | 9 | 2 - 7.5 |
| Ph-3 | No | 2, 3, 5 | 100 | 450 | 9.5 | 2 - 7.5 |
| CN-3 | No | 3, 5 | 100 | 450 | 14 | 2 - 7.5 |
| NH2 | No | 3, 5 | 100 | 450 | 8 | 2 - 7.5 |
| Diol | No | 3, 5 | 100 | 450 | 22 | 2 - 7.5 |
| HILIC | No | 3, 5 | 100 | 450 | 20 | 2 - 7.5 |
| Amide | No | 3, 5 | 100 | 450 | 18 | 2 - 7.5 |
| AX | No | 5 | 100 | 450 | 17 | 2 - 7.5 |
| CX | No | 5 | 100 | 450 | 14 | 2 - 7.5 |

*Inertsil phases are known to provide excellent results at pH levels of 9 – 10. However, optimum column life may be achieved at a pH between 2 and 7.5.

**Inertsil ODS-3V** is a highly efficient validated HPLC column. Each lot of packing undergoes a series of critical QC analytical tests, consistent with the demands of GLP/GMP compliance.

**Inertsil ODS-P** is a polymerically bonded C18 phase, particularly suited to the separation of polycyclic aromatic hydrocarbons and shows additional retentivity for planar compounds.

**Inertsil ODS-EP** is a C18 phase with a polar embedded group, which creates a phase which is stable in 100% aqueous eluents. It provides highly reproducible separations for acids and bases in organic eluents as well as acidified eluents typically used in LC-MS.

**Inertsil ODS-Sprint** is super base deactivated and optimally bonded to retain polar compounds without excessive retention of non-polar compounds. It operates at low back pressures, even at high flow rates, and shows excellent robustness and reproducibility.

**Inertsil Sulfa C18** is designed specifically for the analysis of sulpha drugs. It is also effective in the screening of synthetic antibacterial agents and residues in products of animal origin.

**Inertsil Diol** is a dihydroxypropyl bonded silica which can be used in both reversed-phase and normal-phase modes.

## Inertsil® 3 Series (continued)

**Inertsil® HILIC** is a propyl alcohol bonded silica phase, suitable for the analysis of polar compounds in the HILIC mode. The high organic solvent composition of the eluent leads to high sensitivity LC-MS analyses, due to a decrease in ion suppression. Figure 1 shows a fast and sensitive LC-MS/MS method for the analysis of melamine and cyanuric acid contaminants.

**Inertsil Amide** is a carbamoyl bonded silica phase designed for the separation of highly polar compounds by HILIC.

**Inertsil AX and CX** are silica based anion- and cation-exchange phases respectively. They show good reproducibility, exceptional stability and long column lifetime.

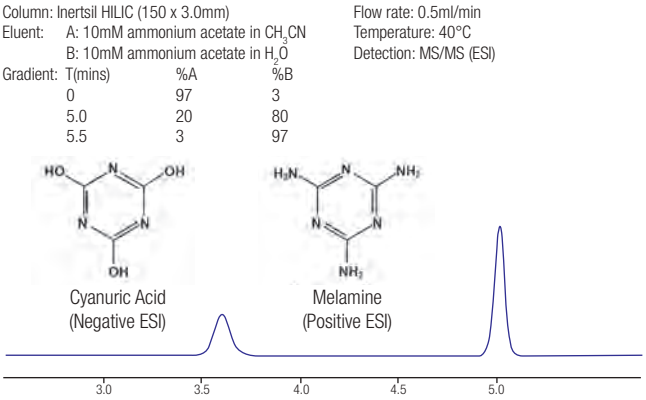



Figure 1. Analysis of melamine and cyanuric acid on Inertsil HILIC

### Ordering Information – Hichrom Manufactured Columns

#### Microbore (2.1mm i.d.) Columns

| Inertsil Phase | Column Dimensions (mm) | | | |
|---|---|---|---|---|
| **3µm** | **50 x 2.1** | **100 x 2.1** | **150 x 2.1** | **250 x 2.1** |
| ODS-3 | INODS3-3-50DM | INODS3-3-100DM | INODS3-3-150DM | - |
| **5µm** | | | | |
| ODS-3 | INODS3-50DM | INODS3-100DM | INODS3-150DM | INODS3-250DM |
| C8-3 | INC83-50DM | INC83-100DM | INC83-150DM | INC83-250DM |
| Ph-3 | INP3-50DM | INP3-100DM | INP3-150DM | INP3-250DM |
| CN-3 | INCN3-50DM | INCN3-100DM | INCN3-150DM | INCN3-250DM |

#### Medium Bore (3.0mm i.d.) Columns

| Inertsil Phase | Column Dimensions (mm) | | | |
|---|---|---|---|---|
| **3µm** | **50 x 3.0** | **100 x 3.0** | **150 x 3.0** | **250 x 3.0** |
| ODS-3 | INODS3-3-50DS | INODS3-3-100DS | INODS3-3-150DS | INODS3-3-250DS |
| **5µm** | | | | |
| ODS-3 | INODS3-50DS | INODS3-100DS | INODS3-150DS | INODS3-250DS |
| C8-3 | INC83-50DS | INC83-100DS | INC83-150DS | INC83-250DS |
| Ph-3 | INP3-50DS | INP3-100DS | INP3-150DS | INP3-250DS |
| CN-3 | INCN3-50DS | INCN3-100DS | INCN3-150DS | INCN3-250DS |

#### Analytical (4.6mm i.d.) Columns

| Inertsil Phase | Column Dimensions (mm) | | | |
|---|---|---|---|---|
| **3µm** | **50 x 4.6** | **100 x 4.6** | **150 x 4.6** | **250 x 4.6** |
| ODS-3 | INODS3-3-50D | INODS3-3-100D | INODS3-3-150D | INODS3-3-250D |
| C8-3 | INC83-3-50D | INC83-3-100D | INC83-3-150D | - |
| Ph-3 | INP3-3-50D | INP3-3-100D | INP3-3-150D | - |
| **5µm** | | | | |
| ODS-3 | INODS3-50D | INODS3-100D | INODS3-150D | INODS3-250D |
| C8-3 | INC83-50D | INC83-100D | INC83-150D | INC83-250D |
| Ph-3 | INP3-50D | INP3-100D | INP3-150D | INP3-250D |
| CN-3 | INCN3-50D | INCN3-100D | INCN3-150D | INCN3-250D |

Please enquire for ordering information for other 3 Series phases as well as 4 Series and InertSustain products.

## Inertsil 2 Series and Inertsil ODS

### Inertsil 2 Series and Inertsil ODS Phases

| Inertsil Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| ODS-2 | Octadecyl | Yes | 5 | 150 | 320 | 18.5 |
| C8 | Octyl | Yes | 5 | 150 | 320 | 10.5 |
| C4 | Butyl | Yes | 5 | 150 | 320 | 7.5 |
| Phenyl | Phenyl | Yes | 5 | 150 | 320 | 10 |
| ODS* | Octadecyl | Yes | 5 | 100 | 350 | 14 |

* From original Inertsil series

The well established Inertsil 2 Series phases are based on high purity silica which is robust and shows high reproducibility from batch-to-batch and column-to-column.

## Inertsil® 2 Series (continued)

### Ordering Information – Hichrom Manufactured Columns

### Microbore (1.0mm i.d.) Columns

| Inertsil Phase 5µm | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 1.0mm i.d. Columns) | |
|---|---|---|---|---|---|---|
| | 50 x 1.0 | 100 x 1.0 | 150 x 1.0 | 250 x 1.0 | | |
| ODS-2 | INODS2-50M | INODS2-100M | INODS2-150M | INODS2-250M | INODS2-10CE5 | (5/pk)[3] |
| C4 | INC4-50M | INC4-100M | INC4-150M | INC4-250M | INC4-10CE5 | (5/pk)[3] |
| Phenyl | INP-50M | INP-100M | INP-150M | INP-250M | INP-10CE5 | (5/pk)[3] |

### Microbore (2.1mm i.d.) Columns

| Inertsil Phase 5µm | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 2.1mm i.d. Columns) | |
|---|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | | |
| ODS-2 | INODS2-50AM | INODS2-100AM | INODS2-150AM | INODS2-250AM | INODS2-10CM5 | (5/pk)[3] |
| C4 | INC4-50AM | INC4-100AM | INC4-150AM | INC4-250AM | INC4-10CM5 | (5/pk)[3] |
| Phenyl | INP-50AM | INP-100AM | INP-150AM | INP-250AM | INP-10CM5 | (5/pk)[3] |

### Medium Bore (3.2mm i.d.) Columns

| Inertsil Phase 5µm | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 3.2mm i.d. Columns) | |
|---|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | | |
| ODS-2 | INODS2-50AS | INODS2-100AS | INODS2-150AS | INODS2-250AS | INODS2-10C5 | (5/pk)[3] |
| C4 | INC4-50AS | INC4-100AS | INC4-150AS | INC4-250AS | INC4-10C5 | (5/pk)[3] |
| Phenyl | INP-50AS | INP-100AS | INP-150AS | INP-250AS | INP-10C5 | (5/pk)[3] |

### Analytical (4.0mm i.d.) Columns

| Inertsil Phase 5µm | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 4.0mm i.d. Columns) | |
|---|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | | |
| ODS-2 | INODS2-50AF | INODS2-100AF | INODS2-150AF | INODS2-250AF | INODS2-10C5 | (5/pk)[3] |
| C4 | INC4-50AF | INC4-100AF | INC4-150AF | INC4-250AF | INC4-10C5 | (5/pk)[3] |
| Phenyl | INP-50AF | INP-100AF | INP-150AF | INP-250AF | INP-10C5 | (5/pk)[3] |

### Analytical (4.6mm i.d.) Columns

| Inertsil Phase 5µm | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 4.6mm i.d. Columns) | |
|---|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | | |
| ODS-2 | INODS2-50A | INODS2-100A | INODS2-150A | INODS2-250A | INODS2-10C5 | (5/pk)[3] |
| C4 | INC4-50A | INC4-100A | INC4-150A | INC4-250A | INC4-10C5 | (5/pk)[3] |
| Phenyl | INP-50A | INP-100A | INP-150A | INP-250A | INP-10C5 | (5/pk)[3] |

[1] Other column dimensions available – please enquire [2] Use with free-standing holder HI-161 and column coupler HI-081 – see p.20 [3] Starter kits also available – see p. 21

### Semi-Preparative and Preparative (7.75 – 21.2mm i.d.) Columns

Please contact Hichrom for further details of 7.75 – 21.2mm i.d. Inertsil columns.

## Titansphere®

Titansphere® consists of porous spherical particles of titanium dioxide, with a smooth and alkaline surface that displays amphoteric ion-exchange properties. Titansphere products are specifically designed for biological applications involving the selective isolation of low level phosphopeptides. Capillary columns, extraction cartridges, bulk material and PhosTiO extraction kits are all available. It is particularly useful in 2D chromatographic separations, where it is used as a precolumn or trap column prior to on-line column switching to a reversed-phase or ion-exchange analytical column.

### Titansphere Phase

| | |
|---|---|
| **Nature of Phase** | Spherical $TiO_2$ |
| **Particle Size (µm)** | 5, 10 |
| **Pore Size (Å)** | 100 |
| **Surface Area ($m^2/g$)** | 100 |
| **pH Range** | 2 - 12 |

Please contact us for ordering information on Titansphere products.

# GRACE®

Grace Materials Technologies / Discovery Sciences manufacture a number of silica based products for analytical, preparative and process scale chromatography. These products are suitable for a wide range of applications, including drug discovery and purification for the pharmaceutical and biotechnology industries, environmental analysis, forensics, petrochemical analysis, food, cosmetics and vitamins.

## Vydac® Columns for Proteins and Peptides

Vydac columns were developed for bioseparations and became the benchmark for the reversed-phase separation of proteins and peptides. Vydac 300Å wide pore phases are available in a wide range of dimensions from nano and capillary, to micro and analytical, to preparative and process scale.

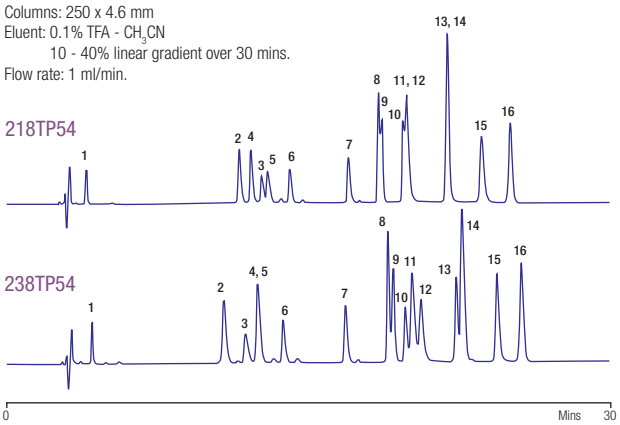
The Vydac TP range of phases was the first generation of wide pore media phases developed in the Vydac range. The large pores of the 300Å TP silica give polypeptide molecules complete access to the interior of the silica pores.

Vydac MS phases were developed from the original Vydac TP phases, by incorporating a surface treatment and proprietary bonding process. The MS product line is recommended for the LC-MS analysis of small peptides to large intact, undenatured proteins. Only low levels of ion-pairing reagents, such as trifluoroacetic acid (TFA) may be required.

Vydac TP and MS phases include:

- **208MS and 208TP** – C8 bonding, ideal for biomolecules of 5 -10,000 Da
- **214MS and 214TP** – C4 bonding, suitable for hydrophobic polypeptides larger than 4-5,000 Da
- **218MS, 218TP, 238MS and 238TP** – C18 bonding, suitable for small polypeptides < 4-5,000 Da
- **219MS and 219TP** – Diphenyl bonding, suitable for polypeptides with aromatic side chains
- **238EV (Everest)** – C18 bonding, monomeric, suitable for hydrophilic and hydrophobic peptides from complex protein digests

Subtle differences in selectivity may be observed between analyses using 218MS or 218TP polymeric bonded and 238MS or 238TP monomeric bonded C18 phases. Figure 1 shows a comparison between the selectivity of Vydac 218TP and 238TP for a mixture of peptides.




1. GY
2. VYV
3. Neurotensin fragment 1-8
4. AcRGGGGLGLGK-$NH_2$
5. RGAGGLGLGK-$NH_2$
6. AcRGAGGLGLGL-$NH_2$
7. AcRGVGGLGLGK-$NH_2$
8. Oxytocin
9. Met-enkephalin
10. Bradykinin
11. AcRGVVGLGLGK-$NH_2$
12. Neurotensin fragment 8-13
13. Angiotensin II
14. Leu-enkephalin
15. Neurotensin
16. Angiotensin I

Figure 1. Separation of synthetic peptides on Vydac 218TP and 238TP phases

In addition to Vydac TP and MS capillary and analytical columns, Vydac TP preparative and process scale columns and bulk material are also available.

## Vydac 201TP and 202TP

Vydac 201TP and 202TP columns were developed specifically for the separation and quantification of polyaromatic hydrocarbons (PAHs). Vydac 201TP columns also have application in the analysis of carotenoids, retinoids and vitamins.

## VisionHT™

The VisionHT™ media platform has the mechanical strength required for ultra-high pressure use and the flexibility to expand seamlessly to larger particle sizes and multiple phase chemistries. The identical base silica is used for bonding 1.5, 3, 5 and 10µm particles. Method transfer between UHPLC, traditional and preparative HPLC systems is simple and quick. For 1.5µm phases, the exceptionally rigid silica structure withstands routine use at pressures of 16,000psi. Six VisionHT high purity phases are available, each with unique separation benefits. These include C18 High Load, C18 Basic, C18 Classic, C18 Polar, HILIC and Silica.

## Genesis®

Genesis® phases are based on high purity metal-free spherical silica. They are suitable for the analysis of a wide range of analytes. Columns are supplied in conventional column hardware. Available phases include: Silica, C18, C8, C8e/c, AQ, Phenyl, CN and NH2.


## GraceSmart™

GraceSmart™ C18 is based on high purity silica which is monomerically bonded with uniform coverage. This phase is ideal for general use.

Please contact us for ordering information on Grace products.

# HALO® FUSED-CORE®



HALO® UHPLC and HPLC columns were developed by Advanced Materials Technology using innovative Fused-Core® particle technology, whereby a porous silica layer is 'fused' onto solid spherical silica particles, producing 'superficially porous' or 'core-shell' particles. HALO 2.7µm particle phases for UHPLC applications were the first to be developed using this technology. The success of HALO UHPLC columns led to the development of HALO-5 HPLC columns packed with 4.6µm Fused-Core particles. These are well suited for use with legacy HPLC instruments.

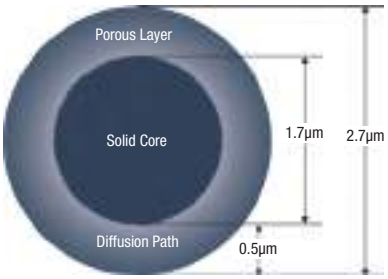
## HALO UHPLC Phases

The HALO 2.7µm UHPLC phases comprise a 1.7µm solid core and a 0.5µm porous outer shell (see Figure 1). HALO columns provide the efficiency and separation speed of sub 2µm particles but at approximately half the back pressure.

## HALO-5 HPLC Phases

HALO-5 phases are based on the same Fused-Core technology as the original 2.7µm phases. They consist of solid core particles having a 0.6µm porous silica layer fused to the surface. HALO-5 columns show extremely high plate numbers compared with conventional porous 5µm phases and the equivalent or higher plate numbers compared with conventional 3µm phases, but with half the pressure.

Figure 1. Fused-Core particle technology




## HALO Protein and HALO Peptide Phases

HALO Protein and Peptide UHPLC columns are specifically designed for fast, high resolution separations of proteins and peptides. HALO Protein C4 is based on a 3.4µm particle with a thin (0.2µm) porous silica shell having pores of 400Å. It is optimum for the separation of larger proteins up to 500kDa. HALO Peptide columns (160Å) are recommended for peptides in the range of 3 to 20kDa.

## HALO and HALO-5 Phases

| HALO and HALO-5 Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | pH Range |
|---|---|---|---|---|---|---|
| C18 | 2.7[1], 4.6[2] | 90 | 135, 90 | 7.7, 5.5 | Yes | 2 - 9 |
| C8 | 2.7[1], 4.6[2] | 90 | 135, 90 | 5.4, 3.7 | Yes | 2 - 9 |
| HILIC (Silica) | 2.7[1], 4.6[2] | 90 | 135, 90 | - | No | 1 - 8 |
| RP-Amide | 2.7[1] | 90 | 135 | 8.2 | Yes | 2 - 9 |
| Phenyl-Hexyl | 2.7[1], 4.6[2] | 90 | 135, 90 | 7.1, 5.2 | Yes | 2 - 9 |
| PFP | 2.7[1], 4.6[2] | 90 | 135, 90 | 5.5, 3.9 | Yes | 2 - 8 |
| Penta-HILIC | 2.7[1], 4.6[2] | 90 | 135, 90 | 3.2, 2.2 | No | 2 - 9 |
| ES-CN | 2.7[1], 4.6[2] | 90 | 135, 90 | 3.5, 2.5 | Yes | 2 - 9 |
| Peptide ES-C18 | 2.7[1], 4.6[2] | 160 | 80, 60 | 4.6, 4.0 | No | 1 - 8 |
| Peptide ES-CN | 2.7[1], 4.6[2] | 160 | 80, 60 | 2.0, 1.5 | Yes | 1 - 8 |
| Protein (C4) | 3.4[3] | 400 | 15 | 0.4 | Yes | 2 - 9 |

[1] 1.7µm solid core particle with 0.5µm porous silica layer fused to surface
[2] 3.4µm solid core particle with 0.6µm porous silica layer fused to surface
[3] 3.0µm solid core particle with 0.2µm porous silica layer fused to surface

# Advantages of HALO UHPLC and HPLC Columns

## More Separating Power

HPLC and UHPLC columns packed with HALO particles deliver significantly more separating power compared to columns packed with traditional totally porous particles of the same size.

## Hyper-fast Separations

The high efficiency of HALO Fused-Core particles permits the use of shorter columns to reduce analysis time without sacrificing resolution. In addition, HALO columns are designed to excel when operated at high eluent velocity. Therefore, short HALO columns operating at high eluent velocity can be used to achieve hyper-fast, high resolution separations.


## Higher Efficiency at Lower Back Pressure

Columns packed with HALO particles deliver approximately 50% higher efficiency than typically expected based on particle size, but the back pressure is as expected for particle size. This means that HALO columns can generate the same separating power as sub 2µm totally porous particles, but at reduced back pressures. This lower pressure also enables many HALO UHPLC columns to be used on HPLC instruments, simplifying the transfer of methods from UHPLC to HPLC instruments.

## Super-rugged Separations

The narrow particle size distribution of HALO particles enables rugged and reliable columns to be packed. It also enables the use of larger porosity inlet frits than required with other HPLC and UHPLC columns, thereby making them less prone to plugging from particulates.

Please contact us for ordering information on HALO Fused-Core products.

# HICHROM

- Capillary to preparative scale dimensions
- ISO 9001:2008 (Quality) and ISO 14001:2004 (Environmental) standards
- Every column exceeds stringent quality test criteria
- Exceptional reproducibility and efficiency
- See also Partisil/Partisphere and Ultrasphere sections

Hichrom has been firmly established in the field of chromatography for over 30 years, specialising in the development, manufacture and supply of high quality HPLC products, both in the UK and throughout the entire world. During this time Hichrom has built up a strong reputation amongst chromatographers for technical expertise, speed of delivery and competitive pricing. Hichrom is accredited to both ISO 9001:2008 (Quality) and ISO 14001:2004 (Environmental) standards. Every Hichrom manufactured column exceeds stringent quality criteria and all columns are supplied with documentation enabling a complete audit trail from the time of manufacture to the point of use. In addition to the following range of Hichrom branded columns (pages 92-100) Hichrom offer a wide range of HPLC columns packed with any commercially available silica, an OEM manufacturing and custom bonding service and also manufacture the Partisil and Partisphere (see pages 121-125) and the Ultrasphere (see pages 156-157) brands.

## Hichrom C8 and C18

- Ultra pure, base deactivated porous silica
- Suitable for acidic, basic and neutral molecules
- 3.5 and 5µm particle sizes
- Excellent reproducibility

Hichrom's C8 and C18 columns offer high performance in order to tackle the most challenging reversed-phase applications. The use of ultra pure silica, advanced bonding technology, superior column specification and comprehensive batch validation all contribute to the columns' excellent reproducibility.

### Hichrom Phases

| Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| C18 | Octadecyl | Yes | 3.5, 5 | 150 | 250 | 15 |
| C8 | Octyl | Yes | 3.5, 5 | 150 | 250 | 8 |

### Excellent Batch Reproducibility

Combining ultra pure silica with advanced bonding technology results in a densely bonded silica that is both highly robust and highly reproducible. Unpredictable interactions between residual silanol sites on the silica surface and the analyte are effectively eliminated. Comprehensive batch validation ensures absolute batch to batch reproducibility is maintained with acidic, basic and neutral molecules. Figures 1 and 2 show two of the tests used during the validation of Hichrom C8 and C18 phases.

Test Conditions
Column: Hichrom 5 C18 (250 x 4.6 mm)
Eluent: $CH_3CN$ - 0.05M $KH_2PO_4$, pH 3.2 (60:40)
Flow rate: 1 ml/min
Temperature: Ambient

Test Components
1. Uracil
2. Pyridine
3. Phenol
4. Dimethyl phthalate
5. N,N-Dimethylaniline
6. 4-Butylbenzoic acid
7. Toluene

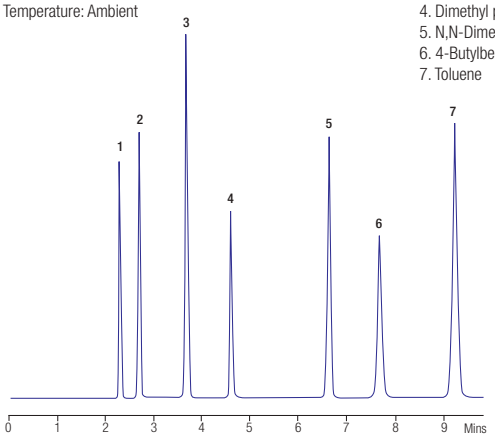



Figure 1. Validation test - Selectivity with acidic, basic and neutral molecules

Test Conditions
Column: Hichrom 5 C18 (250 x 4.6 mm)
Eluent: $CH_3OH$ - 0.025M $KH_2PO_4$, pH 6.0 (80:20)
Flow rate: 1 ml/min
Temperature: Ambient

Test Components
1. Norephedrine
2. Nortriptyline
3. Toluene
4. Imipramine
5. Amitriptyline

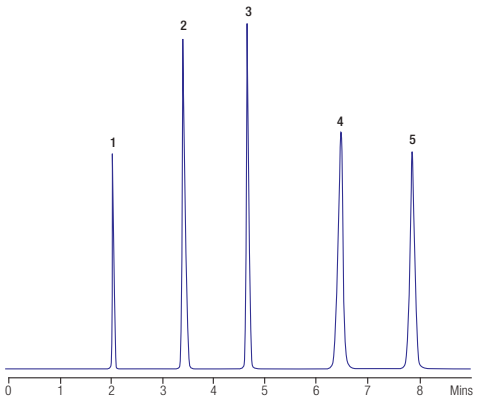



Figure 2. Validation test - Tricyclic antidepressants

## Hichrom C8 and C18 (continued)

### Excellent Batch Reproducibility (continued)

Figure 3 demonstrates the excellent selectivity match obtained with four Hichrom 5 C18 columns when subjected to the tricyclic antidepressants validation test. Each of these four columns is packed from a different batch of silica.

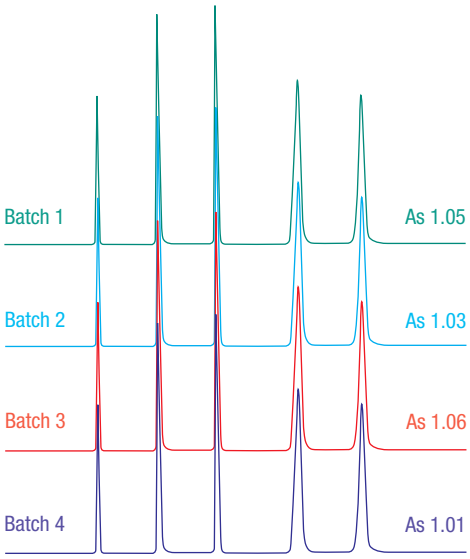




Figure 3. Batch reproducibilty – Tricyclic antidepressants

### Excellent Column Efficiency

- 3.5µm columns > 150,000 plates/metre
- 5µm columns > 90,000 plates/metre

Careful control of all stages during the manufacturing process results in a high purity silica with a tight particle size distribution. This results in excellent column efficiencies with both 3.5 and 5µm materials.


Every Hichrom C8 and C18 column is individually manufactured and meticulously tested in the Hichrom laboratory. Efficiency measurements and two peak asymmetry calculations are recorded for each component of the quality control test mixture. Samples of the quality control test mixture are available on request. Only columns exceeding the most stringent efficiency and peak shape specifications are approved for sale.

All Hichrom C8 and C18 columns are supplied with a Test Chromatogram and Batch Validation Certificate. Information regarding column care and use is displayed on the reverse of the Test Chromatogram.

Figure 4 shows the analysis of toluene using twenty Hichrom 5 C18 columns under standard quality control conditions. Excellent efficiency, peak asymmetry and column-to-column reproducibility are demonstrated.

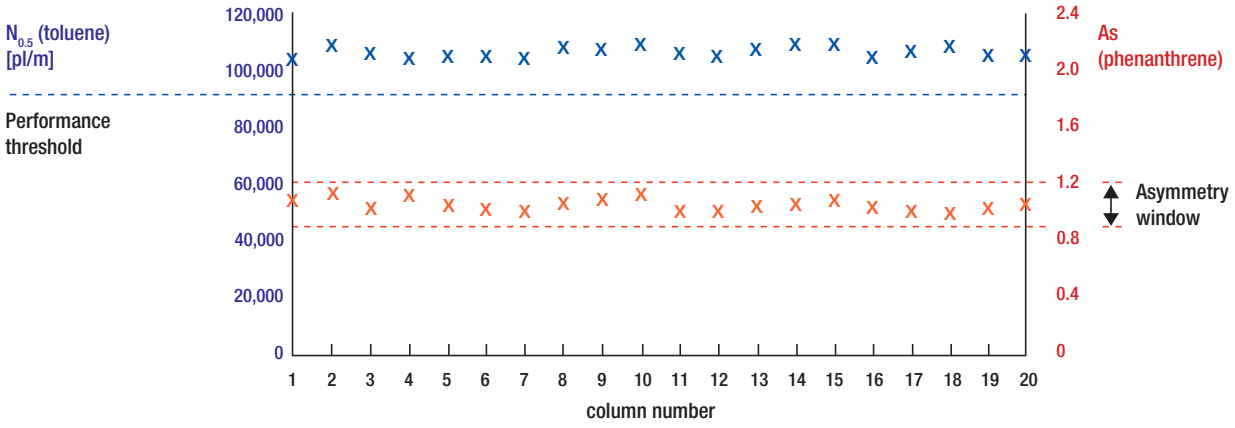



Figure 4. Excellent Hichrom 5 C18 column-to-column reproducibility

## Hichrom C8 and C18 (continued)

### Extended Column Lifetime

Hichrom C8 and C18 columns exhibit extended lifetimes – the result of intensive research and development.

### Bonding Stability

Combining a unique dense bonding process (inhibiting ligand cleavage at low pH) with a revolutionary capping process (shielding the silica from dissolution at high pH) enables Hichrom C8 and C18 columns to offer exceptional stability across an extended pH range. Figure 5 demonstrates the acid robustness of a Hichrom 5 C18 column.

### Column Robustness

A stable packed silica bed is critical to the long term performance of the column. Hichrom C8 and C18 columns are packed to extremely high efficiencies with excellent peak asymmetries, ensuring that high performance is maintained throughout the column's lifetime. Figure 6 shows that the performance of both Hichrom 3.5µm C18 and Hichrom 5µm C18 columns remains unaffected after extended use.

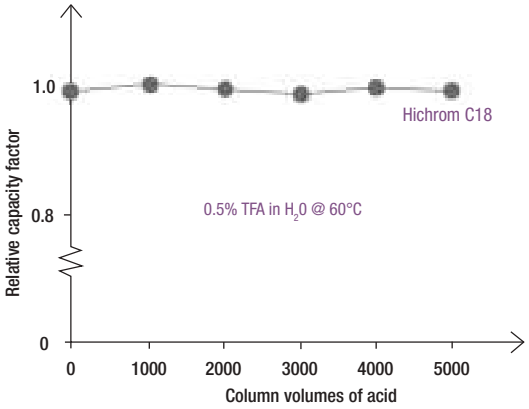



Figure 5. Acid robustness testing

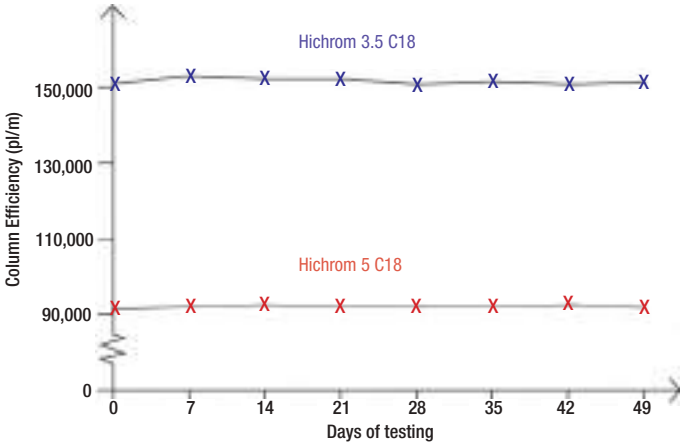



Figure 6. Extended column testing

### Guard Cartridges

Guard cartridges are available for all Hichrom C8 and C18 column dimensions, to further extend column lifetime by preventing irreversible adsorption and frit blockage at the top of the column. As shown in Figure 7, a fingertight column coupler (HI-081) is used to connect the guard holder (HI-161) to a Hichrom 5 C18 column. Further information on the guard cartridge range is listed on pages 20 and 21.

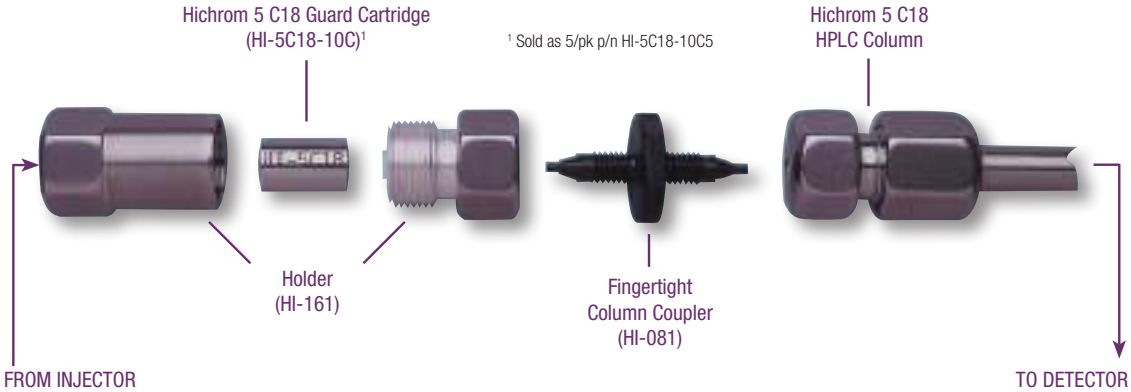



Figure 7. Stand alone guard cartridge system

## Ordering Information - Hichrom C8 and C18

### Microbore (1.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 1.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 1.0 | 100 x 1.0 | 150 x 1.0 | 250 x 1.0 | |
| **3.5µm** | | | | | |
| C8 | HI-3.5C8-50M | HI-3.5C8-100M | HI-3.5C8-150M | - | HI-3.5C8-10CE5 |
| C18 | HI-3.5C18-50M | HI-3.5C18-100M | HI-3.5C18-150M | - | HI-3.5C18-10CE5 |
| **5µm** | | | | | |
| C8 | HI-5C8-50M | HI-5C8-100M | HI-5C8-150M | HI-5C8-250M | HI-5C8-10CE5 |
| C18 | HI-5C18-50M | HI-5C18-100M | HI-5C18-150M | HI-5C18-250M | HI-5C18-10CE5 |

### Microbore (2.1mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| **3.5µm** | | | | | |
| C8 | HI-3.5C8-50AM | HI-3.5C8-100AM | HI-3.5C8-150AM | - | HI-3.5C8-10CM5 |
| C18 | HI-3.5C18-50AM | HI-3.5C18-100AM | HI-3.5C18-150AM | - | HI-3.5C18-10CM5 |
| **5µm** | | | | | |
| C8 | HI-5C8-50AM | HI-5C8-100AM | HI-5C8-150AM | HI-5C8-250AM | HI-5C8-10CM5 |
| C18 | HI-5C18-50AM | HI-5C18-100AM | HI-5C18-150AM | HI-5C18-250AM | HI-5C18-10CM5 |

### Medium Bore (3.2mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **3.5µm** | | | | | |
| C8 | HI-3.5C8-50AS | HI-3.5C8-100AS | HI-3.5C8-150AS | - | HI-3.5C8-10C5 |
| C18 | HI-3.5C18-50AS | HI-3.5C18-100AS | HI-3.5C18-150AS | - | HI-3.5C18-10C5 |
| **5µm** | | | | | |
| C8 | HI-5C8-50AS | HI-5C8-100AS | HI-5C8-150AS | HI-5C8-250AS | HI-5C8-10C5 |
| C18 | HI-5C18-50AS | HI-5C18-100AS | HI-5C18-150AS | HI-5C18-250AS | HI-5C18-10C5 |

### Analytical (4.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| **3.5µm** | | | | | |
| C8 | HI-3.5C8-50AF | HI-3.5C8-100AF | HI-3.5C8-150AF | - | HI-3.5C8-10C5 |
| C18 | HI-3.5C18-50AF | HI-3.5C18-100AF | HI-3.5C18-150AF | - | HI-3.5C18-10C5 |
| **5µm** | | | | | |
| C8 | HI-5C8-50AF | HI-5C8-100AF | HI-5C8-150AF | HI-5C8-250AF | HI-5C8-10C5 |
| C18 | HI-5C18-50AF | HI-5C18-100AF | HI-5C18-150AF | HI-5C18-250AF | HI-5C18-10C5 |

### Analytical (4.6mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **3.5µm** | | | | | |
| C8 | HI-3.5C8-50A | HI-3.5C8-100A | HI-3.5C8-150A | - | HI-3.5C8-10C5 |
| C18 | HI-3.5C18-50A | HI-3.5C18-100A | HI-3.5C18-150A | - | HI-3.5C18-10C5 |
| **5µm** | | | | | |
| C8 | HI-5C8-50A | HI-5C8-100A | HI-5C8-150A | HI-5C8-250A | HI-5C8-10C5 |
| C18 | HI-5C18-50A | HI-5C18-100A | HI-5C18-150A | HI-5C18-250A | HI-5C18-10C5 |

### Semi-Preparative (7.75mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 7.75mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 7.75 | 100 x 7.75 | 150 x 7.75 | 250 x 7.75 | |
| **5µm** | | | | | |
| C8 | HI-5C8-50SP | HI-5C8-100SP | HI-5C8-150SP | HI-5C8-250SP | HI-5C8-10CP3 |
| C18 | HI-5C18-50SP | HI-5C18-100SP | HI-5C18-150SP | HI-5C18-250SP | HI-5C18-10CP3 |

[1] Other column dimensions available – please enquire
[2] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20
[3] Starter kits available – see p. 21
[4] 3/pk – Use with free-standing holder HI-150 and column coupler HI-081 – see p. 20

## Ordering Information - Hichrom C8 and C18 (continued)

### Semi-Preparative (10.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 10.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 10.0 | 100 x 10.0 | 150 x 10.0 | 250 x 10.0 | |
| **5µm** | | | | | |
| C8 | HI-5C8-50SP1 | HI-5C8-100SP1 | HI-5C8-150SP1 | HI-5C8-250SP1 | HI-5C8-10CP3 |
| C18 | HI-5C18-50SP1 | HI-5C18-100SP1 | HI-5C18-150SP1 | HI-5C18-250SP1 | HI-5C18-10CP3 |

### Preparative (21.2mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2] (For 21.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 21.2 | 100 x 21.2 | 150 x 21.2 | 250 x 21.2 | |
| **5µm** | | | | | |
| C8 | HI-5C8-50P | HI-5C8-100P | HI-5C8-150P | HI-5C8-250P | HI-5C8-10CP3 |
| C18 | HI-5C18-50P | HI-5C18-100P | HI-5C18-150P | HI-5C18-250P | HI-5C18-10CP3 |

### Preparative (30.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[3] (For 30.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 30.0 | 100 x 30.0 | 150 x 30.0 | 250 x 30.0 | |
| **5µm** | | | | | |
| C8 | HI-5C8-50P3 | HI-5C8-100P3 | HI-5C8-150P3 | HI-5C8-250P3 | HI-5C8-20CP |
| C18 | HI-5C18-50P3 | HI-5C18-100P3 | HI-5C18-150P3 | HI-5C18-250P3 | HI-5C18-20CP |

[1] Other column dimensions available – please enquire
[2] 3/pk – Use with free-standing holder HI-150 and column coupler HI-081
[3] 1/pk – Use with free-standing holder HI-183 and column coupler HI-083

## Hichrom RPB

- High purity base deactivated silica
- 3.5, 5 and 10µm particle sizes
- Unique C8/C18 multi-alkyl phase
- Excellent chromatography

Hichrom RPB is a high purity base deactivated silica which offers a unique selectivity due to the proprietary C8/C18 multi-alkyl bonding and exhaustive endcapping employed. The range includes 3.5, 5 and 10µm phases, which are suitable for LC-MS applications and preparative separations respectively.

| Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| RPB | C8/C18 multi-alkyl | Yes | 3.5, 5, 10 | 110 | 340 | 14 |

Hichrom RPB is specifically designed for the **R**eversed-**P**hase separation of **B**asic molecules. The use of organic modifiers is minimised.

Residual acidic sites have been eliminated whilst monolayer coverage of the high purity silica surface has been retained to obtain optimum plate efficiency. Both octyl- and octadecylsilanes are bonded to give a material which combines the robustness of a C18 phase with the high coverage of a C8 phase.

Excellent peak shapes are obtained for basic drug molecules. In addition, acids and bases can be similarly chromatographed under suitably buffered conditions (see Figure 1).

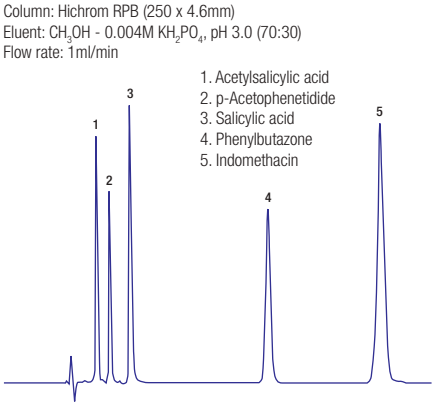



Figure 1. Chromatography of acidic and basic molecules

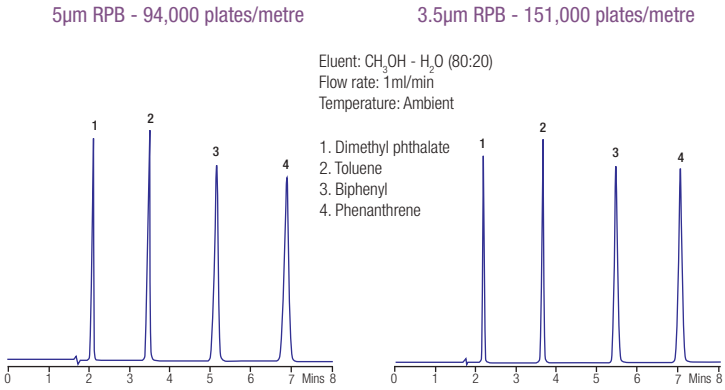



Figure 2. Reduce particle size to increase column efficiency

### High Efficiency Columns:

- 3.5µm > 145,000 plates/metre
- 5µm > 90,000 plates/metre
- 10µm > 50,000 plates/metre

Hichrom 3.5µm RPB allows the chromatographer to decrease column length without sacrificing efficiency. This results in increased productivity due to reduced analysis time. Figure 2 compares the efficiency obtained under standard quality control conditions for 150 x 4.6mm i.d. Hichrom 5µm RPB and Hichrom 3.5µm RPB columns.

### Ordering Information - Hichrom RPB
### Microbore (1.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 1.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 1.0 | 100 x 1.0 | 150 x 1.0 | 250 x 1.0 | |
| 3.5µm RPB | HI-3.5RPB-50M | HI-3.5RPB-100M | HI-3.5RPB-150M | - | HI-3.5RPB-10CE5 |
| 5µm RPB | HIRPB-50M | HIRPB-100M | HIRPB-150M | HIRPB-250M | HIRPB-10CE5 |

[1] Other column dimensions available – please enquire [2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] 5/pk – Starter kits available – see p. 21

## Ordering Information - Hichrom RPB (continued)

### Microbore (2.1mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| 3.5µm RPB | HI-3.5RPB-50AM | HI-3.5RPB-100AM | HI-3.5RPB-150AM | - | HI-3.5RPB-10CM5 |
| 5µm RPB | HIRPB-50AM | HIRPB-100AM | HIRPB-150AM | HIRPB-250AM | HIRPB-10CM5 |

### Medium Bore (3.2mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| 3.5µm RPB | HI-3.5RPB-50AS | HI-3.5RPB-100AS | HI-3.5RPB-150AS | - | HI-3.5RPB-10C5 |
| 5µm RPB | HIRPB-50AS | HIRPB-100AS | HIRPB-150AS | HIRPB-250AS | HIRPB-10C5 |

### Analytical (4.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 4.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| 3.5µm RPB | HI-3.5RPB-50AF | HI-3.5RPB-100AF | HI-3.5RPB-150AF | - | HI-3.5RPB-10C5 |
| 5µm RPB | HIRPB-50AF | HIRPB-100AF | HIRPB-150AF | HIRPB-250AF | HIRPB-10C5 |

### Analytical (4.6mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| 3.5µm RPB | HI-3.5RPB-50A | HI-3.5RPB-100A | HI-3.5RPB-150A | - | HI-3.5RPB-10C5 |
| 5µm RPB | HIRPB-50A | HIRPB-100A | HIRPB-150A | HIRPB-250A | HIRPB-10C5 |
| 10µm RPB | HI-10RPB-50A | HI-10RPB-100A | HI-10RPB-150A | HI-10RPB-250A | HI-10RPB-10C5 |

### Semi-Preparative (7.75mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 7.75mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 7.75 | 100 x 7.75 | 150 x 7.75 | 250 x 7.75 | |
| 5µm RPB | HIRPB-50SP | HIRPB-100SP | HIRPB-150SP | HIRPB-250SP | HIRPB-10CP3 |
| 10µm RPB | HI-10RPB-50SP | HI-10RPB-100SP | HI-10RPB-150SP | HI-10RPB-250SP | HI-10RPB-10CP3 |

### Semi-Preparative (10.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 10.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 10.0 | 100 x 10.0 | 150 x 10.0 | 250 x 10.0 | |
| 5µm RPB | HIRPB-50SP1 | HIRPB-100SP1 | HIRPB-150SP1 | HIRPB-250SP1 | HIRPB-10CP3 |
| 10µm RPB | HI-10RPB-50SP1 | HI-10RPB-100SP1 | HI-10RPB-150SP1 | HI-10RPB-250SP1 | HI-10RPB-10CP3 |

### Preparative (21.2mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 21.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 21.2 | 100 x 21.2 | 150 x 21.2 | 250 x 21.2 | |
| 5µm RPB | HIRPB-50P | HIRPB-100P | HIRPB-150P | HIRPB-250P | HIRPB-10CP3 |
| 10µm RPB | HI-10RPB-50P | HI-10RPB-100P | HI-10RPB-150P | HI-10RPB-250P | HI-10RPB-10CP3 |

### Preparative (30.0mm i.d.) Columns

| Hichrom Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[5] (For 30.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 30.0 | 100 x 30.0 | 150 x 30.0 | 250 x 30.0 | |
| 5µm RPB | HIRPB-50P3 | HIRPB-100P3 | HIRPB-150P3 | HIRPB-250P3 | HIRPB-20CP[6] |
| 10µm RPB | HI-10RPB-50P3 | HI-10RPB-100P3 | HI-10RPB-150P3 | HI-10RPB-250P3 | HI-10RPB-20CP[6] |

[1] Other column dimensions available – please enquire
[2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20
[3] 5/pk – Starter kits available – see p. 21
[4] 3/pk – Use with free-standing holder HI-150 and column coupler HI-081
[5] 1/pk – Use with free-standing holder HI-183 and column coupler HI-083
[6] A newer improved holder HI-655 for use with new guards HIRPB-10CB3 or HI-10RPB-10CB3 is now available

## Hichrom PAH2

- Optimised for EPA Method 610
- Excellent separation of 16 PAHs
- Wide range of column dimensions

### Hichrom PAH2 Phase

| | |
|---|---|
| **Particle Size (µm)** | 5 |
| **Pore Size (Å)** | 120 |
| **Endcapped** | Yes |

Polynuclear aromatic hydrocarbons (PAHs) are important environmental pollutants due to their ubiquitous presence and their carcinogenicity. They are produced by the incomplete combustion and pyrolysis of fossil fuels and other organic material eg. coal-tar pitch. These compounds are routinely analysed in industrial waste water, drinking water and ground water, solid waste, air, particulate matter and food samples.

Due to government legislation, environmental laboratories are required to perform an increasing number of PAH analyses, from a variety of matrices. Many countries have their own government legislation. In England and Wales, all water intended for human consumption must be screened for five indicator PAHs (compounds 11, 12, 13, 15 and 16 shown below). The US Environmental Protection Agency (EPA) has designated 16 priority pollutant PAHs that are indicative of PAH contamination or pollution. Methods based on these compounds, such as EPA 610 for PAH analysis in waste water and EPA 550 (drinking water), are universally accepted.

Hichrom PAH2 is based on an alkyl bonded silica material with a high carbon loading, designed specifically for the analysis of PAHs. Figure 1 shows the separation of 16 priority pollutants in just over 30 minutes.

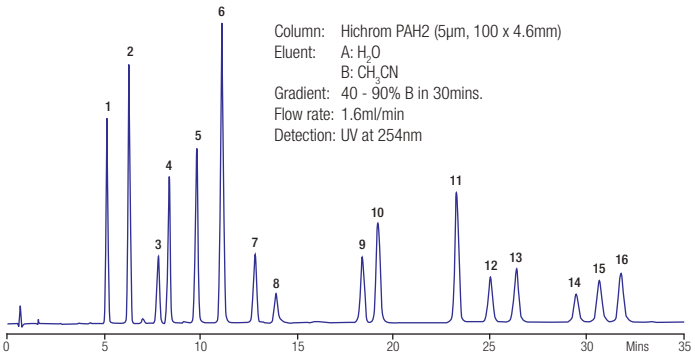
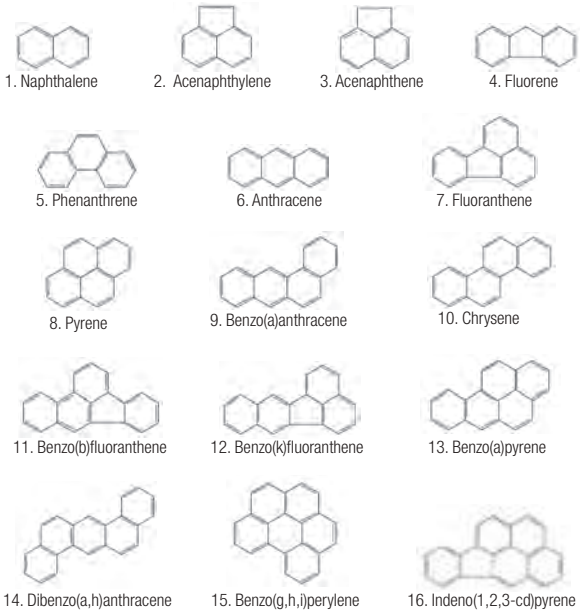



Figure 1. Separation of 16 EPA PAHs on Hichrom PAH2




### Ordering Information – Hichrom PAH2

| Column i.d. | Column Length[1] (mm) | | | Guard Cartridges[2,3] |
|---|---|---|---|---|
| | 100 | 150 | 250 | |
| 2.1 | HI-5PAH2-100AM | HI-5PAH2-150AM | HI-5PAH2-250AM | HI-5PAH2-10CM5 |
| 3.2 | HI-5PAH2-100AS | HI-5PAH2-150AS | HI-5PAH2-250AS | HI-5PAH2-10C5 |
| 4.6 | HI-5PAH2-100A | HI-5PAH2-150A | HI-5PAH2-250A | HI-5PAH2-10C5 |

[1] Other column dimensions available – please enquire [2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] 5/pk – Starter kits also available – see p. 21

## HICHROM CHIRAL COLUMNS

- Chiral 'Pirkle-type'
- Unique covalent bonding
- Enantiomer elution order inversion
- Competitively priced
- Hichrom high efficiency

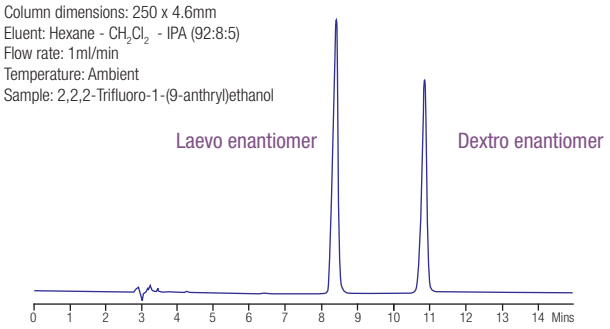
Hichrom manufactures two ranges of 'Pirkle-type' columns for the analysis of a wide range of enantiomers of pharmaceutical or agrochemical origin. CHIRA-chrom 1 type columns contain a stationary phase in which the hydroxyl groups on the silica surface have been modified by a nitrobenzoyl derivative of an optically active amino acid. In the CHIRA-chrom 2 type columns a chiral dinitrophenyltartramide moiety is bonded to the silica surface through a propyl spacer group.

### Pirkle Chiral Phases

| Phase | Type | Endcapped | Particle Size (µm) |
|---|---|---|---|
| D-Phenylglycine | CHIRA-chrom-1 | No | 5 |
| L-Phenylglycine | CHIRA-chrom-1 | No | 5 |
| DL-Phenylglycine | CHIRA-chrom-1 | No | 5 |
| L-Leucine | CHIRA-chrom-1 | No | 5 |
| Dinitrophenyltartramide | CHIRA-chrom-2 | No | 5 |

### Inversion of enantiomer elution order

By substituting L-phenylglycine for D-phenylglycine, the order of elution of the chiral peaks can be reversed. Such a procedure can be useful in assigning peak identity or ensuring prior elution of the minor enantiomer, thus allowing its more accurate determination. The availability of the DL-phenylglycine further aids peak assignment by removing chiral separations whilst maintaining background peak retention profile.




D-Phenylglycine column evaluation using 2,2,2-Trifluoro-1-(9-anthryl)ethanol

### Ordering Information

Please contact Hichrom for details of additional column dimensions not listed.

| Chiral Phase | Column Dimensions (mm) | | | Guard Cartridge[1,2] (For 3.2-4.6mm i.d. Columns) | Guard Cartridge[3] (For 7.75mm i.d. Columns) |
|---|---|---|---|---|---|
| | 250 x 3.2 | 250 x 4.6 | 250 x 7.75 | | |
| D-Phenylglycine | CHI-D-PGC-250AS | CHI-D-PGC-250A | CHI-D-PGC-250SP | CHI-D-PGC-10C5 | CHI-D-PGC-10CP3 |
| L-Phenylglycine | CHI-L-PGC-250AS | CHI-L-PGC-250A | CHI-L-PGC-250SP | CHI-L-PGC-10C5 | CHI-L-PGC-10CP3 |
| DL-Phenylglycine | CHI-DL-PGC-250AS | CHI-DL-PGC-250A | CHI-DL-PGC-250SP | CHI-DL-PGC-10C5 | CHI-DL-PGC-10CP3 |
| L-Leucine | CHI-L-LEUC-250AS | CHI-L-LEUC-250A | CHI-L-LEUC-250SP | CHI-L-LEU-10C5 | CHI-L-LEU-10CP3 |
| Phenyltartramide | CHI-TA-250AS | CHI-TA-250A | CHI-TA-250SP | CHI-TA-10C5 | CHI-TA-10CP3 |

[1] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20
[2] Starter kits also available – see p. 21
[3] 3/pk – Use with free-standing holder HI-150 and column coupler HI-081

# L-COLUMN



- C18 and C8 phases
- Super endcapped
- ODS-V validated phase
- L-column2 phases with improved endcapping

The L-column and L-column2 series of columns are manufactured by CERI (Chemicals Evaluation and Research Institute, Japan).

| Series | Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped |
|---|---|---|---|---|---|---|
| L-column2[1] | L-column2 ODS | 2, 3, 5 | 120 | 340 | 17 | Yes |
| | L-column2 C8 | 5 | 120 | 340 | 10 | Yes |
| L-column | ODS | 3, 5 | 120 | 340 | 17 | Yes |
| | ODS-V | 5 | 120 | 340 | 17 | Yes |
| | C8 | 5 | 120 | 340 | 10 | Yes |

[1] L-column2 C6-Phenyl also available – please enquire

L-column packing materials are endcapped by high temperature gaseous phase silylation (super endcapping; US Patent No. 5,134,110, Japan Patent No. 2611545), which allows a nearly complete deactivation of residual silanols. As a result, L-columns show no peak tailing of basic compounds and high durability (pH range of C18 is 2 - 9, pH range of C8 is 2 – 7.5).

## L-column ODS-V

L-column ODS-V has been developed for complying with method validation required by GLP/GMP. Batch-to-batch and column-to-column reproducibility are controlled by strict quality control of manufacturing and testing procedures.

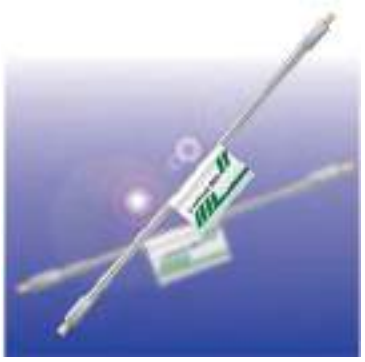
## L-column2 ODS and C8

L-column2 ODS and C8 columns form part of a newer generation of columns, showing higher performance than their L-column counterparts, due to advanced endcapping methodology. They show sharper peaks for acidic, basic and chelating compounds.

## Ordering Information

| Phase | Column Dimensions[1,2] (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 |
| **3µm** | | | | | | | | |
| L-column2 ODS | 711140 | 711170 | 711020 | 711220 | 721150 | 721180 | 721070 | 721080 |
| L-column ODS | 611140 | 611170 | 611020 | 611220 | 621150 | 621180 | 621070 | 621080 |
| **5µm** | | | | | | | | |
| L-column2 ODS | 712140 | 712170 | 712020 | 712220 | 722150 | 722180 | 722070 | 722080 |
| L-column2 C8 | 712141 | 712171 | 712021 | 712221 | 722151 | 722181 | 722071 | 722081 |
| L-column ODS | 612140 | 612170 | 612020 | 612220 | 622150 | 622180 | 622070 | 622080 |
| L-column C8 | 612141 | 612171 | 612021 | 612221 | 622151 | 622181 | 622071 | 622081 |
| L-column ODS-V | - | - | - | - | - | - | 622078 | 622088 |

[1] Other dimensions available, including capillary and preparative [2] Guard cartridges available



# MACHEREY-NAGEL

Macherey-Nagel GmbH & Co. KG manufactures a wide range of HPLC products for different techniques and applications. NUCLEOSIL® was one of the first commercially available spherical silicas, but is still used in many analytical and preparative applications. NUCLEODUR® is Macherey-Nagel's newer range of HPLC and UHPLC phases and NUCLEOSHELL® is a range of phases based on core shell (superficially porous) technology. Other Macherey-Nagel LC products include NUCLEOGEN® columns for bioanalysis, NUCLEOGEL® columns for food analysis and several types of chiral phases. Please see later sections or contact us for other Macherey-Nagel products including GC, SPE, TLC and Flash.

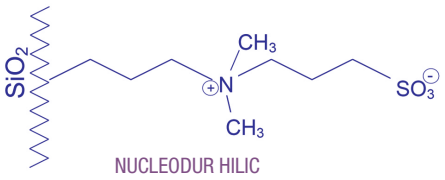
## NUCLEOSIL®

NUCLEOSIL is a classical silica, available in a wide range of pore sizes. Please see page 103 for specifications of available phases and ordering information for Hichrom packed NUCLEOSIL columns.

## NUCLEODUR®

The NUCLEODUR range of UHPLC and HPLC phases is based on state-of-the-art silica, offering outstanding particle surface smoothness, high pressure stability and low metal content. Phases have a pore size of 110Å, surface area of 340m²/g and are available with particle sizes of 1.8, 3 and 5µm. All phases show excellent mechanical and chemical stability and are LC-MS compatible. Phases include:

- **NUCLEODUR C18 Isis** – C18 with special cross-linked surface modification, leading to high steric selectivity
- **NUCLEODUR Sphinx RP** – a bifunctional phase consisting of C18 and propylphenyl groups
- **NUCLEODUR C18 Pyramid** – C18 with hydrophilic endcapping
- **NUCLEODUR PolarTec** – a C18 phase with an embedded polar group
- **NUCLEODUR Phenyl-Hexyl** – suitable for aromatic, unsaturated and polar compounds
- **NUCLEODUR HILIC** – a zwitterionic modified phase with ammonium-sulphonic acid ligands



NUCLEODUR HILIC

Other NUCLEODUR phases include:
C18 Gravity, C8 Gravity, C18ec, C8ec, C18 PAH, C18 HTec, PFP, CN and CN-RP, NH2 and NH2-RP and Silica

## NUCLEOSHELL®

NUCLEOSHELL phases consist of a solid silica core (1.7µm) with a homogeneous shell of porous silica, giving an overall particle size of 2.7µm. These superficially porous particles produce higher efficiencies compared with traditional totally porous 3µm materials. The lower back pressure achieved enables the columns to be used with both UHPLC and HPLC instruments. Four phases are available:

- **NUCLEOSHELL RP 18** – suitable for LC-MS and HPLC at pH extremes (1 - 11)
- **NUCLEOSHELL HILIC** – a zwitterionic phase based on ammonium-sulphonic acid modified silica
- **NUCLEOSHELL PFP** – separates components due to H-bonding, dipole-dipole interactions, $\pi - \pi$ interactions and hydrophobic interactions
- **NUCLEOSHELL Phenyl-Hexyl** - suitable for aromatic, unsaturated and polar compounds

## NUCLEOGEN® Columns for Bioanalysis

These are 7µm silica based anion-exchange phases, with diethylaminoethyl (DEAE) bonding. Phases are available with pore sizes of 60, 500 and 4000Å.

- **NUCLEOGEN 60-7 DEAE** – for separation of oligonucleotides up to chain length of 40 bases
- **NUCLEOGEN 500-7 DEAE** – for separation of RNA, viroids in molecular weight range 25,000 to 1,000,000 Da
- **NUCLEOGEN 4000-7 DEAE** – for separation of plasmids, DNA restriction fragments, RNA

## NUCLEOGEL® Columns for Food Analysis

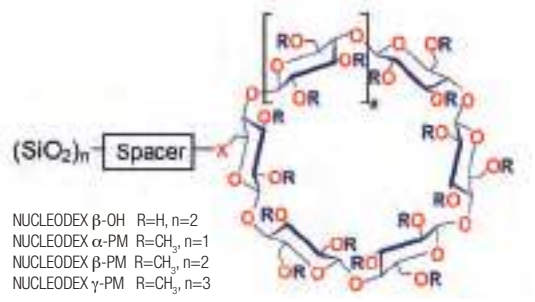
The NUCLEOGEL Sugar 810 series is based on spherical sulphonated polystyrene-divinylbenzene resin in different ionic forms. Separations of carbohydrates, organic acids and other polar small molecules are achieved by a combination of ion exclusion, ion-exchange, size exclusion, ligand exchange and partition mechanisms. Available phases include:

- **NUCLEOGEL Sugar 810H** – Hydrogen ion form (USP L17) for separation of sugars, sugar alcohols and organic acids
- **NUCLEOGEL Sugar 810Ca** – Calcium ion form (USP L19) for separation of mono-, di- and oligosaccharides

## Chiral Phases

Macherey-Nagel manufacture a number of chiral LC phases. These include NUCLEODEX , RESOLVOSIL, NUCLEOSIL CHIRAL-1 and NUCLEOCEL.

- **NUCLEODEX®** phases are based on NUCLEOSIL silica bonded with modified cyclodextrins as chiral selectors. Four different phases are available. In addition to determining enantiomeric purity, NUCLEODEX phases are also useful for the analysis of positional and cis/trans isomers.
- **RESOLVOSIL® BSA-7** was one of the first commercially available protein-bound columns to demonstrate optical resolution. It is based on 7µm NUCLEOSIL silica with a pore size of 300Å.
- **NUCLEOSIL® CHIRAL-1** is chemically bonded with an L-hydroxyproline $Cu^{2+}$ complex and separates racemic molecules by ligand exchange HPLC.
- **NUCLEOCEL®** ***DELTA*** is a cellulose coated phase conforming to USP L40. Columns for normal-phase and reversed-phase applications are available and can separate a wide range of chiral compounds.



NUCLEODEX β-OH R=H, n=2
NUCLEODEX α-PM R=$CH_3$, n=1
NUCLEODEX β-PM R=$CH_3$, n=2
NUCLEODEX γ-PM R=$CH_3$, n=3

## NUCLEOSIL®

- Spherical porous silica
- Wide range of bonded phases
- Excellent mechanical stability
- Hichrom high efficiency

NUCLEOSIL®, a classical silica manufactured by Macherey-Nagel GmbH & Co. KG, is available in a wide range of pore sizes. NUCLEOSIL 100Å and 120Å have similar relative retention characteristics. NUCLEOSIL 100Å has the greater capacity whilst the 120Å material exhibits the lower column back pressure. The wide pore NUCLEOSIL 300Å silica is used as the base material for the reversed-phase bioanalytical columns. NUCLEOSIL 100-5C18 AB was the first of the newer generation NUCLEOSIL materials which include the HD high density phases (please enquire for further details), NUCLEOSIL PROTECT 1 and Nautilus, for use with up to 100% aqueous eluents (see p.37).

### NUCLEOSIL Silicas

| Material | Pore Size (Å) | Surface Area ($m^2/g$) | Particle Size (µm) |
|---|---|---|---|
| NUCLEOSIL 100 | 100 | 350 | 3, 5, 7, 10 |
| NUCLEOSIL 120 | 120 | 200 | 3, 5, 7, 10 |
| NUCLEOSIL 300 | 300 | 100 | 5, 7, 10 |

### NUCLEOSIL Phases

| NUCLEOSIL Phase | Functional Group | Pore Size (Å) | Particle Size (µm) | Carbon Load (%) | Endcapped |
|---|---|---|---|---|---|
| C18 | Octadecyl | 100 | 3, 5, 7, 10 | 15 | Yes |
| | | 120 | 3, 5, 7, 10 | 11 | Yes |
| | | 300 | 5 | 6.5 | Yes |
| C18 AB | Octadecyl, crosslinked | 100 | 5 | 25 | Yes |
| C8 | Octyl | 100 | 5, 7, 10 | 8.5 | No |
| | | 120 | 3, 5, 7, 10 | 6.5 | No |
| | | 300 | 5 | 3 | No |
| C4 | Butyl | 300 | 5 | 2 | Yes |
| C2 | Dimethyl | 100 | 7 | 3.5 | No |
| Phenyl | Phenyl | 100 | 5, 7 | 8 | No |
| | | 120 | 5, 7 | 6.5 | No |
| CN | Cyano | 100 | 5, 10 | 5 | No |
| | | 120 | 7 | | |
| NH2 | Amino | 100 | 5 | 3.5 | No |
| | | 120 | 7 | | |
| NO2 | Nitro | 100 | 5 | 4.5 | No |
| OH | Diol | 100 | 5, 7 | 5 | No |
| SA | Sulphonic acid | 100 | 5, 10 | 6.5 | No |
| SB | Quaternary ammonium | 100 | 5, 10 | 10 | No |

### Ordering Information – NUCLEOSIL 100Å – Hichrom Manufactured Columns

#### Microbore (1.0 – 2.1mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm and 2.1mm i.d. NUCLEOSIL 100Å columns.

#### Medium Bore (3.2mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **3µm** | | | | | |
| 100 3 Silica | NC100-3-50AS | NC100-3-100AS | NC100-3-150AS | NC100-3-250AS | NC100-3-10C5 |
| 100 3 C18 | NC100-3C18-50AS | NC100-3C18-100AS | NC100-3C18-150AS | - | NC100-3C18-10C5 |
| **5µm** | | | | | |
| 100 5 Silica | NC100-5-50AS | NC100-5-100AS | NC100-5-150AS | NC100-5-250AS | NC100-5-10C5 |
| 100 5 C18 | NC100-5C18-50AS | NC100-5C18-100AS | NC100-5C18-150AS | NC100-5C18-250AS | NC100-5C18-10C5 |
| 100 5 C8 | NC100-5C8-50AS | NC100-5C8-100AS | NC100-5C8-150AS | NC100-5C8-250AS | NC100-5C8-10C5 |
| 100 5 CN | NC100-5CN-50AS | NC100-5CN-100AS | NC100-5CN-150AS | NC100-5CN-250AS | NC100-5CN-10C5 |
| 100 5 NH2 | NC100-5NH-50AS | NC100-5NH-100AS | NC100-5NH-150AS | NC100-5NH-250AS | NC100-5NH-10C5 |
| 100 5 NO2 | NC100-5NO-50AS | NC100-5NO-100AS | NC100-5NO-150AS | NC100-5NO-250AS | NC100-5NO-10C5 |
| 100 5 SA | NC100-5SA-50AS | NC100-5SA-100AS | NC100-5SA-150AS | NC100-5SA-250AS | NC100-5SA-10C5 |
| 100 5 SB | NC100-5SB-50AS | NC100-5SB-100AS | NC100-5SB-150AS | NC100-5SB-250AS | NC100-5SB-10C5 |
| 100 5 C18 AB | NC100-5C18AB-50AS | NC100-5C18AB-100AS | NC100-5C18AB-150AS | NC100-5C18AB-250AS | NC100-5C18AB-10C5 |

[1] Other column dimensions available – please enquire [2] 5/pk - Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] Starter kits also available – see p. 21

## Ordering Information – NUCLEOSIL® 100Å – Hichrom Manufactured Columns (continued)

### Analytical (4.0mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| **3µm** | | | | | |
| 100 3 Silica | NC100-3-50AF | NC100-3-100AF | NC100-3-150AF | NC100-3-250AF | NC100-3-10C5 |
| 100 3 C18 | NC100-3C18-50AF | NC100-3C18-100AF | NC100-3C18-150AF | NC100-3C18-250AF | NC100-3C18-10C5 |
| **5µm** | | | | | |
| 100 5 Silica | NC100-5-50AF | NC100-5-100AF | NC100-5-150AF | NC100-5-250AF | NC100-5-10C5 |
| 100 5 C18 | NC100-5C18-50AF | NC100-5C18-100AF | NC100-5C18-150AF | NC100-5C18-250AF | NC100-5C18-10C5 |
| 100 5 C8 | NC100-5C8-50AF | NC100-5C8-100AF | NC100-5C8-150AF | NC100-5C8-250AF | NC100-5C8-10C5 |
| 100 5 CN | NC100-5CN-50AF | NC100-5CN-100AF | NC100-5CN-150AF | NC100-5CN-250AF | NC100-5CN-10C5 |
| 100 5 NH2 | NC100-5NH-50AF | NC100-5NH-100AF | NC100-5NH-150AF | NC100-5NH-250AF | NC100-5NH-10C5 |
| 100 5 NO2 | NC100-5NO-50AF | NC100-5NO-100AF | NC100-5NO-150AF | NC100-5NO-250AF | NC100-5NO-10C5 |
| 100 5 SA | NC100-5SA-50AF | NC100-5SA-100AF | NC100-5SA-150AF | NC100-5SA-250AF | NC100-5SA-10C5 |
| 100 5 SB | NC100-5SB-50AF | NC100-5SB-100AF | NC100-5SB-150AF | NC100-5SB-250AF | NC100-5SB-10C5 |
| 100 5 C18 AB | NC100-5C18AB-50AF | NC100-5C18AB-100AF | NC100-5C18AB-150AF | NC100-5C18AB-250AF | NC100-5C18AB-10C5 |

### Analytical (4.6mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **3µm** | | | | | |
| 100 3 Silica | NC100-3-50A | NC100-3-100A | NC100-3-150A | NC100-3-250A | NC100-3-10C5 |
| 100 3 C18 | NC100-3C18-50A | NC100-3C18-100A | NC100-3C18-150A | - | NC100-3C18-10C5 |
| **5µm** | | | | | |
| 100 5 Silica | NC100-5-50A | NC100-5-100A | NC100-5-150A | NC100-5-250A | NC100-5-10C5 |
| 100 5 C18 | NC100-5C18-50A | NC100-5C18-100A | NC100-5C18-150A | NC100-5C18-250A | NC100-5C18-10C5 |
| 100 5 C8 | NC100-5C8-50A | NC100-5C8-100A | NC100-5C8-150A | NC100-5C8-250A | NC100-5C8-10C5 |
| 100 5 CN | NC100-5CN-50A | NC100-5CN-100A | NC100-5CN-150A | NC100-5CN-250A | NC100-5CN-10C5 |
| 100 5 NH2 | NC100-5NH-50A | NC100-5NH-100A | NC100-5NH-150A | NC100-5NH-250A | NC100-5NH-10C5 |
| 100 5 NO2 | NC100-5NO-50A | NC100-5NO-100A | NC100-5NO-150A | NC100-5NO-250A | NC100-5NO-10C5 |
| 100 5 SA | NC100-5SA-50A | NC100-5SA-100A | NC100-5SA-150A | NC100-5SA-250A | NC100-5SA-10C5 |
| 100 5 SB | NC100-5SB-50A | NC100-5SB-100A | NC100-5SB-150A | NC100-5SB-250A | NC100-5SB-10C5 |
| 100 5 C18 AB | NC100-5C18AB-50A | NC100-5C18AB-100A | NC100-5C18AB-150A | NC100-5C18AB-250A | NC100-5C18AB-10C5 |
| **7µm** | | | | | |
| 100 7 Silica | NC100-7-50A | NC100-7-100A | NC100-7-150A | NC100-7-250A | NC100-7-10C5 |
| 100 7 C18 | NC100-7C18-50A | NC100-7C18-100A | NC100-7C18-150A | NC100-7C18-250A | NC100-7C18-10C5 |
| 100 7 C8 | NC100-7C8-50A | NC100-7C8-100A | NC100-7C8-150A | NC100-7C8-250A | NC100-7C8-10C5 |
| 100 7 C2 | NC100-7C2-50A | NC100-7C2-100A | NC100-7C2-150A | NC100-7C2-250A | NC100-7C2-10C5 |
| 100 7 Phenyl | NC100-7P-50A | NC100-7P-100A | NC100-7P-150A | NC100-7P-250A | NC100-7P-10C5 |
| 100 7 Diol | NC100-7DIOL-50A | NC100-7DIOL-100A | NC100-7DIOL-150A | NC100-7DIOL-250A | NC100-7DIOL-10C5 |
| **10µm** | | | | | |
| 100 10 Silica | NC100-10-50A | NC100-10-100A | NC100-10-150A | NC100-10-250A | NC100-10-10C5 |
| 100 10 C18 | NC100-10C18-50A | NC100-10C18-100A | NC100-10C18-150A | NC100-10C18-250A | NC100-10C18-10C5 |
| 100 10 C8 | NC100-10C8-50A | NC100-10C8-100A | NC100-10C8-150A | NC100-10C8-250A | NC100-10C8-10C5 |
| 100 10 CN | NC100-10CN-50A | NC100-10CN-100A | NC100-10CN-150A | NC100-10CN-250A | NC100-10CN-10C5 |
| 100 10 SA | NC100-10SA-50A | NC100-10SA-100A | NC100-10SA-150A | NC100-10SA-250A | NC100-10SA-10C5 |
| 100 10 SB | NC100-10SB-50A | NC100-10SB-100A | NC100-10SB-150A | NC100-10SB-250A | NC100-10SB-10C5 |

[1] Other column dimensions available – please enquire [2] 5/pk - Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] Starter kits also available – see p. 21

### Semi-Preparative and Preparative (7.75 – 21.2mm i.d.) Columns

Please contact Hichrom for further details of 7.75-21.2mm i.d. NUCLEOSIL 100Å columns.

## Ordering Information - NUCLEOSIL® 120Å – Hichrom Manufactured Columns

### Microbore (1.0 – 2.1mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm and 2.1mm i.d. NUCLEOSIL 120Å columns.

### Medium Bore (3.2mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **3µm** | | | | | |
| 120 3 Silica | NC120-3-50AS | NC120-3-100AS | NC120-3-150AS | NC120-3-250AS | NC120-3-10C5 |
| 120 3 C18 | NC120-3C18-50AS | NC120-3C18-100AS | NC120-3C18-150AS | - | NC120-3C18-10C5 |
| 120 3 C8 | NC120-3C8-50AS | NC120-3C8-100AS | NC120-3C8-150AS | - | NC120-3C8-10C5 |
| **5µm** | | | | | |
| 120 5 Silica | NC120-5-50AS | NC120-5-100AS | NC120-5-150AS | NC120-5-250AS | NC120-5-10C5 |
| 100 5 C18 | NC120-5C18-50AS | NC120-5C18-100AS | NC120-5C18-150AS | NC120-5C18-250AS | NC120-5C18-10C5 |
| 100 5 C8 | NC120-5C8-50AS | NC120-5C8-100AS | NC120-5C8-150AS | NC120-5C8-250AS | NC120-5C8-10C5 |

### Analytical (4.0mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| **3µm** | | | | | |
| 120 3 Silica | NC120-3-50AF | NC120-3-100AF | NC120-3-150AF | NC120-3-250AF | NC120-3-10C5 |
| 120 3 C18 | NC120-3C18-50AF | NC120-3C18-100AF | NC120-3C18-150AF | - | NC120-3C18-10C5 |
| 120 3 C8 | NC120-3C8-50AF | NC120-3C8-100AF | NC120-3C8-150AF | - | NC120-3C8-10C5 |
| **5µm** | | | | | |
| 120 5 Silica | NC120-5-50AF | NC120-5-100AF | NC120-5-150AF | NC120-5-250AF | NC120-5-10C5 |
| 100 5 C18 | NC120-5C18-50AF | NC120-5C18-100AF | NC120-5C18-150AF | NC120-5C18-250AF | NC120-5C18-10C5 |
| 100 5 C8 | NC120-5C8-50AF | NC120-5C8-100AF | NC120-5C8-150AF | NC120-5C8-250AF | NC120-5C8-10C5 |

### Analytical (4.6mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **3µm** | | | | | |
| 120 3 Silica | NC120-3-50A | NC120-3-100A | NC120-3-150A | NC120-3-250A | NC120-3-10C5 |
| 120 3 C18 | NC120-3C18-50A | NC120-3C18-100A | NC120-3C18-150A | - | NC120-3C18-10C5 |
| 120 3 C8 | NC120-3C8-50A | NC120-3C8-100A | NC120-3C8-150A | - | NC120-3C8-10C5 |
| **5µm** | | | | | |
| 120 5 Silica | NC120-5-50A | NC120-5-100A | NC120-5-150A | NC120-5-250A | NC120-5-10C5 |
| 120 5 C18 | NC120-5C18-50A | NC120-5C18-100A | NC120-5C18-150A | NC120-5C18-250A | NC120-5C18-10C5 |
| 120 5 C8 | NC120-5C8-50A | NC120-5C8-100A | NC120-5C8-150A | NC120-5C8-250A | NC120-5C8-10C5 |
| **7µm** | | | | | |
| 120 7 C18 | NC120-7C18-50A | NC120-7C18-100A | NC120-7C18-150A | NC120-7C18-250A | NC120-7C18-10C5 |
| 120 7 C8 | NC120-7C8-50A | NC120-7C8-100A | NC120-7C8-150A | NC120-7C8-250A | NC120-7C8-10C5 |
| 120 7 Phenyl | NC120-7P-50A | NC120-7P-100A | NC120-7P-150A | NC120-7P-250A | NC120-7CP-10C5 |
| 120 7 CN | NC120-7CN-50A | NC120-7CN-100A | NC120-7CN-150A | NC120-7CN-250A | NC120-7CN-10C5 |
| 120 7 NH2 | NC120-7NH-50A | NC120-7NH-5100A | NC120-7NH-150A | NC120-7NH-250A | NC120-7NH-10C5 |
| **10µm** | | | | | |
| 120 10 C18 | NC120-10C18-50A | NC120-10C18-100A | NC120-10C18-150A | NC120-10C18-250A | NC120-10C18-10C5 |
| 120 10 C8 | NC120-10C8-50A | NC120-10C8-100A | NC120-10C8-150A | NC120-10C8-250A | NC120-10C8-10C5 |

[1] Other column dimensions available – please enquire [2] 5/pk - Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] Starter kits also available – see p. 21

## Ordering Information - NUCLEOSIL® 300Å – Hichrom Manufactured Columns

### Microbore (1.0mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm i.d. NUCLEOSIL 300Å columns.

### Microbore (2.1mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| **5µm** | | | | | |
| 300 5 C4 | NC300-5C4-50AM | NC300-5C4-100AM | NC300-5C4-150AM | NC300-5C4-250AM | NC300-5C4-10CM5 |
| 300 5 C8 | NC300-5C8-50AM | NC300-5C8-100AM | NC300-5C8-150AM | NC300-5C8-250AM | NC300-5C8-10CM5 |
| 300 5 C18 | NC300-5C18-50AM | NC300-5C18-100AM | NC300-5C18-150AM | NC300-5C18-250AM | NC300-5C18-10CM5 |

### Medium Bore (3.2mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **5µm** | | | | | |
| 300 5 C4 | NC300-5C4-50AS | NC300-5C4-100AS | NC300-5C4-150AS | NC300-5C4-250AS | NC300-5C4-10C5 |
| 300 5 C8 | NC300-5C8-50AS | NC300-5C8-100AS | NC300-5C8-150AS | NC300-5C8-250AS | NC300-5C8-10C5 |
| 300 5 C18 | NC300-5C18-50AS | NC300-5C18-100AS | NC300-5C18-150AS | NC300-5C18-250AS | NC300-5C18-10C5 |

### Analytical (4.0mm i.d.) Columns

Please contact Hichrom for further details of 4.0mm i.d. NUCLEOSIL 300Å columns.

### Analytical (4.6mm i.d.) Columns

| NUCLEOSIL Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2, 3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **5µm** | | | | | |
| 300 5 C4 | NC300-5C4-50A | NC300-5C4-100A | NC300-5C4-150A | NC300-5C4-250A | NC300-5C4-10C5 |
| 300 5 C8 | NC300-5C8-50A | NC300-5C8-100A | NC300-5C8-150A | NC300-5C8-250A | NC300-5C8-10C5 |
| 300 5 C18 | NC300-5C18-50A | NC300-5C18-100A | NC300-5C18-150A | NC300-5C18-250A | NC300-5C18-10C5 |

[1] Other column dimensions available – please enquire [2] 5/pk - Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] Starter kits also available – see p. 21

### Semi-Preparative and Preparative Columns (7.75 – 21.2mm i.d.)

Please contact Hichrom for further details of 7.75 – 21.2mm i.d. NUCLEOSIL 300Å columns.

**Please contact us for ordering details on all Machery-Nagel columns not listed**

# MCI GEL®



- Polymeric phases
- Reversed-phase, ion-exchange, SEC phases
- Columns and bulk resins
- High chemical stability

The MCI GEL® range of polymeric phases, manufactured by Mitsubishi Chemicals, includes reversed-phase, ion-exchange, ion chromatography, chiral and size exclusion materials. These products are available as packed columns and bulk materials. The features of these MCI GEL phases are summarised below. Please contact Hichrom for ordering information for the bulk resins and chiral columns. Please see page 3 for new MCI GEL CHK40/C04 mixed-mode columns for polar molecule separation.

## Columns for Low Molecular Weight Separations (eg. MW < 2000)

### Columns for ion-exchange (CK and CA Series) – for MW < 2000

Matrix: styrene-divinylbenzene copolymer

| MCI GEL Phase | Cross Link-age % | Counter Ion | Particle Size (µm) | Applications | Column Dimensions[1] (mm) | Cat. No. |
|---|---|---|---|---|---|---|
| CK10U | 10 | $Na^+$ | 5 | Amino acids, amines | 120 x 6.0 | 001901 |
| CK08S | 8 | $Na^+$ | 11 | General sugar separations | 500 x 8.0 | 000901 |
| CK08E | 8 | $Na^+$ | 9 | General sugar separations | 300 x 8.0 | 001001 |
| CK08EC | 8 | $Ca^{2+}$ | 9 | General sugar separations, sugar alcohols | 300 x 8.0 | 001002 |
| CK08ES | 8 | $Ag^+$ | 9 | - | 300 x 8.0 | 001003 |
| CK08EH | 8 | $H^+$ | 9 | Carboxylic acids, sugar alcohols | 300 x 8.0 | 001005 |
| CK04S | 4 | $Na^+$ | 11 | Oligosaccharides | 200 x 10 | 000301 |
| CK04SS | 4 | $Ag^+$ | 11 | Oligosaccharides | 200 x 10 | 000302 |
| CK02A | 2 | $Na^+$ | 20 | Oligosaccharides | 250 x 20 | 000101 |
| CK02AS | 2 | $Ag^+$ | 20 | Oligosaccharides | 250 x 20 | 000102 |
| CA08F | 8 | $Cl^-$ | 7 | Sugars, carboxylic acids, nucleotides | 250 x 4.6 | 011101 |
| CDR10 | porous | $AcO^-$ | 7 | Sugars, nucleotides from physiological fluids | 250 x 4.6 | 011901 |

[1] Guard columns available

### Columns for ion chromatography (SCK and SCA)

The MCI GEL ion chromatography columns are designed for non-suppressed ion chromatography applications.
Matrix: SCK01 – styrene-divinylbenzene copolymer, SCA04 – polyhydroxymethacrylate

| MCI GEL Phase | Functional Group | Counter Ion | Particle Size (µm) | Applications | Column Dimensions[1] (mm) | Cat. No. |
|---|---|---|---|---|---|---|
| SCK01 | $-SO_3^-$ | $Na^+$ | 11 | Mono- and divalent cations, transition metals, nucleosides | 50 x 6.0 | 003401 |
| | | | | | 150 x 4.6 | 003404 |
| SCA04 | Quaternary ammonium | $Cl^-$ | 5 | Inorganic anions | 150 x 4.6[2] | 013302 |

[1] Guard columns available [2] PEEK hardware

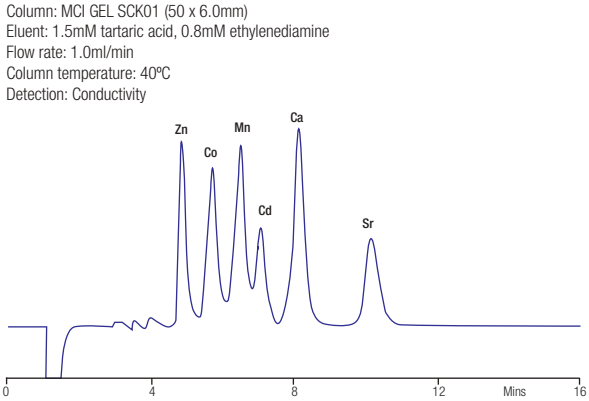



Figure 1. Transition metal analysis on MCI GEL SCK01

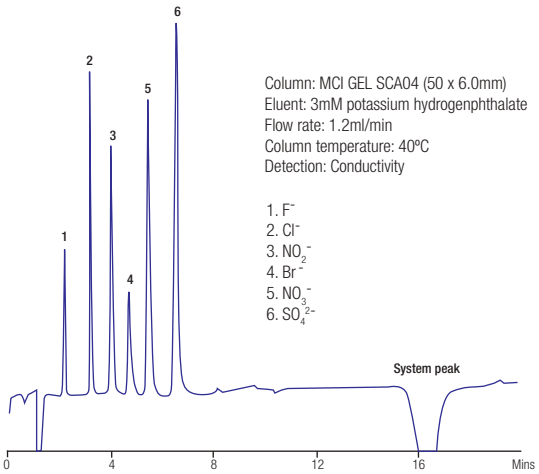



Figure 2. Anion analysis on MGI GEL SCA04

## Columns for Low Molecular Weight Separations (eg. MW < 2000) (continued)

### Columns for reversed-phase (CHP Series)

| MCI GEL Phase | Matrix | Particle Size (µm) | Pore Size (Å) | pH Range | Column Dimensions (mm) | Cat. No. |
|---|---|---|---|---|---|---|
| CHP20/C04 | PS-DVB | 4 | proprietary | 1 - 13 | 150 x 4.6 | 040105 |
| CHP20/C10 | PS-DVB | 10 | 250 | 1 - 13 | 250 x 4.6 | 040301 |
| CMG20/C04 | Hydroxypolymethacrylate | 4 | proprietary | 2 - 12 | 150 x 4.6 | 040205 |
| CMG20/C10 | Hydroxypolymethacrylate | 10 | 250 | 2 - 12 | 250 x 4.6 | 020205 |

## Columns for Biopolymer Separations (eg. MW > 2000)

### Columns for gel filtration (CQP Series)

Matrix: polyhydroxymethacrylate

| MCI GEL Phase | Particle Size (µm) | Pore Size (Å) | Exclusion Limit PEG | Applications | Column Dimensions[1] (mm) | Cat. No. |
|---|---|---|---|---|---|---|
| CQP06 | 10 | 120 | $10^3$ | Proteins, peptides, enzymes and other biomolecules | 600 x 7.5 | 021301 |
| CQP10 | 10 | 200 | $10^4$ | | 600 x 7.5 | 021401 |
| CQP30 | 10 | 600 | $10^6$ | | 600 x 7.5 | 021501 |

[1] Guard columns available

### Columns for ion-exchange (CQA and CQK Series) – for MW > 2000

Matrix: polyhydroxymethacrylate

| MCI GEL Phase | Functional Group | Counter Ion | Particle Size (µm) | Pore Size (Å) | pH Range | Applications | Column Dimensions (mm) | Cat. No. |
|---|---|---|---|---|---|---|---|---|
| CQA31S | Diethylaminoethyl | $Cl^-$ | 10 | 600 | 2 - 12 | Proteins and peptides | 75 x 7.5 | 012601 |
| CQA35S | Quaternary ammonium | $Cl^-$ | 10 | 600 | 2 - 12 | | 75 x 7.5 | 013001 |
| CQK30S | Sulphopropyl | $Na^+$ | 10 | 600 | 1 - 13 | | 75 x 7.5 | 003601 |
| CQK31S | Carboxymethyl | $Na^+$ | 10 | 600 | 4 - 13 | | 75 x 7.5 | 003801 |

### Columns for hydrophobic interaction chromatography (CQH Series) – for MW > 2000

Matrix: polyhydroxymethacrylate

| MCI GEL Phase | Functional Group | Particle Size (µm) | Pore Size (Å) | Applications | Column Dimensions (mm) | Cat. No. |
|---|---|---|---|---|---|---|
| CQH3ES | Ether | 10 | 600 | Proteins and peptides | 75 x 7.5 | 021701 |
| CQH3BS | Butyl | 10 | 600 | | 75 x 7.5 | 021601 |
| CQH3PS | Phenyl | 10 | 600 | | 75 x 7.5 | 021801 |

### MCI GEL® Bulk Media

MCI GEL® bulk packing materials include ion-exchange resins, size exclusion materials and non-functionalised polymer for reversed-phase separations. The availability of resins with particle sizes from 4 to greater than 100µm makes MCI GEL materials suitable for analytical to preparative applications.

Please contact Hichrom for further details of these materials.

Merck Millipore, a division of Merck KGaA, Darmstadt, Germany, manufactures a number of products for use in the life science industry. A brief overview of the Merck SeQuant® speciality HILIC phases, Chromolith® columns, Purospher® and Purospher STAR HPLC columns is given on the next couple of pages. Specifications and ordering details for LiChrosorb® and LiChrospher® columns are found on pages 111-113. Merck TLC plates are described on page 181. Please contact us for ordering information on any products not listed.

## SeQuant ZIC-HILIC and ZIC-*p*HILIC

Merck SeQuant ZIC-HILIC and ZIC-*p*HILIC phases have been developed for the separation of polar and hydrophilic compounds in HILIC (Hydrophilic Interaction Liquid Chromatography) mode.

| Phase | Base Material | Particle Size (µm) | Surface Area ($m^2/g$) | pH Stability | Max. Temp. (°C) |
|---|---|---|---|---|---|
| ZIC-HILIC 100Å | Spherical silica | 3.5 | 180 | 3 - 8 | 70 |
| ZIC-HILIC 200Å | Spherical silica | 3.5, 5 | 130 | 3 - 8 | 70 |
| ZIC-*p*HILIC | Polymeric | 5 | - | 2 - 10 | 50 |

The ZIC-HILIC and ZIC-*p*HILIC phases have a bonded stationary phase consisting of a highly polar, permanent zwitterionic sulphobetaine structure (see Figure 1).

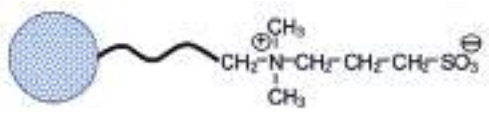


Figure 1. ZIC-HILIC phase

Separation selectivity is favoured by the 1:1 zwitterionic charge balance, which makes the column overall neutral, but with weak ionic interactions. Figure 2 shows the separation of morphine and its glucuronated metabolites on a ZIC-HILIC column.

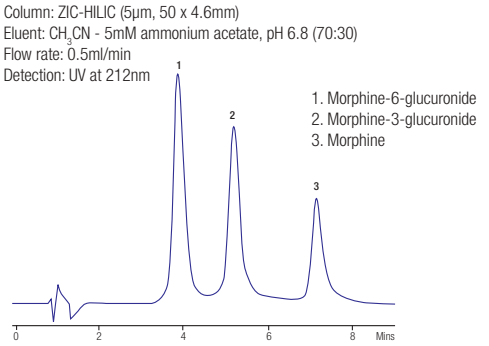



Figure 2. Separation of morphine and glucuronated metabolites

### SeQuant ZIC-HILIC

ZIC-HILIC shows the highest separation efficiency and is suitable for most HILIC applications. Its key features include:

**1) Orthogonality** Polar and hydrophilic compounds generally experience stronger retention on ZIC-HILIC than on reversed-phase columns.
**2) LC-MS Compatibility** Since typical eluents for HILIC consist of 40-97% acetonitrile in water or volatile buffer, ZIC-HILIC columns are ideal for LC-MS analyses. By changing from reversed-phase to HILIC, a large increase in sensitivity is often observed for hydrophilic analytes.
**3) Scalability** In addition to standard analytical and semi-preparative columns, the ZIC-HILIC range includes fused silica nano columns and glass-lined stainless steel capillary columns for down-scaling of methods. Methods developed on analytical ZIC-HILIC columns can also be successfully scaled up to preparative dimensions.
**4) Rapid Resolution and High Throughput** A 3.5µm 20 x 2.1mm PEEK column is available for high throughput analyses.

### SeQuant ZIC-*p*HILIC

The polymer-based ZIC-*p*HILIC phase comprises the same sulphobetaine type zwitterionic functional group as the ZIC-HILIC phase and offers similar selectivity to the ZIC-HILIC columns. However, it can be used in a wider pH range, thereby extending the classes of analytes that can be separated by HILIC. In addition, the selection of optimum ionisation conditions for MS sensitivity may be enhanced.

#### SeQuant ZIC-cHILIC

SeQuant ZIC-cHILIC is a newer zwitterionic stationary phase in which phosphorylcholine functional groups (rather than sulphobetaine groups) are bonded to the silica surface (see Figure 3).

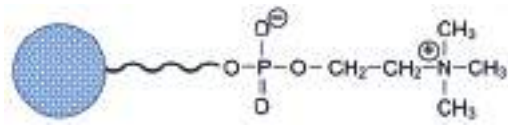


Figure 3. ZIC-cHILIC phase

This phase offers complementary selectivity to the ZIC-HILIC phase as well as to reversed-phase and other HILIC columns. The selectivity features are especially beneficial for analysing negatively charged polar compounds such as nucleosides and organic acids, but are also beneficial for the separation of positively charged hydrophilic molecules, including aminoglycosides and cations. Figure 4 illustrates the gradient separation of a mixture of five aminoglycoside antibiotics.

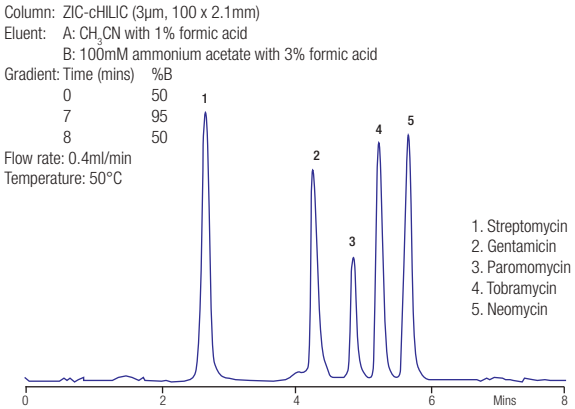



Figure 4. LC-MS of aminoglycoside antibiotics

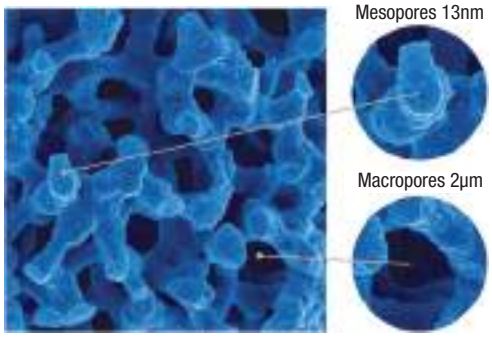
ZIC-cHILIC and ZIC-HILIC, which have differently orientated zwitterionic functional groups, show different selectivity towards polar hydrophilic compounds.

Please contact us for ordering information on all Merck SeQuant HILIC phases.

## Chromolith®

Chromolith® HPLC columns consist of monolithic rods made of highly porous metal-free silica. The rods are mechanically stable and chemically resistant. After derivatisation and endcapping the silica rods are clad in PEEK. Chromolith silica has a unique bimodal pore structure, consisting of a dense network of macropores, each 2µm in diameter, which reduces the column back-pressure and enables the use of faster flow rates. Within the skeletal structure of the rod is a further network of mesopores, each 13nm in diameter, which provides the large active surface area for high efficiency separations.

Chromolith phases include RP-18e, RP-18e HR (High Resolution), RP-8e, NH2 and Si.




### Key Features of Chromolith Columns

**1) Selectivity** Chromolith columns show comparable selectivity to conventional reversed-phase columns, so existing methods can be easily transferred with only minimal method development. However, retention times are shorter on Chromolith columns.

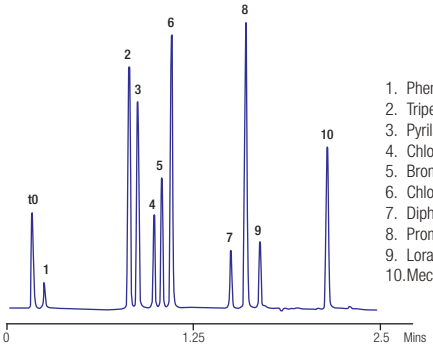
**2) Speed of analysis** Separations twice as fast and at half the column back pressure compared to conventional reversed-phase 5µm columns can be achieved. Figure 5 shows the separation of a range of antihistamines on a Chromolith FastGradient RP-18e column.

**3) Column coupling** Several Chromolith columns can be linked in series producing a column with a theoretical plate count significantly higher than particulate columns.

**4) Flow programming** Chromolith columns are very responsive to changes in flow rate, due to fast re-equilibration. Flow rates can be changed in mid flow to shorten separation time once the target compound has successfully eluted.

**5) Reduced sample preparation** Chromolith columns are very resistant to blocking, even with biological samples.

**6) Scalability** Chromolith SemiPrep 10mm i.d. columns have the same bimodal porous silica rod structure as Chromolith analytical columns and are ideal for direct scale-up. For Chromolith Prep columns (100 x 25mm i.d.) the macropore size is 3µm and mesopore size is 12nm.




Column: Chromolith FastGradient RP-18e (50 x 2mm)
Eluent: A: 0.1% TFA in $H_2O$
B: 0.1% TFA in $CH_3CN$
Gradient: 5% to 90% B in 3.4 mins
Flow rate: 1.0ml/min
Detection: UV, 230nm

Figure 5. Ultra-fast separation of antihistamines

## Purospher® and Purospher STAR Phases

**The Purospher® range** of HPLC columns is based on high purity metal-free silica. Phases include:
RP-18 – an amino endcapped C18 for analysis of hydrophilic compounds, pore size 90Å
RP-18e – C18 with hydrophobic endcapping, pore size 120Å
RP-18 HC – non endcapped C18, suitable for analysis of explosives and related compounds

**Purospher STAR phases** are based on high purity silica for the separation of basic, neutral and metal-chelating samples. Available phases include:
RP-18e and RP-8e – C18 and C8 respectively, both with hydrophobic endcapping, pore size of 120Å and particle sizes of 2, 3 and 5µm.
Other phases – NH2 and Si

## Speciality Phases (Aluspher® RP-select B and ChiraDex® Chiral)

**Aluspher® RP-select B** is a reversed-phase material based on porous spherical alumina, the surface of which has been modified by polymer coating with polybutadiene. It is stable over the pH range 2 – 12, enabling the use of basic eluents such as NaOH. Working at high pH, the ionisation of basic compounds is suppressed and peak tailing is avoided.

**ChiraDex®** is based on β-cyclodextrin covalently linked to spherical silica particles. The phase shows broad enantioselectivity, with molecules separating depending on the extent of their complexation within the cyclodextrin cavities.

## Merck Cartridge System

LiChrosorb, LiChrospher and Superspher are available in Merck's LiChroCART cartridge system used with manu-CART end fittings. These columns can be used with or without an integral guard cartridge. Please contact us for further details of available cartridges.

Please contact us for ordering information for Chromolith, Purospher and the other Merck phases listed.

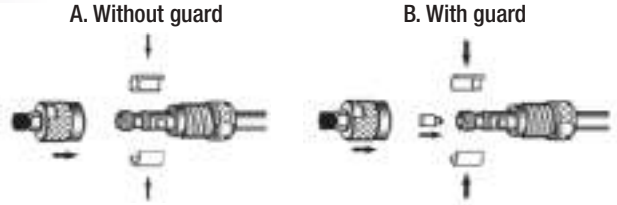



Figure 6. manu-CART cartridge end fittings

## LiChrosorb®

- Irregular porous silica
- 5 and 10µm particle sizes
- Hichrom high efficiency packed columns

LiChrosorb® is a traditional silica manufactured by Merck Millipore and is available as 60 and 100Å pore size particles. It has been used in HPLC for over 25 years and is well documented in the literature. All Hichrom packed columns use industry standard compression fittings.

### LiChrosorb Phases

| LiChrosorb Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Silica | - | - | 5, 10 | 60 | 500 | - |
| | | | 10 | 100 | 300 | - |
| RP-8 | Octyl | No | 5, 10 | 100 | 300 | 9.5 |
| RP-18 | Octadecyl | No | 5, 10 | 100 | 300 | 16.2 |
| CN | Cyano | No | 5 | 100 | 300 | 7 |
| NH2 | Amino | No | 5, 10 | 100 | 300 | 4 |
| DIOL | Hydroxyl | No | 5, 10 | 100 | 300 | 8 |

### Ordering Information – Hichrom Manufactured Columns

### Analytical (4.0mm i.d.) Columns

| 5µm LiChrosorb Phase | Column Dimensions[1] (mm) | | | | | Guard Cartridges[2, 3] (For 4.0mm i.d. columns) |
|---|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 125 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| Silica (60Å) | LI60-5-50AF | LI60-5-100AF | LI60-5-125AF | LI60-5-150AF | LI60-5-250AF | LI60-5-10C5 |
| Silica (100Å) | LI100-5-50AF | LI100-5-100AF | LI100-5-125AF | LI100-5-150AF | LI100-5-250AF | LI100-5-10C5 |
| RP-8 | LIRP8-5-50AF | LIRP8-5-100AF | LIRP8-5-125AF | LIRP8-5-150AF | LIRP8-5-250AF | LIRP8-5-10C5 |
| RP-18 | LIRP18-5-50AF | LIRP18-5-100AF | LIRP18-5-125AF | LIRP18-5-150AF | LIRP18-5-250AF | LIRP18-5-10C5 |
| CN | LICN-5-50AF | LICN-5-100AF | LICN-5-125AF | LICN-5-150AF | LICN-5-250AF | LICN-5-10C5 |
| NH2 | LINH-5-50AF | LINH-5-100AF | LINH-5-125AF | LINH-5-150AF | LINH-5-250AF | LINH-5-10C5 |
| DIOL | LIOH-5-50AF | LIOH-5-100AF | LIOH-5-125AF | LIOH-5-150AF | LIOH-5-250AF | LIOH-5-10C5 |

### Analytical (4.6mm i.d.) Columns

| 5µm LiChrosorb Phase | Column Dimensions[1] (mm) | | | | | Guard Cartridges[2, 3] (For 4.6mm i.d. columns) |
|---|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 125 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| Silica (60Å) | LI60-5-50A | LI60-5-100A | LI60-5-125A | LI60-5-150A | LI60-5-250A | LI60-5-10C5 |
| Silica (100Å) | LI100-5-50A | LI100-5-100A | LI100-5-125A | LI100-5-150A | LI100-5-250A | LI100-5-10C5 |
| RP-8 | LIRP8-5-50A | LIRP8-5-100A | LIRP8-5-125A | LIRP8-5-150A | LIRP8-5-250A | LIRP8-5-10C5 |
| RP-18 | LIRP18-5-50A | LIRP18-5-100A | LIRP18-5-125A | LIRP18-5-150A | LIRP18-5-250A | LIRP18-5-10C5 |
| CN | LICN-5-50A | LICN-5-100A | LICN-5-125A | LICN-5-150A | LICN-5-250A | LICN-5-10C5 |
| NH2 | LINH-5-50A | LINH-5-100A | LINH-5-125A | LINH-5-150A | LINH-5-250A | LINH-5-10C5 |
| DIOL | LIOH-5-50A | LIOH-5-100A | LIOH-5-125A | LIOH-5-150A | LIOH-5-250A | LIOH-5-10C5 |

### Analytical (4.0 and 4.6mm i.d.) Columns

| 10µm LiChrosorb Phase | Column Dimensions[1] (mm) | | Guard Cartridges[2, 3] (For 4.0 and 4.6mm i.d. columns) |
|---|---|---|---|
| | 250 x 4.0 | 250 x 4.6 | |
| Silica (60Å) | LI60-10-250AF | LI60-10-250A | LI60-10-10C5 |
| Silica (100Å) | LI100-10-250AF | LI100-10-250A | LI100-10-10C5 |
| RP-8 | LIRP8-10-250AF | LIRP8-10-250A | LIRP8-10-10C5 |
| RP-18 | LIRP18-10-250AF | LIRP18-10-250A | LIRP18-10-10C5 |
| NH2 | LINH-10-250AF | LINH-10-250A | LINH-10-10C5 |
| DIOL | LIOH-10-250AF | LIOH-10-250A | LIOH-10-10C5 |

[1] Other column dimensions available – please enquire

[2] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 – see p.20

[3] Starter kits also available – see p.21

## LiChrospher®

- Spherical porous silica
- Base deactivated LiChrospher RP-select B
- Endcapping options for RP-8 and RP-18
- Hichrom high efficiency packed columns

LiChrospher® is a traditional silica manufactured by Merck Millipore. The availability of both endcapped and non-endcapped C8 and C18 phases significantly enhances the chromatographer's selectivity options. LiChrospher RP-select B was Merck's first generation base deactivated material. A range of 4µm materials has been marketed under the Superspher® tradename in Europe.

### LiChrospher Phases

| LiChrospher Phase | Functional Group | Endcapped | Particle Size[1,2] (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Si 60 | - | - | 5 | 60 | 700 | - |
| Si 100 | - | - | 5 | 100 | 400 | - |
| 100RP-8 | Octyl | No | 5 | 100 | 350 | 12.5 |
| 100RP-8e | Octyl | Yes | 5 | 100 | 350 | 13.0 |
| 100RP-18 | Octadecyl | No | 5 | 100 | 350 | 21.0 |
| 100RP-18e | Octadecyl | Yes | 5 | 100 | 350 | 21.6 |
| 100NH2 | Amino | No | 5 | 100 | 350 | 4.6 |
| 100CN | Cyano | No | 5 | 100 | 350 | 6.6 |
| 100DIOL | Hydroxyl | No | 5 | 100 | 350 | 8.0 |
| 60RP-select B | Octyl | Yes | 5 | 60 | 360 | 11.5 |

[1] Superspher 4µm columns available

[2] LiChrospher 10µm particle size materials available

### Superspher Phases

| Superspher Phase[1] | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Si 60 | - | - | 4 | 60 | 700 | - |
| 60RP-8 | Octyl | No | 4 | 60 | 350 | 12.5 |
| 60RP-8e | Octyl | Yes | 4 | 60 | 350 | 13.0 |
| 100RP-18 | Octadecyl | No | 4 | 100 | 350 | 21.0 |
| 100RP-18e | Octadecyl | Yes | 4 | 100 | 350 | 21.6 |
| 60RP-select B | Octyl | Yes | 4 | 60 | 360 | 11.5 |

[1] Please contact us for ordering information on Superspher columns

### Ordering Information – Hichrom Manufactured LiChrospher Columns

### Microbore (1.0mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm i.d. LiChrospher columns.

### Microbore (2.1mm i.d.) Columns

| LiChrospher Phase | Column Dimensions (mm) | | | | | Guard Cartridges[1,2] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 125 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| **5µm** | | | | | | |
| Si60 | LISP60-5-50AM | LISP60-5-100AM | LISP60-5-125AM | LISP60-5-150AM | LISP60-5-250AM | LISP60-5-10CM5 |
| Si100 | LISP100-5-50AM | LISP100-5-100AM | LISP100-5-125AM | LISP100-5-150AM | LISP100-5-250AM | LISP100-5-10CM5 |
| 100RP-8 | LISPRP8-5-50AM | LISPRP8-5-100AM | LISPRP8-5-125AM | LISPRP8-5-150AM | LISPRP8-5-250AM | LISPRP8-5-10CM5 |
| 100RP-8e | LISPRP8E-5-50AM | LISPRP8E-5-100AM | LISPRP8E-5-125AM | LISPRP8E-5-150AM | LISPRP8E-5-250AM | LISPRP8E-5-10CM5 |
| 100RP-18 | LISPRP18-5-50AM | LISPRP18-5-100AM | LISPRP18-5-125AM | LISPRP18-5-150AM | LISPRP18-5-250AM | LISPRP18-5-10CM5 |
| 100RP-18e | LISPRP18E-5-50AM | LISPRP18E-5-100AM | LISPRP18E-5-125AM | LISPRP18E-5-150AM | LISPRP18E-5-250AM | LISPRP18E-5-10CM5 |
| 100NH2 | LISPNH-5-50AM | LISPNH-5-100AM | LISPNH-5-125AM | LISPNH-5-150AM | LISPNH-5-250AM | LISPNH-5-10CM5 |
| 100CN | LISPCN-5-50AM | LISPCN-5-100AM | LISPCN-5-125AM | LISPCN-5-150AM | LISPCN-5-250AM | LISPCN-5-10CM5 |
| 100DIOL | LISPOH-5-50AM | LISPOH-5-100AM | LISPOH-5-125AM | LISPOH-5-150AM | LISPOH-5-250AM | LISPOH-5-10CM5 |
| 60RP-select B | LISPRPB-5-50AM | LISPRPB-5-100AM | LISPRPB-5-125AM | LISPRPB-5-150AM | LISPRPB-5-250AM | LISPRPB-5-10CM5 |

[1] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 - see p.20

[2] Starter kits also available - see p.21

## Ordering Information – Hichrom Manufactured LiChrospher® Columns (continued)

### Medium Bore (3.2mm i.d.) Columns

| LiChrospher Phase | Column Dimensions (mm) | | | | | Guard Cartridges[1,2] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 125 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **5µm** | | | | | | |
| Si60 | LISP60-5-50AS | LISP60-5-100AS | LISP60-5-125AS | LISP60-5-150AS | LISP60-5-250AS | LISP60-5-10C5 |
| Si100 | LISP100-5-50AS | LISP100-5-100AS | LISP100-5-125AS | LISP100-5-150AS | LISP100-5-250AS | LISP100-5-10C5 |
| 100RP-8 | LISPRP8-5-50AS | LISPRP8-5-100AS | LISPRP8-5-125AS | LISPRP8-5-150AS | LISPRP8-5-250AS | LISPRP8-5-10C5 |
| 100RP-8e | LISPRP8E-5-50AS | LISPRP8E-5-100AS | LISPRP8E-5-125AS | LISPRP8E-5-150AS | LISPRP8E-5-250AS | LISPRP8E-5-10C5 |
| 100RP-18 | LISPRP18-5-50AS | LISPRP18-5-100AS | LISPRP18-5-125AS | LISPRP18-5-150AS | LISPRP18-5-250AS | LISPRP18-5-10C5 |
| 100RP-18e | LISPRP18E-5-50AS | LISPRP18E-5-100AS | LISPRP18E-5-125AS | LISPRP18E-5-150AS | LISPRP18E-5-250AS | LISPRP18E-5-10C5 |
| 100NH2 | LISPNH-5-50AS | LISPNH-5-100AS | LISPNH-5-125AS | LISPNH-5-150AS | LISPNH-5-250AS | LISPNH-5-10C5 |
| 100CN | LISPCN-5-50AS | LISPCN-5-100AS | LISPCN-5-125AS | LISPCN-5-150AS | LISPCN-5-250AS | LISPCN-5-10C5 |
| 100DIOL | LISPOH-5-50AS | LISPOH-5-100AS | LISPOH-5-125AS | LISPOH-5-150AS | LISPOH-5-250AS | LISPOH-5-10C5 |
| 60RP-select B | LISPRPB-5-50AS | LISPRPB-5-100AS | LISPRPB-5-125AS | LISPRPB-5-150AS | LISPRPB-5-250AS | LISPRPB-5-10C5 |

### Analytical (4.0mm i.d.) Columns

| LiChrospher Phase | Column Dimensions (mm) | | | | | Guard Cartridges[1,2] (For 4.0mm i.d. Columns) |
|---|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 125 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| **5µm** | | | | | | |
| Si60 | LISP60-5-50AF | LISP60-5-100AF | LISP60-5-125AF | LISP60-5-150AF | LISP60-5-250AF | LISP60-5-10C5 |
| Si100 | LISP100-5-50AF | LISP100-5-100AF | LISP100-5-125AF | LISP100-5-150AF | LISP100-5-250AF | LISP100-5-10C5 |
| 100RP-8 | LISPRP8-5-50AF | LISPRP8-5-100AF | LISPRP8-5-125AF | LISPRP8-5-150AF | LISPRP8-5-250AF | LISPRP8-5-10C5 |
| 100RP-8e | LISPRP8E-5-50AF | LISPRP8E-5-100AF | LISPRP8E-5-125AF | LISPRP8E-5-150AF | LISPRP8E-5-250AF | LISPRP8E-5-10C5 |
| 100RP-18 | LISPRP18-5-50AF | LISPRP18-5-100AF | LISPRP18-5-125AF | LISPRP18-5-150AF | LISPRP18-5-250AF | LISPRP18-5-10C5 |
| 100RP-18e | LISPRP18E-5-50AF | LISPRP18E-5-100AF | LISPRP18E-5-125AF | LISPRP18E-5-150AF | LISPRP18E-5-250AF | LISPRP18E-5-10C5 |
| 100NH2 | LISPNH-5-50AF | LISPNH-5-100AF | LISPNH-5-125AF | LISPNH-5-150AF | LISPNH-5-250AF | LISPNH-5-10C5 |
| 100CN | LISPCN-5-50AF | LISPCN-5-100AF | LISPCN-5-125AF | LISPCN-5-150AF | LISPCN-5-250AF | LISPCN-5-10C5 |
| 100DIOL | LISPOH-5-50AF | LISPOH-5-100AF | LISPOH-5-125AF | LISPOH-5-150AF | LISPOH-5-250AF | LISPOH-5-10C5 |
| 60RP-select B | LISPRPB-5-50AF | LISPRPB-5-100AF | LISPRPB-5-125AF | LISPRPB-5-150AF | LISPRPB-5-250AF | LISPRPB-5-10C5 |

### Analytical (4.6mm i.d.) Columns

| LiChrospher Phase | Column Dimensions (mm) | | | | | Guard Cartridges[1,2] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 125 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **5µm** | | | | | | |
| Si60 | LISP60-5-50A | LISP60-5-100A | LISP60-5-125A | LISP60-5-150A | LISP60-5-250A | LISP60-5-10C5 |
| Si100 | LISP100-5-50A | LISP100-5-100A | LISP100-5-125A | LISP100-5-150A | LISP100-5-250A | LISP100-5-10C5 |
| 100RP-8 | LISPRP8-5-50A | LISPRP8-5-100A | LISPRP8-5-125A | LISPRP8-5-150A | LISPRP8-5-250A | LISPRP8-5-10C5 |
| 100RP-8e | LISPRP8E-5-50A | LISPRP8E-5-100A | LISPRP8E-5-125A | LISPRP8E-5-150A | LISPRP8E-5-250A | LISPRP8E-5-10C5 |
| 100RP-18 | LISPRP18-5-50A | LISPRP18-5-100A | LISPRP18-5-125A | LISPRP18-5-150A | LISPRP18-5-250A | LISPRP18-5-10C5 |
| 100RP-18e | LISPRP18E-5-50A | LISPRP18E-5-100A | LISPRP18E-5-125A | LISPRP18E-5-150A | LISPRP18E-5-250A | LISPRP18E-5-10C5 |
| 100NH2 | LISPNH-5-50A | LISPNH-5-100A | LISPNH-5-125A | LISPNH-5-150A | LISPNH-5-250A | LISPNH-5-10C5 |
| 100CN | LISPCN-5-50A | LISPCN-5-100A | LISPCN-5-125A | LISPCN-5-150A | LISPCN-5-250A | LISPCN-5-10C5 |
| 100DIOL | LISPOH-5-50A | LISPOH-5-100A | LISPOH-5-125A | LISPOH-5-150A | LISPOH-5-250A | LISPOH-5-10C5 |
| 60RP-select B | LISPRPB-5-50A | LISPRPB-5-100A | LISPRPB-5-125A | LISPRPB-5-150A | LISPRPB-5-250A | LISPRPB-5-10C5 |

[1] 5/pk – Use with free-standing holder HI-161 and column coupler HI-081 - see p.20

[2] Starter kits also available - see p.21

### Semi-Preparative and Preparative (7.75 - 21.2mm i.d.) Columns

Please contact Hichrom for further details of 7.75 - 21.2mm i.d. LiChrospher columns.

## LiChroprep®

- Irregular preparative silica
- Three particle size ranges
- Guaranteed reproducibility from analytical (LiChrosorb and LiChrospher) to preparative phases
- Competitive price

LiChroprep® is a proven, highly successful packing material providing fast, effective and reproducible separations in HPLC and medium pressure chromatography. Merck offer three different particle size ranges of LiChroprep silica, available as a base silica or C18 bonded phase. The smaller particle size range, 15-25µm, is the most suitable for HPLC. LiChroprep RP-8, amino-, cyano- and diol-bonded phases are available in larger particle size materials.

### Preparative Scale-Up

Figure 1A shows an analytical scouting separation of a prostaglandin sample performed at 1.5ml/min on a 10µm 250 x 4mm LiChrosorb Si60 column. A scaled up separation of the same prostaglandin sample (Figure 1B), but with higher loading, was performed at 2.0litre/min on a 600 x 200mm LiChroprep Si60 column packed with 25-40µm particles. In this example, four different fractionation zones were identified from which the prostaglandin purity was identified.

The lack of silanol groups means that endcapping is not required. In addition, unlike conventional silica (Si-OH) columns, TYPE-C (Si-H) columns do not suffer from column phase bleed (eg. Phenyl Hydride is more stable than a conventional silica phenyl phase).

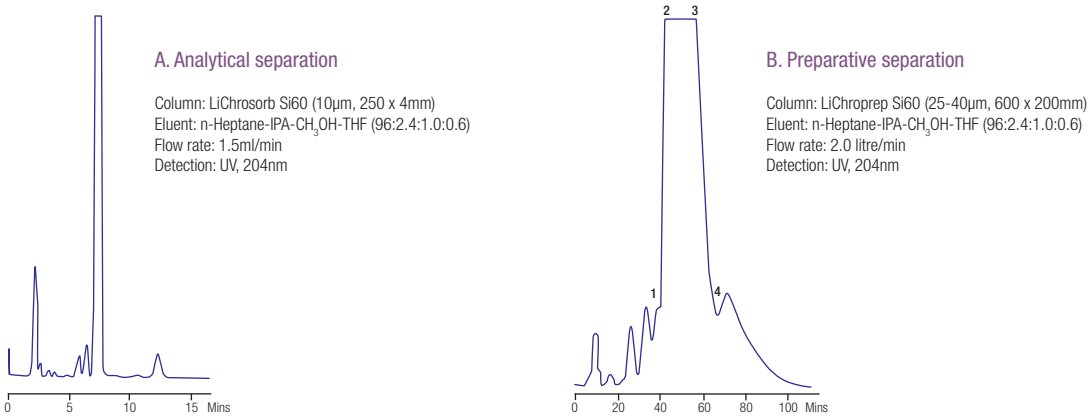



Figure 1. Example of preparative scale-up

### LiChroprep RP-18 Phase

| | | | |
|---|---|---|---|
| **Particle Size Range (µm)** | 15-25 | 25-40 | 40-63 |
| **Mean Particle Size (µm)** | 22 | 32 | 54 |
| **Minimum Theoretical Plates (plates/m)[1]** | 10,000 | 5,500 | 1500 |
| **Typical Column Back Pressure (psi)[1]** | 150 | 90 | 75 |
| **Surface Area ($m^2/g$)** | | 320 | |
| **Pore Size (Å)** | | 100 | |
| **Carbon Load (%)** | | 17-19 | |

[1] 250 x 4 mm, $CH_3CN$-$H_2O$ (75:25) @ 1ml/min.

### Ordering Information

| LiChroprep Phase | Particle Size (µm) | Bulk Material[1] | | | |
|---|---|---|---|---|---|
| | | **100g** | **250g** | **500g** | **1kg** |
| Si 60 | 15-25 | - | - | - | 09336.1000 |
| | 25-40 | - | - | - | 09390.1000 |
| | 40-63 | - | 13905.0250 | - | 13905.1000 |
| RP-18 | 15-25 | - | - | 13901.0500 | - |
| | 25-40 | 09303.0100 | - | 09303.0500 | - |
| | 40-63 | - | 13900.0250 | - | 13900.1000 |
| RP-8 | 40-63 | - | 09362.0250 | - | 09362.1000 |
| DIOL | 40-63 | - | 13973.0250 | - | - |
| NH2 | 40-63 | - | 13974.0250 | - | 13974.1000 |
| CN | 40-63 | - | 13959.0250 | - | - |

[1] Larger pack sizes also available

- Si-OH bonds replaced by Si-H bonds
- Highly stable, low bleed columns
- Fast re-equilibration for fast cycle time
- Increased retention of polar compounds
- Operable in RP, NP and ANP modes

MicroSolv Technology Corporation, USA manufacture the Cogent™ TYPE-C™ silica phases. They are formed from a high purity Type B silica backbone which is modified to cover the silica surface with silicon-hydride (Si-H) bonds, thus replacing >95% of surface silanols (Si-OH), as indicated in Figure 1. Using patented technology, bonded phases such as C18, C8, Phenyl, Cholesterol and Diamond Hydride are produced from the unique silica hydride surface with direct silicon-carbon bonds (see Figure 8 on page 117). These extremely strong bonds offer increased stability over traditional silica based phases.

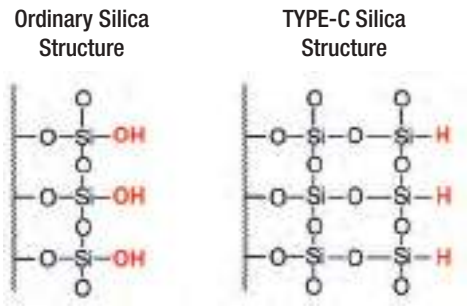



Figure 1. TYPE-C silica surface

The lack of silanol groups means that endcapping is not required. In addition, unlike conventional silica (Si-OH) columns, TYPE-C (Si-H) columns do not suffer from column phase bleed (eg. Phenyl Hydride is more stable than a conventional silica phenyl phase).

## Cogent™ Phases[1]

| Cogent Phase | Particle Size[2] (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | pH Range | Max. Temp. (°C) |
|---|---|---|---|---|---|---|---|
| Silica-C | 4 | 100 | 350 | - | - | 1 - 7 | 60 |
| Bidentate C18 | 4 | 100 | 350 | 16.5 | No | 1 - 10 | 80 |
| Bidentate C8 | 4 | 100 | 350 | 7 | No | 1 - 8 | 80 |
| Phenyl Hydride | 4 | 100 | 350 | 10.8 | No | 1 - 8 | 80 |
| UDC-Cholesterol | 4 | 100 | 350 | 12 | No | 1 - 8 | 80 |
| Diamond Hydride | 4 | 100 | 350 | > 0.5 | - | 2.5 - 7 | 60 |
| Bidentate C8 300 | 5 | 300 | 150 | 5 | No | 1 - 8 | 80 |

[1] UDA and Diol phases also available - please see pages 2 and 4 [2] 2.2µm phases also available - see page 116

The surface silanols that are present in all Type A and B silicas, even after bonding and extensive endcapping, form a strong association with water resulting in a 'hydration shell' surrounding the silica (see Figure 2). However, the silica hydride particles of TYPE-C silica are slightly hydrophobic and have only a weak attraction for water. TYPE-C columns can be operated in 3 modes of chromatography: reversed-phase, normal-phase and aqueous normal-phase. Unlike HILIC, aqueous normal-phase (ANP) does not require a 'water-rich' environment in order to operate.

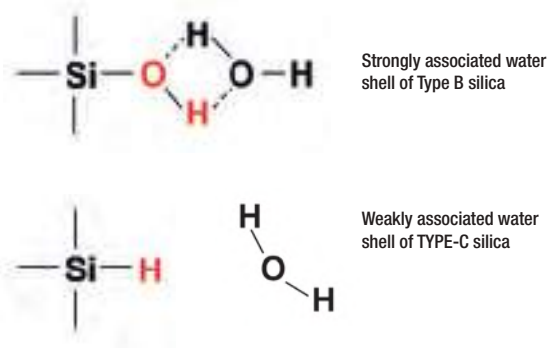



Figure 2. Type B vs TYPE-C silica

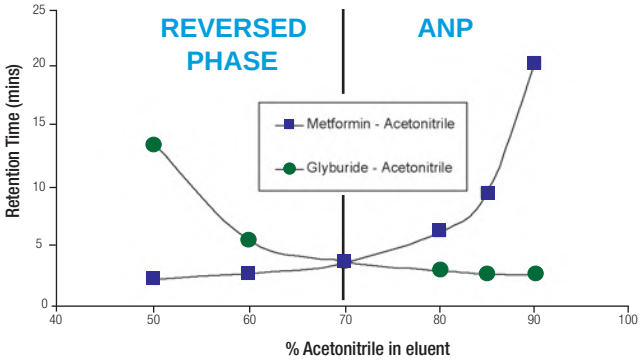



Figure 3. Dual RP and ANP retention capability

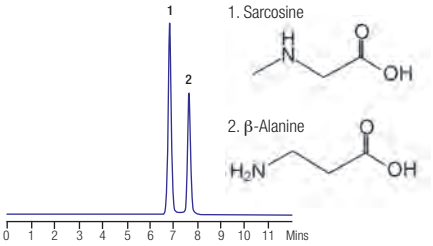
## Aqueous Normal-Phase (ANP)

Cogent TYPE-C silica based phases (Bidentate C18, Bidentate C8, UDC-Cholesterol, Diamond Hydride, Phenyl Hydride, UDA and Silica-C) have the ability to operate in ANP mode which enables the retention of polar solutes at high concentrations of the organic component whilst maintaining an aqueous component in the eluent. The exact point in the composition of the eluent where ANP retention begins depends on the solute as well as the stationary phase. In addition, TYPE-C columns can also retain non-polar compounds based on a typical reversed-phase mechanism. Figure 3 illustrates the dual retention capability for both polar (metformin) and non-polar (glyburide) compounds. In this case, with an eluent composition of less than 70% acetonitrile, glyburide and metformin are both retained by a reversed-phase mechanism, with the metformin eluting first. With increasing percentages of acetonitrile, the retention of metformin increases significantly due to ANP mechanisms and now elutes after glyburide (see Figure 9 on page 117).

## Cogent Diamond Hydride™ (The 'Metabolomics' Column)

- Excellent peak shape
- Useful for high polarity compounds
- Retain amino acids without derivatisation
- Excellent choice for metabolomics

Cogent Diamond Hydride™, containing a low level of carbon on the surface, is a unique phase for the analysis of extremely polar compounds by aqueous normal-phase (see page 115). It is a popular choice for the analysis of metabolites, underivatized amino acids, carbohydrates and small organic acids. Figure 4 shows the separation of sarcosine, a potential urine biomarker, from isobaric β-alanine on a Cogent Diamond Hydride LC-MS column. Figure 5 shows the separation of four nucleotide bases on Cogent Diamond Hydride under 100% aqueous conditions.

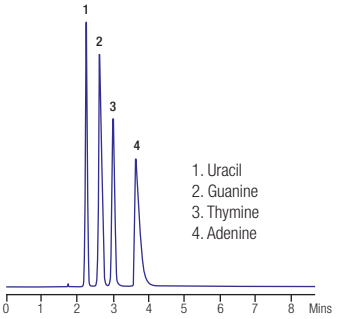



Column: Cogent Diamond Hydride (4µm, 150 x 2.1mm)
Eluent: A. IPA – DI $H_2O$ (50:50) with 0.1% acetic acid
B. $CH_3CN$ – DI water (97:3) with 0.1% acetic acid

| Gradient: | Time (mins) | %B | Time (mins) | %B |
|---|---|---|---|---|
| | 0 | 75 | 5 | 65 |
| | 3 | 75 | 10 | 20 |
| | 4 | 65 | 12 | 75 |

Flow rate: 0.6ml/min Detection: ESI (+ve) Temperature: 50°C

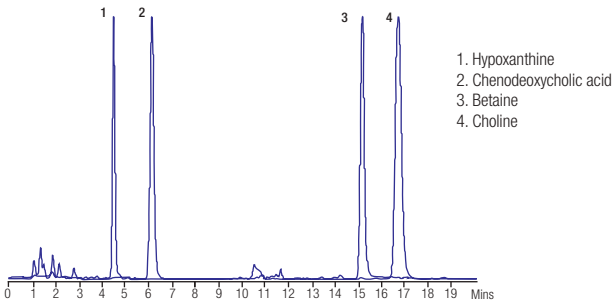
Figure 4. Separation of sarcosine and β-alanine




Column: Cogent Diamond Hydride (4µm, 75 x 4.6mm)
Eluent: 0.1% acetic acid in DI water
Flow rate: 1ml/min
Detection: UV, 254nm
Temperature: 25°C

Figure 5. Separation of nucleotide bases

Figure 6 shows the separation of metabolites in human urine and Figure 7 shows the analysis of sumatriptan tablets using Cogent Diamond Hydride columns.

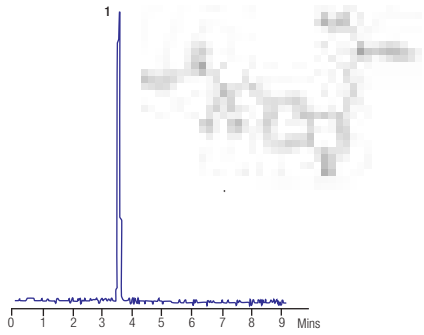



Column: Cogent Diamond Hydride (4µm, 150 x 2.1mm)
Eluent: A: 0.1% formic acid in $H_2O$
B: 0.1% formic acid in $CH_3CN$

| Gradient: Time (mins) | 0 | 0.2 | 30 | 35 | 35.1 | 40 |
|---|---|---|---|---|---|---|
| %B | 95 | 95 | 50 | 50 | 95 | 95 |

Flow rate: 0.4ml/min
Detection: ESI(+)

Figure 6. Analysis of metabolites in human urine




Column: Cogent Diamond Hydride (2.2µm, 50 x 2.1mm)
Eluent: A: 0.1% formic acid in $H_2O$
B: 0.1% formic acid in $CH_3CN$

| Gradient: Time (mins) | 0 | 4 | 6 | 7 |
|---|---|---|---|---|
| %B | 90 | 30 | 30 | 90 |

Flow rate: 0.4ml/min
Detection: ESI (+)

Figure 7. Analysis of sumatriptan tablets

## Metabolomics Kits

Metabolomics kits are ideal for LC-MS when searching for very polar and very hydrophobic compounds from serum, plasma, urine, saliva or any other biological fluid. They consist of one Cogent Diamond Hydride and one Cogent Bidentate C18 column of the same dimensions. Using both aqueous normal-phase and reversed-phase with these two columns, both positive and negative species can be separated and very accurate 'metabolomes' can be produced. Please enquire for further details.

## Cogent 2.ō™ HPLC Columns

Cogent 2.o silica hydride columns have a particle size of 2.2µm and show increased efficiencies compared to the 4µm phases. They have an upper pressure limit of 9,500 psi. The part numbers for Cogent Diamond Hydride and Bidentate C18 2.o HPLC columns are shown on page 120. Please enquire for the availability of alternative dimensions and further phases.

Please see page 120 for ordering information.

## Cogent Bidentate C18™

- Use in RP, NP and ANP modes
- Robust and reliable
- Useful up to pH 10

The C18 in Cogent Bidentate C18™ is bonded directly to the TYPE-C™ silica surface with two separate points of attachment (Figure 8). Columns are highly durable and suitable for use with 100% water or 100% organic solvent. These columns will retain the most polar compounds with ≥ 70% organic content. Figure 9 shows the separation of polar metformin and non-polar glyburide by aqueous normal-phase (see page 115) and reversed-phase methods.

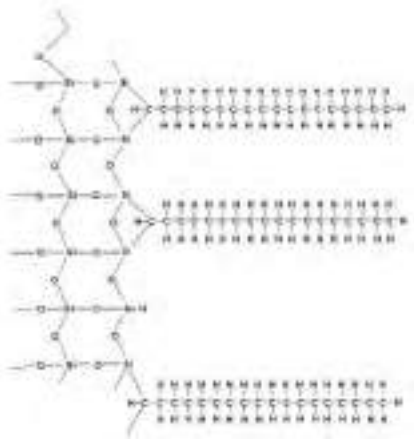


Figure 8. Bonding in Cogent Bidentate C18

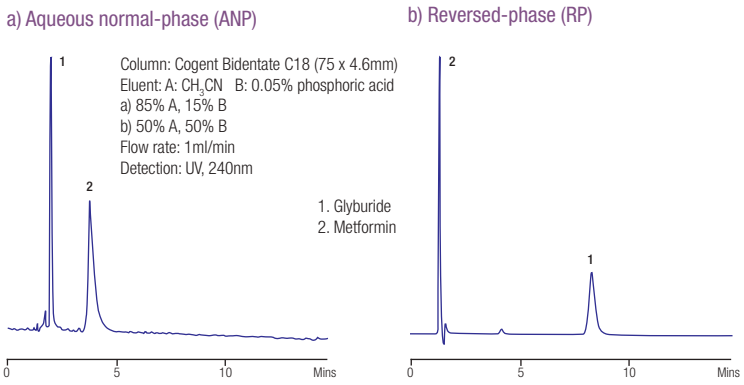
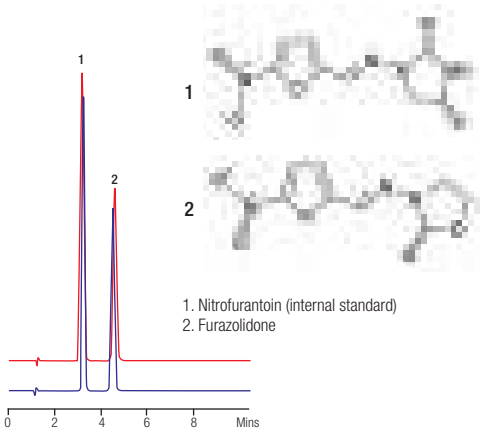



Figure 9. Separation of polar and non-polar compounds

## Cogent Bidentate C8™

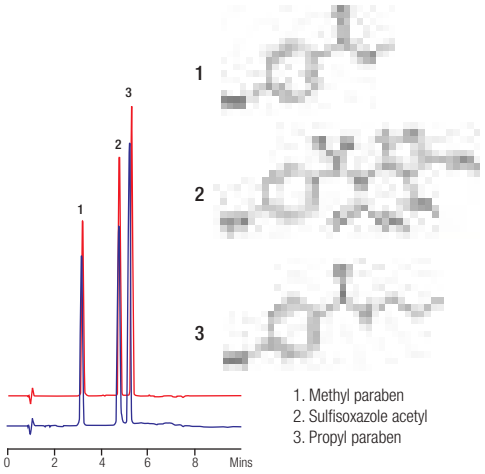
- Good starter column for ANP
- Polar and non-polar analytes
- Useful for complex mixtures

As with Cogent Bidentate C18, the Cogent Bidentate C8™ phase is bonded directly to the TYPE-C silica surface with two separate points of attachment. Columns are stable and efficient and can operate in RP, NP and ANP modes. Figure 10 shows the separation of furazolidone and nitrofurantoin. Figure 11 shows the separation of sulfisoxazole acetyl from the preservatives methyl paraben and propyl paraben. A 300Å phase, as well as the 100Å phase, is available.




Column: Cogent Bidentate C8 (4µm, 75 x 4.6mm)
Eluent: $H_2O$ – $CH_3CN$ (80:20) with 0.1% formic acid
Flow rate: 1ml/min
Detection: UV, 367nm

Figure 10. Analysis of furazolidone on Cogent Bidentate C8




Column: Cogent Bidentate C8 (4µm, 75 x 4.6mm)
Eluent: A: 0.1% TFA in $H_2O$
B: 0.1% TFA in $CH_3CN$
Gradient: : Time (mins) 0 1 6 7
%B 30 30 60 30
Flow rate: 1ml/min
Detection: UV, 254nm

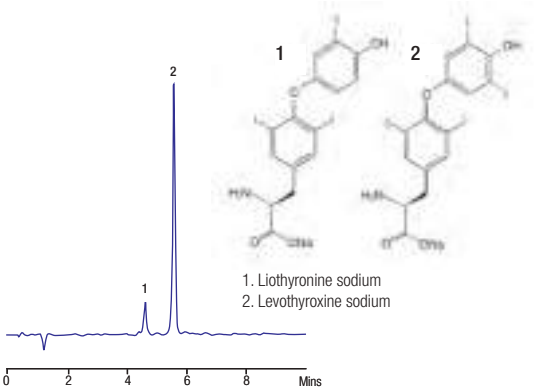
Figure 11. Analysis of sulfisoxazole acetyl

Please see page 120 for ordering information.

## Cogent Phenyl Hydride™

- Good selectivity for aromatic compounds
- Use in RP or ANP modes
- Long column lifetime

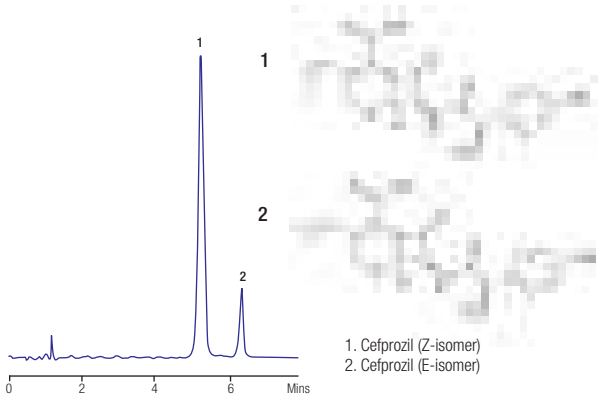
Cogent Phenyl Hydride™ columns, based on TYPE-C silica, are an ideal choice for difficult to separate aromatic compounds. Due to the lack of endcapping, these columns do not exhibit 'bleed', making them ideal for LC-MS analyses. Direct silicon-carbon bonds and the hydride surface enable long column lifetimes to be achieved. Figure 12 shows the separation of levothyroxine and liothyronine on a Cogent Phenyl Hydride column. Figure 13 shows the separation of the cis and trans isomers of the antibiotic cefprozil.




1. Liothyronine sodium
2. Levothyroxine sodium

Column: Cogent Phenyl Hydride (4µm, 75 x 4.6mm)
Eluent: A: 0.1% formic acid in $H_2O$
B: $CH_3CN$ – $H_2O$ – formic acid (97:3:0.1)
Gradient: Time (mins) 0 6 7
%B 20 50 20
Flow rate: 1ml/min Temperature: 35°C

Figure 12. Analysis of levothyroxine




1. Cefprozil (Z-isomer)
2. Cefprozil (E-isomer)

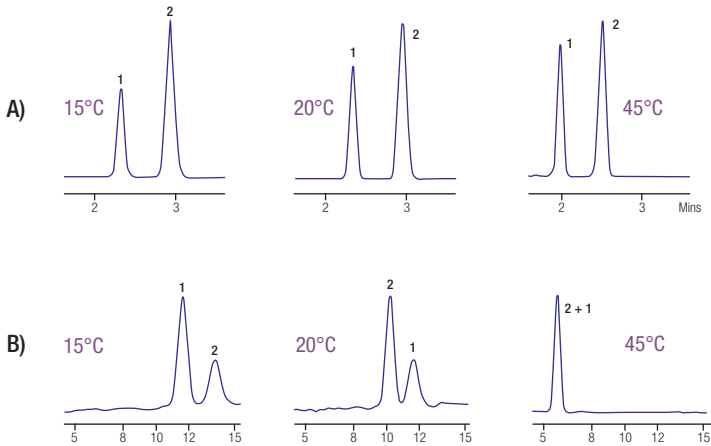
Column: Cogent Phenyl Hydride (4µm, 75 x 4.6mm)
Eluent: A: 0.1% formic acid in $H_2O$
B: 0.1% formic acid in $CH_3CN$
Gradient: Time (mins) 0 6 7
%B 5 20 5
Flow rate: 1ml/min
Temperature: 45°C
Detection: UV, 280nm

Figure 13. Separation of cefprozil isomers

## Cogent UDC-Cholesterol™

- Operable in RP, NP and ANP modes
- Good shape recognition (eg. for steroids)
- Unique selectivity
- Medium range of hydrophobicity

The Cogent UDC-Cholesterol™ stationary phase is a liquid crystal attached to a silica-hydride particle and produces unique selectivity for many compounds, especially at lower temperatures. Figure 14 shows that Cogent UDC-Cholesterol with acetonitrile as organic modifier produced similar results for the separation of the steroids ethinyl estradiol and norgestrel at the three different temperatures shown, with negligible effect on selectivity and retention. However, using methanol as modifier relative retention times differ considerably and elution order is reversed. This indicates that when used with methanol, Cogent UDC-Cholesterol has a selectivity mechanism based on shape recognition.




1. Ethinyl estradiol
2. Norgestrel

Column: Cogent UDC-Cholesterol (4µm, 75 x 4.6mm)
Eluent: (A) $CH_3CN$ - 0.1% TFA (50:50)
(B) $CH_3OH$ - 0.1% TFA (55:45)
Flow rate: 1ml/min
Detection: UV, 240nm

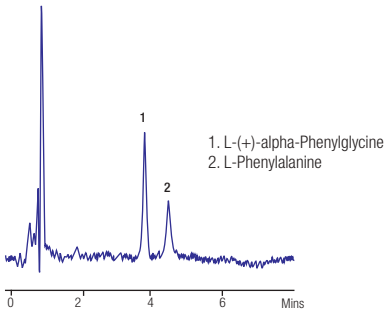
Figure 14. Molecular shape recognition of Cogent UDC-Cholesterol

Please see page 120 for ordering information.

## Cogent Silica-C™

- Useful for highly polar compounds
- Very rugged and reproducible
- Suitable for NP and ANP applications

For the retention of very polar compounds, Cogent Silica-C™ is the best choice from the TYPE-C™ suite of columns. Since this unbonded phase has virtually no silanols, it does not have a strong association with water and other solvents and will not have the expected hydration shell of other silica based columns. Cogent Silica-C is suitable for NP and ANP applications and for preparative chromatography. Figure 15 shows the separation of underivatised L-(+)-alpha-phenylglycine and L-phenylalanine under LC-MS conditions.




Column: Cogent Silica-C (4µm, 75 x 4.6mm)
Eluent: 0.1% formic acid in $H_2O$ – 0.1% formic acid in $CH_3CN$ (20:80)
Flow rate: 1ml/min
Detection: UV, 254nm


Figure 15. Separation of aromatic amino acids

## Other Cogent (High Purity Type B) Columns

In addition to their unique TYPE-C silica phases, MicroSolv also manufacture a range of phases based on 'conventional' Type B silica. These phases include C18, C8, Amino, Phenyl, Cyano, Silica, UPHOLD C27 and C30. The specifications of the longer chain phases are shown below.

### Longer Chain Hydrophobic Columns

| Cogent Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | pH Range | Max. Temp. (°C) |
|---|---|---|---|---|---|---|---|
| C30 | 3, 5 | 200 | n/a | 18 | No | 2 - 8 | 40 |
| UPHOLD C27 | 5 | 120 | 300 | 17 | Yes | 2 - 8 | 60 |

Cogent C30 shows good selectivity characteristics for the separation of complex carotenoid mixtures. The longer C30 ligands show enhanced interactions with lipophilic long chain carotenoid compounds. In addition to reversed-phase interactions, geometrical and positional isomers of the conjugated double bond systems can be differentiated by analyte shape. At lower temperatures, the long alkyl chains become more rigid and steric effects become significant, leading to greater selectivity. This is illustrated in Figure 16 for a complex carotenoid mixture. Cogent C30 columns are also suitable for the analysis of peptides and some proteins.

1. Astaxanthin
2. Capsanthin
3. Lutein
4. Zeaxanthin
5. Canthaxanthin
6. β-Cryptoxanthin
7. Echinenone
8. 15-cis-β-carotene
9. 13-cis-β-carotene
10. α-Carotene
11. trans-β-Carotene
12. 9-cis-β-Carotene
13. δ-Carotene
14. Lycopene

Column: Cogent C30 (5µm, 200Å, 250 x 4.6mm)
Eluent: A: $CH_3OH$ – MTBE – $H_2O$ (81:15:4)
B: $CH_3OH$ – MTBE – $H_2O$ (6:90:4)
Gradient: 0 to 100% B over 90 mins.
Temperature: 20°C
Detection: UV, 450nm

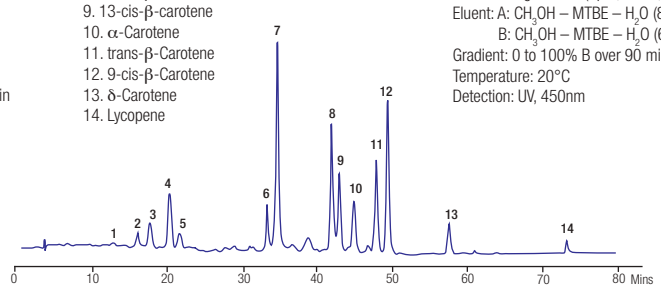



Figure 16. Separation of carotenoids

Please contact us for ordering information on all Cogent Type B columns.

## Ordering Information – Cogent TYPE-C™ Silica Analytical Columns

| Cogent Phase 4µm | Column Dimensions (mm) | | | | | Guard Columns[1, 2] (20 x 2mm, 5/pk) |
|---|---|---|---|---|---|---|
| | 50 x 2.1 | 75 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| Silica-C | 40000-05P-2 | 40000-7.5P-2 | 40000-10P-2 | 40000-15P-2 | 40000-25P-2 | 40000-GC2 |
| Bidentate C18 | 40018-05P-2 | 40018-75P-2 | 40018-10P-2 | 40018-15P-2 | 40018-25P-2 | 40018-GC2 |
| Bidentate C8 | 40008-05P-2 | 40008-75P-2 | 40008-10P-2 | 40008-15P-2 | 40008-25P-2 | 40008-GC2 |
| Phenyl Hydride | 69020-05P-2 | - | 69020-10P-2 | 69020-15P-2 | 69020-25P-2 | 69020-GC1 |
| UDC-Cholesterol | 69069-05P-2 | 69069-7.5P-2 | 69069-10P-2 | 69069-15P-2 | 69069-25P-2 | 69069-GC2 |
| Diamond Hydride | 70000-05P-2 | 70000-7.5P-2 | 70000-10P-2 | 70000-15P-2 | 70000-25P-2 | 70000-GC1 |
| Bidentate C8 300 | 40008-05P-2-3M | 40008-75P-2-3M | 40008-10P-2-3M | 40008-15P-2-3M | 40008-25P-2-3M | - |

| Cogent Phase 4µm | Column Dimensions (mm) | | | | | Guard Columns[1, 2] (20 x 2mm, 5/pk) |
|---|---|---|---|---|---|---|
| | 50 x 3.0 | 75 x 3.0 | 100 x 3.0 | 150 x 3.0 | 250 x 3.0 | |
| Silica-C | 40000-05P-3 | - | 40000-10P-3 | 40000-15P-3 | - | 40000-GC2 |
| Bidentate C18 | 40018-05P-3 | - | 40018-10P-3 | 40018-15P-3 | - | 40018-GC2 |
| Bidentate C8 | 40008-05P-3 | - | 40008-10P-3 | 40008-15P-3 | - | 40008-GC2 |
| Phenyl Hydride | 69020-05P-3 | - | 69020-10P-3 | 69020-15P-3 | 69020-25P-3 | 69020-GC1 |
| UDC-Cholesterol | 69069-05P-3 | - | 69069-10P-3 | 69069-15P-3 | 69069-25P-3 | 69069-GC2 |
| Diamond Hydride | 70000-05P-3 | - | 70000-10P-3 | 70000-15P-3 | 70000-25P-3 | 70000-GC1 |
| Bidentate C8 300 | - | - | - | - | - | - |

| Cogent Phase 4µm | Column Dimensions (mm) | | | | | Guard Columns[1, 2] (20 x 4mm, 5/pk) |
|---|---|---|---|---|---|---|
| | 50 x 4.6 | 75 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| Silica-C | 40000-05P | 40000-7.5P | 40000-10P | 40000-15P | 40000-25P | 40000-GC3 |
| Bidentate C18 | 40018-05P | 40018-75P | 40018-10P | 40018-15P | 40018-25P | 40018-GC3 |
| Bidentate C8 | 40008-05P | 40008-75P | 40008-10P | 40008-15P | 40008-25P | 40008-GC3 |
| Phenyl Hydride | 69020-05P | 69020-75P | 69020-10P | 69020-15P | 69020-25P | 69020-GC2 |
| UDC-Cholesterol | 69069-05P | 69069-7.5P | 69069-10P | 69069-15P | 69069-25P | 69069-GC3 |
| Diamond Hydride | 70000-05P | 70000-7.5P | 70000-10P | 70000-15P | 70000-25P | 70000-GC2 |
| Bidentate C8 300 | - | 40008-75P-3M | 40008-10P-3M | 40008-15P-3M | 40008-25P-3M | - |

[1] Requires universal guard holder 81000-GD [2] 1mm guards also available. Alternative holder required

## High Throughput Columns

| Cogent Phase 4µm | Column Dimensions (mm) | |
|---|---|---|
| | 20 x 2.1 | 30 x 2.1 |
| Diamond Hydride | 70000-02P-2 | 70000-03P-2 |
| Phenyl Hydride | 69020-02P-2 | 69020-03P-2 |

## Semi-preparative and Preparative Columns

| Cogent Phase 4µm | Column Dimensions (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 100 x 10.0 | 150 x 10.0 | 250 x 10.0 | 100 x 21.2 | 150 x 21.2 | 250 x 21.2 |
| Silica-C | - | 40000-SP150 | - | 40000-P21-100 | 40000-P21-150 | 40000-P21-250 |
| Bidentate C18 | 40018-SP100 | 40018-SP150 | 40018-SP250 | 40018-P21-100 | 40018-P21-150 | 40018-P21-250 |
| Bidentate C8 | 40008-SP100 | 40008-SP150 | 40008-SP250 | 40008-P21-100 | 40008-P21-150 | 40008-P21-250 |
| UDC-Cholesterol | 69069-SP100 | 69069-SP150 | - | 69069-P21-100 | 69069-P21-150 | 69069-P21-250 |
| Diamond Hydride | 70000-SP100 | 70000-SP150 | 70000-SP250 | 70000-P21-100 | 70000-P21-150 | 70000-P21-250 |

## Cogent 2.ō Phase Columns

| Cogent 2.ō Phase | Column Dimensions[1] (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 20 x 2.1 | 30 x 2.1 | 50 x 2.1 | 75 x 2.1 | 100 x 2.1 | 150 x 2.1 |
| Silica-C | 40200-02P-2 | 40200-03P-2 | 40200-05P-2 | 40200-75P-2 | 40200-10P-2 | 40200-15P-2 |
| Bidentate C18 | 40218-02P-2 | 40218-03P-2 | 40218-05P-2 | 40218-75P-2 | 40218-10P-2 | 40218-15P-2 |
| Diamond Hydride | 70200-02P-2 | 70200-03P-2 | 70200-05P-2 | 70200-75P-2 | 70200-10P-2 | 70200-15P-2 |

[1] Other column dimensions available – please enquire

# PARTISIL® and PARTISPHERE®



- Acquired by Hichrom from Whatman, a division of GE Healthcare, in 2012
- Choice of spherical or irregular silica
- Wide range of surface chemistries

### Partisil® and Partisphere® Acquisition

Hichrom acquired the Partisil and Partisphere HPLC column range from Whatman, a division of GE Healthcare, in 2012. Hichrom worked closely with GE Healthcare during the manufacture of these products for a number of years prior to acquisition and, as such, manufacturing protocols, product specifications and part numbers remain unaffected. All column dimensions previously offered by Whatman/GE Healthcare remain available and are detailed on the following pages.

### Hichrom Partisil Columns – Non Standard Whatman/GE Healthcare Dimensions

Hichrom have a deserved reputation for the ability to manufacture and supply high efficiency columns packed into non standard dimensions not originally offered by the silica manufacturer. Partisil columns are no exception, as for many years Hichrom have manufactured Partisil columns into a wider range of column dimensions than available from Whatman/GE Healthcare. These additional dimensions will continue to be supported following the acquisition and are detailed on pages 124-125.

## Partisil Phases

- Irregular porous silica
- 5 and 10µm particle sizes
- Hichrom high efficiency

Partisil was one of the first commercially available irregular silicas. A large surface area gives it a high loading capacity. Partisil ion-exchange materials are widely referenced and remain one of the most popular choices for analysts. Partisil 10 ODS3 is similar to Waters µBondapak.

### Partisil Phases

| Partisil Phase | Functional Group | Particle Size (µm) |
|---|---|---|
| Silica | - | 5, 10 |
| C8 | Octyl | 5, 10 |
| ODS | Octadecyl | 10 |
| ODS2 | Octadecyl | 10 |
| ODS3 | Octadecyl | 5, 10 |
| PAC | Amino-cyano | 5, 10 |
| SAX | Tetramethyl ammonium | 5, 10 |
| SCX | Sulphonic acid | 5, 10 |

**Partisil ODS** is a low carbon load C18 phase, with intentionally unbonded surface silanols adding to the selectivity.

**Partisil ODS2** is a high carbon load C18 bonded silica phase which makes it the most non-polar and, therefore, the most retentive of the Partisil reversed-phase columns. The high sample load capacity is suitable for preparative work.

**Partisil ODS3** is an intermediate carbon load C18 phase used for pharmaceutical, natural products, food, biological and environmental pollutants applications. Partisil 10 ODS3 is similar to Waters µBondapak.

**Partisil C8** is recommended for ion-pair chromatography.

**Partisil PAC** is a Polar Amino Cyano bonded phase with secondary amine groups for good thermal and chemical stability. Fast equilibration enables multiple separation mechanisms, including adsorption, reversed-phase and weak anion-exchange, to be used. The phase is particularly suitable for carbohydrate separations.

**Partisil SAX** is a strong anion-exchange phase based on quaternary ammonium groups. It is stable over the pH range 1.5 to 7.5. At intermediate pH, use of a Solvecon eluent conditioning column (see part number 4250-001) is recommended to further enhance column lifetime. Typical applications include nucleic acids, organic acids and inorganic anions.

**Partisil SCX** is a strong cation-exchange phase based on benzenesulphonic acid groups and is stable over the pH range 1.5 to 7.0. At intermediate pH, use of a Solvecon eluent conditioning column (see part number 4251-001) is recommended to further enhance column lifetime. Suitable applications include nucleosides, amino acids, polyamines, drugs and other cationic species.

See page 122 for ordering information for Partisil columns in WCS hardware and dimensions previously offered by Whatman/GE Healthcare.
See pages 124-125 for Hichrom Partisil columns in a wider range of dimensions.

## Partisphere® Phases

- Spherical 5µm porous silica
- Unique void sealing cartridge hardware
- Convenient and easy to use – hand tightened fittings
- Increased column lifetime

Partisphere® was one of the first commercially available spherical silicas and continues to provide reproducible, high efficiency separations. Partisphere columns are available in a wide range of surface chemistries, including two application specific phases: TAC-1 (Taxane Analysis Column) for the analysis of paclitaxel and other taxanes, and WAX (MAX-1) for corn and soy protein.

### Partisphere Phases

| Partisphere Phase | Functional Group | Particle Size (µm) |
|---|---|---|
| Silica | - | 5 |
| C8 | Octyl | 5 |
| C18 | Octadecyl | 5 |
| PAC | Amino-cyano | 5 |
| SAX | Quaternary ammonium | 5 |
| SCX | Sulphonic acid | 5 |
| TAC-1 (PFP) | Pentafluorophenyl | 5 |
| WAX (MAX-1) | Proprietary | 5 |

### Unique Void-sealing Cartridge System

Partisphere columns are available in a unique void sealing (WVS) hardware. If a void eventually forms at the top of the column bed, a simple hand tightening of the inlet fitting moves the frit assembly downwards and recompresses the packed bed, thus removing the void and restoring column efficiency. Large knurled end fittings allow ready hand tightening of the system. All WVS cartridge columns are shipped without end fittings and require end fitting kit, catalogue no. 4631-1001. This item can be interchanged with additional WVS columns.

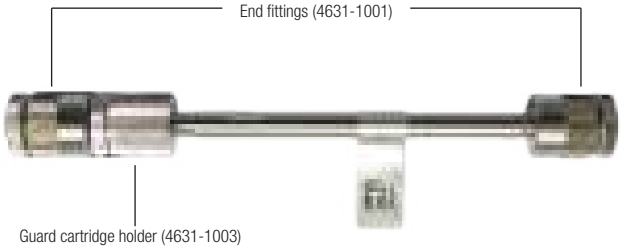



Figure 1. Partisphere WVS hardware cartridge system

### Partisphere Guard Cartridge System

Additional installation of the unique WVS guard cartridge holder (catalogue no. 4631-1003) allows the use of guard cartridges (see Figure 1).

## Ordering Information - Partisil® and Partisphere®

### Partisil 5µm Analytical Columns

| Catalogue No. | Description |
|---|---|
| 4222-225 | Partisil 5µm ODS3 column, 100 x 4.6mm (WCS hardware - RAC-II) |
| 4222-227 | Partisil 5µm SAX column, 100 x 4.6mm (WCS hardware - RAC-II) |
| 4222-228 | Partisil 5µm SCX column, 100 x 4.6mm (WCS hardware - RAC-II) |
| 4222-232 | Partisil 5µm C8 column, 100 x 4.6mm (WCS hardware - RAC-II) |
| 4215-001 | Partisil 5µm SIL column, 250 x 4.6mm (WCS hardware) |
| 4235-001 | Partisil 5µm PAC column, 250 x 4.6mm (WCS hardware) |
| 4238-001 | Partisil 5µm ODS3 column, 250 x 4.6mm (WCS hardware) |
| 4239-001 | Partisil 5µm C8 column, 250 x 4.6mm (WCS hardware) |

### Partisil 10µm Analytical Columns

| Catalogue No. | Description |
|---|---|
| 4216-001 | Partisil 10µm SIL column, 250 x 4.6mm (WCS hardware) |
| 4223-001 | Partisil 10µm ODS column, 250 x 4.6mm (WCS hardware) |
| 4224-001 | Partisil 10µm ODS2 column, 250 x 4.6mm (WCS hardware) |
| 4225-001 | Partisil 10µm PAC column, 250 x 4.6mm (WCS hardware) |
| 4226-001 | Partisil 10µm SAX column, 250 x 4.6mm (WCS hardware) |
| 4227-001 | Partisil 10µm SCX column, 250 x 4.6mm (WCS hardware) |
| 4228-001 | Partisil 10µm ODS3 column, 250 x 4.6mm (WCS hardware) |
| 4229-001 | Partisil 10µm C8 column, 250 x 4.6mm (WCS hardware) |
| 4141-001 | Partisil 10µm SCX column (S-P), 250 x 4.6mm (WCS hardware) |

## Ordering Information - Partisil® and Partisphere® (continued)

### Partisil 10µm Semi-Prep Columns

| Catalogue No. | Description |
|---|---|
| 4230-120 | Partisil 10µm SIL column, 250 x 9.4mm (Magnum-9 hardware) |
| 4230-124 | Partisil 10µm ODS2 column, 250 x 9.4mm (Magnum-9 hardware) |
| 4230-125 | Partisil 10µm ODS3 column, 250 x 9.4mm (Magnum-9 hardware) |
| 4230-126 | Partisil 10µm PAC column, 250 x 9.4mm (Magnum-9 hardware) |
| 4230-220 | Partisil 10µm SIL column, 500 x 9.4mm (Magnum-9 hardware) |
| 4230-224 | Partisil 10µm ODS2 column, 500 x 9.4mm (Magnum-9 hardware) |
| 4230-225 | Partisil 10µm ODS3 column, 500 x 9.4mm (Magnum-9 hardware) |
| 4232-125 | Partisil 10µm ODS3 column, 250 x 20mm (Magnum-20 hardware) |
| 4232-220 | Partisil 10µm SIL column, 500 x 20mm (Magnum-20 hardware) |

### Partisphere 5µm Analytical Columns (note: WVS cartridge columns require end fitting kit p/n 4631-1001)

| Catalogue No. | Description |
|---|---|
| 4120-001 | Partisphere 5µm WAX (MAX-1) column, 250 x 4.6mm (WCS hardware) |
| 4601-1001 | Partisphere 5µm TAC-1 (PFP) cartridge column, 265 x 4.6mm (WVS hardware) |
| 4621-0501 | Partisphere 5µm SIL cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-0502 | Partisphere 5µm C18 cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-0503 | Partisphere 5µm C8 cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-0505 | Partisphere 5µm SAX cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-0507 | Partisphere 5µm SCX cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-0508 | Partisphere 5µm PAC cartridge column, 125 x 4.6mm (WVS hardware) |
| 4621-1501 | Partisphere 5µm SIL cartridge column, 250 x 4.6mm (WVS hardware) |
| 4621-1502 | Partisphere 5µm C18 cartridge column, 250 x 4.6mm (WVS hardware) |
| 4621-1505 | Partisphere 5µm SAX cartridge column, 250 x 4.6mm (WVS hardware) |
| 4621-1507 | Partisphere 5µm SCX cartridge column, 250 x 4.6mm (WVS hardware) |
| 4621-1508 | Partisphere 5µm PAC cartridge column, 250 x 4.6mm (WVS hardware) |

### Partisil Analytical Cartridge Columns (note: WVS cartridge columns require end fitting kit p/n 4631-1001)

| Catalogue No. | Description |
|---|---|
| 4681-0502 | Partisil 5µm ODS3 cartridge column, 125 x 4.6mm (WVS hardware) |
| 4681-0505 | Partisil 5µm SAX cartridge column, 125 x 4.6mm (WVS hardware) |
| 4681-1501 | Partisil 5µm SIL cartridge column, 250 x 4.6mm (WVS hardware) |
| 4681-1502 | Partisil 5µm ODS3 cartridge column, 250 x 4.6mm (WVS hardware) |
| 4681-1505 | Partisil 5µm SAX cartridge column, 250 x 4.6mm (WVS hardware) |
| 4681-1507 | Partisil 5µm SCX cartridge column, 250 x 4.6mm (WVS hardware) |
| 4681-1509 | Partisil 5µm ODS2 cartridge column, 250 x 4.6mm (WVS hardware) |
| 4682-1502 | Partisil 10µm ODS3 cartridge column, 250 x 4.6mm (WVS hardware) |
| 4682-1505 | Partisil 10µm SAX cartridge column, 250 x 4.6mm (WVS hardware) |
| 4682-1507 | Partisil 10µm SCX cartridge column, 250 x 4.6mm (WVS hardware) |

### Accessories

| Catalogue No. | Description |
|---|---|
| 4250-001 | Partisil 10µm SAX column with Solvecon Kit |
| 4251-001 | Partisil 10µm SCX column with Solvecon Kit |
| 4631-1001 | Wrenchless WVS End Fitting Kit |
| 4631-1003 | WVS Guard Cartridge Holder |
| 4631-1004 | WCS Guard Cartridge Holder |
| 4641-0001 | Silica Guard Cartridge (5 pack) |
| 4641-0002 | Reverse Phase Guard Cartridge (5 pack) |
| 4641-0005 | Anion Guard Cartridge (5 pack) |
| 4641-0007 | Cation Phase Guard Cartridge (5 pack) |
| 4641-0008 | PAC Guard Cartridge (5 pack) |
| HI-050X | PEEK Fingertight Fitting (10 pack) – suitable for connection of all Partisil and Partisphere columns to all 1/16″ o.d. tubing types – slip free to 6000psi. |

See also pages 124 and 125 for additional Partisil column dimensions and ordering information.

## Hichrom Partisil® Columns – Non Standard Whatman/GE Healthcare Dimensions

Hichrom have a deserved reputation for the ability to manufacture and supply high efficiency columns packed into non standard dimensions not originally offered by the silica manufacturer. Partisil® columns are no exception and for many years Hichrom have manufactured Partisil columns into a wider range of column dimensions than was originally available from Whatman/GE Healthcare. These additional dimensions continue to be supported following the acquisition and are detailed below. Please contact Hichrom for any column dimension not listed.


### Ordering Information – Partisil Additional Dimensions

### Partisil 2.1mm i.d .Columns

| Partisil Phase | Column Dimensions (mm) | | | | Guard Cartridges[1,2] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| Partisil 5 SIL | P5-50AM | P5-100AM | P5-150AM | P5-250AM | P5-10CM5 |
| Partisil 5 C8 | P5C8-50AM | P5C8-100AM | P5C8-150AM | P5C8-250AM | P5C8-10CM5 |
| Partisil 5 ODS3 | P5ODS3-50AM | P5ODS3-100AM | P5ODS3-150AM | P5ODS3-250AM | P5ODS3-10CM5 |
| Partisil 5 PAC | P5PAC-50AM | P5PAC-100AM | P5PAC-150AM | P5PAC-250AM | P5PAC-10CM5 |
| Partisil 5 SAX | P5SAX-50AM | P5SAX-100AM | P5SAX-150AM | P5SAX-250AM | P5SAX-10CM5 |
| Partisil 5 SCX | P5SCX-50AM | P5SCX-100AM | P5SCX-150AM | P5SCX-250AM | P5SCX-10CM5 |
| Partisil 10 SIL | P10-50AM | P10-100AM | P10-150AM | P10-250AM | P10-10CM5 |
| Partisil 10 C8 | P10C8-50AM | P10C8-100AM | P10C8-150AM | P10C8-250AM | P10C8-10CM5 |
| Partisil 10 ODS | P10ODS-50AM | P10ODS-100AM | P10ODS-150AM | P10ODS-250AM | P10ODS-10CM5 |
| Partisil 10 ODS2 | P10ODS2-50AM | P10ODS2-100AM | P10ODS2-150AM | P10ODS2-250AM | P10ODS2-10CM5 |
| Partisil 10 ODS3 | P10ODS3-50AM | P10ODS3-100AM | P10ODS3-150AM | P10ODS3-250AM | P10ODS3-10CM5 |
| Partisil 10 PAC | P10PAC-50AM | P10PAC-100AM | P10PAC-150AM | P10PAC-250AM | P10PAC-10CM5 |
| Partisil 10 SAX | P10SAX-50AM | P10SAX-100AM | P10SAX-150AM | P10SAX-250AM | P10SAX-10CM5 |
| Partisil 10 SCX | P10SCX-50AM | P10SCX-100AM | P10SCX-150AM | P10SCX-250AM | P10SCX-10CM5 |

[1] Use with holder HI-161 and column coupler HI-081 [2] 5/pk – Starter kits also available – see p.21

### Partisil 3.2mm i.d. Columns

| Partisil Phase | Column Dimensions (mm) | | | | Guard Cartridges[1,2] (For 3.2-4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| Partisil 5 SIL | P5-50AS | P5-100AS | P5-150AS | P5-250AS | P5-10C5 |
| Partisil 5 C8 | P5C8-50AS | P5C8-100AS | P5C8-150AS | P5C8-250AS | P5C8-10C5 |
| Partisil 5 ODS3 | P5ODS3-50AS | P5ODS3-100AS | P5ODS3-150AS | P5ODS3-250AS | P5ODS3-10C5 |
| Partisil 5 PAC | P5PAC-50AS | P5PAC-100AS | P5PAC-150AS | P5PAC-250AS | P5PAC-10C5 |
| Partisil 5 SAX | P5SAX-50AS | P5SAX-100AS | P5SAX-150AS | P5SAX-250AS | P5SAX-10C5 |
| Partisil 5 SCX | P5SCX-50AS | P5SCX-100AS | P5SCX-150AS | P5SCX-250AS | P5SCX-10C5 |
| Partisil 10 SIL | P10-50AS | P10-100AS | P10-150AS | P10-250AS | P10-10C5 |
| Partisil 10 C8 | P10C8-50AS | P10C8-100AS | P10C8-150AS | P10C8-250AS | P10C8-10C5 |
| Partisil 10 ODS | P10ODS-50AS | P10ODS-100AS | P10ODS-150AS | P10ODS-250AS | P10ODS-10C5 |
| Partisil 10 ODS2 | P10ODS2-50AS | P10ODS2-100AS | P10ODS2-150AS | P10ODS2-250AS | P10ODS2-10C5 |
| Partisil 10 ODS3 | P10ODS3-50AS | P10ODS3-100AS | P10ODS3-150AS | P10ODS3-250AS | P10ODS3-10C5 |
| Partisil 10 PAC | P10PAC-50AS | P10PAC-100AS | P10PAC-150AS | P10PAC-250AS | P10PAC-10C5 |
| Partisil 10 SAX | P10SAX-50AS | P10SAX-100AS | P10SAX-150AS | P10SAX-250AS | P10SAX-10C5 |
| Partisil 10 SCX | P10SCX-50AS | P10SCX-100AS | P10SCX-150AS | P10SCX-250AS | P10SCX-10C5 |

[1] Use with holder HI-161 and column coupler HI-081 [2] 5/pk – Starter kits also available – see p.21

## Ordering Information – Partisil® Additional Dimensions (continued)

### Partisil 4.0mm i.d. Columns

| Partisil Phase | Column Dimensions (mm) | | | | Guard Cartridges[1,2] (For 3.2-4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.0 | 100 x 4.0 | 150 x 4.0 | 250 x 4.0 | |
| Partisil 5 SIL | P5-50AF | P5-100AF | P5-150AF | P5-250AF | P5-10C5 |
| Partisil 5 C8 | P5C8-50AF | P5C8-100AF | P5C8-150AF | P5C8-250AF | P5C8-10C5 |
| Partisil 5 ODS3 | P5ODS3-50AF | P5ODS3-100AF | P5ODS3-150AF | P5ODS3-250AF | P5ODS3-10C5 |
| Partisil 5 PAC | P5PAC-50AF | P5PAC-100AF | P5PAC-150AF | P5PAC-250AF | P5PAC-10C5 |
| Partisil 5 SAX | P5SAX-50AF | P5SAX-100AF | P5SAX-150AF | P5SAX-250AF | P5SAX-10C5 |
| Partisil 5 SCX | P5SCX-50AF | P5SCX-100AF | P5SCX-150AF | P5SCX-250AF | P5SCX-10C5 |
| Partisil 10 SIL | P10-50AF | P10-100AF | P10-150AF | P10-250AF | P10-10C5 |
| Partisil 10 C8 | P10C8-50AF | P10C8-100AF | P10C8-150AF | P10C8-250AF | P10C8-10C5 |
| Partisil 10 ODS | P10ODS-50AF | P10ODS-100AF | P10ODS-150AF | P10ODS-250AF | P10ODS-10C5 |
| Partisil 10 ODS2 | P10ODS2-50AF | P10ODS2-100AF | P10ODS2-150AF | P10ODS2-250AF | P10ODS2-10C5 |
| Partisil 10 ODS3 | P10ODS3-50AF | P10ODS3-100AF | P10ODS3-150AF | P10ODS3-250AF | P10ODS3-10C5 |
| Partisil 10 PAC | P10PAC-50AF | P10PAC-100AF | P10PAC-150AF | P10PAC-250AF | P10PAC-10C5 |
| Partisil 10 SAX | P10SAX-50AF | P10SAX-100AF | P10SAX-150AF | P10SAX-250AF | P10SAX-10C5 |
| Partisil 10 SCX | P10SCX-50AF | P10SCX-100AF | P10SCX-150AF | P10SCX-250AF | P10SCX-10C5 |

[1] Use with holder HI-161 and column coupler HI-081 [2] 5/pk – Starter kits also available – see p.21

### Partisil 4.6mm i.d. Columns

| Partisil Phase | Column Dimensions (mm) | | | | Guard Cartridges[1,2] (For 3.2-4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| Partisil 5 SIL | P5-50A | P5-100A | P5-150A | P5-250A | P5-10C5 |
| Partisil 5 C8 | P5C8-50A | P5C8-100A | P5C8-150A | P5C8-250A | P5C8-10C5 |
| Partisil 5 ODS3 | P5ODS3-50A | P5ODS3-100A | P5ODS3-150A | P5ODS3-250A | P5ODS3-10C5 |
| Partisil 5 PAC | P5PAC-50A | P5PAC-100A | P5PAC-150A | P5PAC-250A | P5PAC-10C5 |
| Partisil 5 SAX | P5SAX-50A | P5SAX-100A | P5SAX-150A | P5SAX-250A | P5SAX-10C5 |
| Partisil 5 SCX | P5SCX-50A | P5SCX-100A | P5SCX-150A | P5SCX-250A | P5SCX-10C5 |
| Partisil 10 SIL | P10-50A | P10-100A | P10-150A | P10-250A | P10-10C5 |
| Partisil 10 C8 | P10C8-50A | P10C8-100A | P10C8-150A | P10C8-250A | P10C8-10C5 |
| Partisil 10 ODS | P10ODS-50A | P10ODS-100A | P10ODS-150A | P10ODS-250A | P10ODS-10C5 |
| Partisil 10 ODS2 | P10ODS2-50A | P10ODS2-100A | P10ODS2-150A | P10ODS2-250A | P10ODS2-10C5 |
| Partisil 10 ODS3 | P10ODS3-50A | P10ODS3-100A | P10ODS3-150A | P10ODS3-250A | P10ODS3-10C5 |
| Partisil 10 PAC | P10PAC-50A | P10PAC-100A | P10PAC-150A | P10PAC-250A | P10PAC-10C5 |
| Partisil 10 SAX | P10SAX-50A | P10SAX-100A | P10SAX-150A | P10SAX-250A | P10SAX-10C5 |
| Partisil 10 SCX | P10SCX-50A | P10SCX-100A | P10SCX-150A | P10SCX-250A | P10SCX-10C5 |

[1] Use with holder HI-161 and column coupler HI-081 [2] 5/pk – Starter kits also available – see p.21

Please also see pages 122 and 123 for alternative Partisil columns.
Please contact Hichrom for ordering information on any column dimension not listed.

# PERKIN ELMER

## Brownlee™ MPLC Cartridge Columns

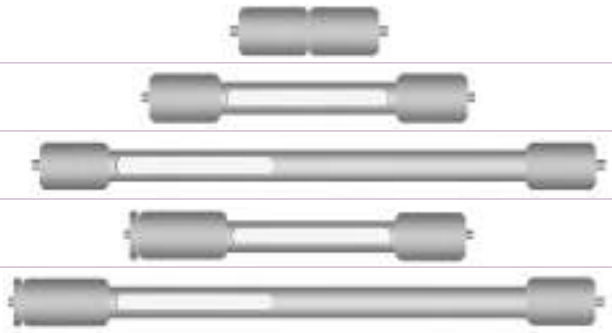
Perkin Elmer's Brownlee™ MPLC cartridge columns provide a range of options for HPLC. They are available in three lengths (3, 10 and 22cm) and two internal diameters (4.6 and 2.1mm). A specific cartridge holder (3, 10 or 22cm) is required for each cartridge length.The MPLC cartridge system allows direct coupling of an analytical and NewGuard cartridge without introduction of any dead volume (available for 10cm and 22cm cartridge column lengths). Changing cartridges is easy with the fingertight cartridge design which does not require any wrench-tightening of the holder or disconnecting of tubing from the LC system.

### Brownlee Spheri- and Aquapore Phases

| Phase | Functional Group | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped |
|---|---|---|---|---|---|---|
| Spheri-RP-8 | C8, monofunctional | 5, 10 | 80 | 180 | 6 | Yes |
| Spheri-RP-18 | C18, monofunctional | 5, 10 | 80 | 180 | 11 | Yes |
| Spheri-ODS | C18, polyfunctional | 5 | 80 | 180 | 14 | Yes |
| Aquapore Butyl, BU-300 | C4 | 7 | 300 | 100 | 3 | Yes |
| Aquapore Octyl, RP-300 | C8 | 7 | 300 | 100 | 5 | Yes |
| Aquapore ODS, OD-300 | C18 | 7 | 300 | 100 | 10 | Yes |

### MPLC Cartridge Hardware

| Description | Part Number |
|---|---|
| 3cm cartridge holder | 0715-0013 |
| 10cm cartridge holder | 0715-0014 |
| 22cm cartridge holder | 0715-0015 |
| 10cm cartridge holder incorporating NewGuard system | 0715-0016 |
| 22cm cartridge holder incorporating NewGuard system | 0715-0017 |



### Ordering Information – Brownlee Spheri- and Aquapore Phases

| Brownlee Phase | Cartridge Dimensions[1] (mm) | | | | | | NewGuard Cartridge[3] 15 x 3.2mm (3/pk) |
|---|---|---|---|---|---|---|---|
| | 30 x 2.1[2] | 30 x 4.6[2] | 100 x 2.1 | 100 x 4.6 | 220 x 2.1 | 220 x 4.6 | |
| Spheri-5, RP-8 | - | 0711-0001 | 0711-0004 | 0711-0003 | 0711-0006 | 0711-0005 | - |
| Spheri-5, RP-18 | 0711-0014 | 0711-0013 | 0711-0016 | 0711-0015 | 0711-0018 | 0711-0017 | - |
| Spheri-5, ODS | 0711-0020 | 0711-0019 | 0711-0022 | 0711-0021 | 0711-0024 | 0711-0023 | - |
| Spheri-10, RP-8 | - | 0711-0121 | - | 0711-0123 | - | - | - |
| Spheri-10, RP-18 | - | 0711-0115 | - | 0711-0117 | - | 0711-0119 | - |
| Aquapore Butyl, BU-300 | 0711-0062 | 0711-0061 | 0711-0064 | - | - | - | 0711-0088 |
| Aquapore Octyl, RP-300 | 0711-0056 | 0711-0055 | 0711-0058 | 0711-0057 | 0711-0060 | 0711-0059 | 0711-0090 |
| Aquapore ODS, OD-300 | 0711-0234 | 0711-0235 | 0711-0233 | 0711-0232 | 0711-0236 | 0711-0231 | 0711-0092 |
| Cartridge holder hardware alone | 0715-0013 | 0715-0013 | 0715-0014 | 0715-0014 | 0715-0015 | 0715-0015 | - |
| Cartridge holder hardware incorporating integral NewGuard system | - | - | 0715-0016 | 0715-0016 | 0715-0017 | 0715-0017 | - |

[1] Use with appropriate length cartridge holder 0715-0013/0014/0015 [2] 2/pk [3] Use with appropriate length integral cartridge holder 0715-0016/0017

**Please contact us for ordering details on all Perkin Elmer columns not listed**

# POLYLC



- Spherical silica, 60 - 1500Å pore size
- Polypeptide covalently bound coating
- High recovery for sensitive, labile proteins

PolyLC of Maryland, USA manufactures a range of unique columns for the more challenging HPLC biochemical applications. Their phases are characterised by the attachment of a polypeptide coating to wide pore silica.

## PolyLC Phases

| PolyLC Phase[1] | Particle Size (µm) | Pore Size (Å) | Functional Group | Chromatography Mode | Applications |
|---|---|---|---|---|---|
| PolyHYDROXYETHYL A™ | 3, 5, 12 | 60, 100, 200, 300, 500, 1000, 1500 | Hydroxyethylaspartamide | 1.Hydrophilic Interaction (HILIC) 2. Size Exclusion | Peptides, proteins, carbohydrates, polar small molecules |
| PolyCAT A™ | 3, 5, 12 | 300, 1000, 1500 | Aspartic acid | Weak cation-exchange | Proteins with isoelectric point >6.0 |
| PolyWAX LP™ | 3, 5, 12 | 100, 300, 1000, 1500 | Linear polyethyleneimine | Weak anion-exchange | Proteins with isoelectric points <6.0, nucleic acids and oligonucleotide analogues |
| PolyGLYCOPLEX™ | 5, 12 | - | - | Hydrophilic Interaction (HILIC) | Complex carbohydrates |
| PolySULFOETHYL A™ | 3, 5, 12 | 200, 300, 1000 | Sulphoethylaspartamide | Strong cation-exchange | Peptides |
| PolyPROPYL A™ | 3, 5, 12 | 300, 1000, 1500 | Propylaspartamide | Hydrophobic Interaction (HIC) | Proteins and peptides |

[1] PolyMETHYL A™ and PolyETHYL A™ materials are also available

## PolyHYDROXYETHYL A™

PolyHYDROXYETHYL A™ is a neutral polar material designed specifically for HILIC. Peptides and proteins are typically eluted with a decreasing gradient of acetonitrile or propanol for peptide mapping or multidimensional purification of synthetic and natural peptides. PolyHYDROXYETHYL A is also used for eliminating detergents, lipids and salts from samples and for the HPLC of solutes that are insoluble in aqueous media, such as membrane proteins. Figure 1 shows a typical chromatogram for the isolation of pure pathogenic prion protein from the brain of a sheep with scrapie.

### Table 1.
### PolyHYDROXYETHYL A - SEC fractionation ranges (Daltons)

| Pore Diameter (Å) | Denaturing Eluent (e.g. 50mM formic acid) | Conventional Eluent (phosphate/sulphate buffer) |
|---|---|---|
| 60 | 40-600 | 40-10,000 |
| 200 | 40-1600 | 200-25,000 |
| 300 | 40-40,000 | 300-100,000 |
| 500 | 40-150,000 | 400-300,000 |
| 1000 | 40-1,000,000 | 1000-2,000,000 |
| 1500 | 40-1,000,000 | 5000-2,000,000 |

In the absence of organic solvent, PolyHYDROXYETHYL A functions in the SEC mode. Using conventional salt buffers, the fractionation range is determined by the pore size of the packing (see Table 1). However, if the eluent contains a denaturing agent (eg. 50mM formic acid), smaller solutes can be separated by size. The 60Å pore size material permits the separation of peptides and other small solutes by SEC (Figure 2).

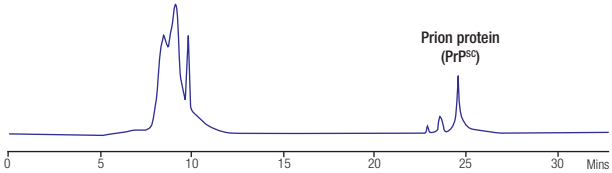



Figure 1. Extract of brain from sheep with scrapie (Proteinase K-treated)

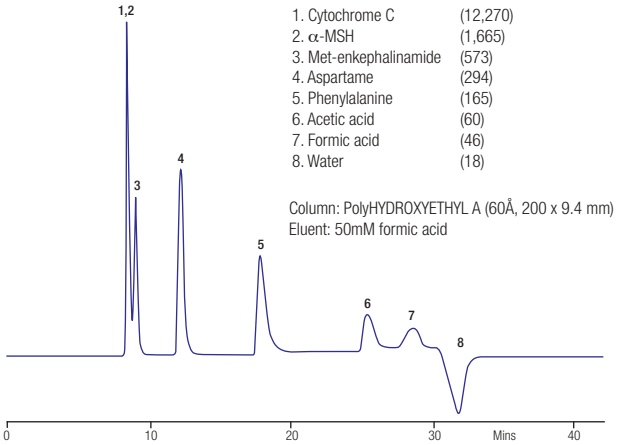
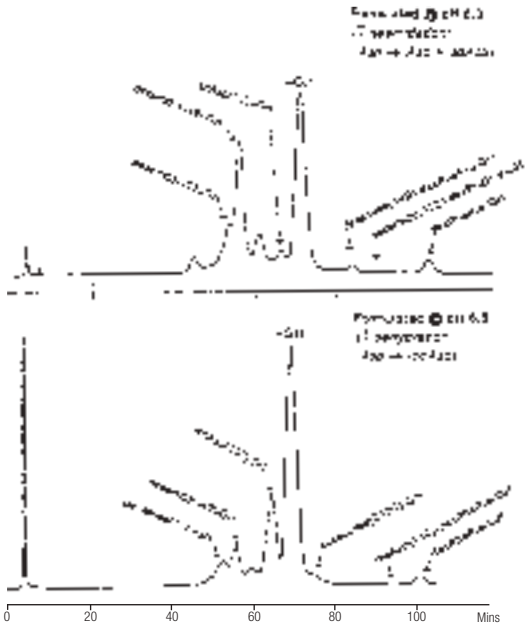



Figure 2. Size exclusion separation of small molecules

## PolyCAT A™

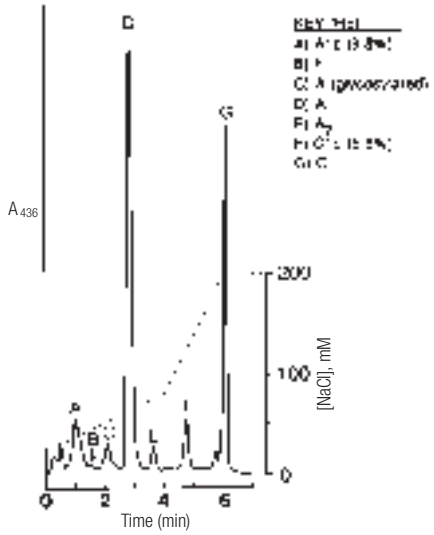
PolyCAT A™ consists of poly(aspartic acid) covalently bonded to silica. Proteins elute from the material by weak cation-exchange chromatography, resulting in high efficiency peaks and high binding capacity and recovery. PolyCAT A columns enable the separation and quantitation of many protein variants that differ by a single residue. Side products from the synthesis or degradation on storage of pharmaceutical proteins can effectively be analysed on a PolyCAT A column. Figure 3 shows the analysis of recombinant human growth hormone (HGH) incubated at two different pHs.

PolyCAT A columns are widely used in haemoglobin analyses, where all major and most minor variants are resolved (see Figure 4).




Column: PolyCAT A, 200 x 4.6mm (1000Å)
Eluent: 130-145mM $NH_4$acetate, pH 4.0 with 40% $CH_3CN$

Figure 3. Recombinant protein variant analysis -Human growth hormone after 6 days at 37°C

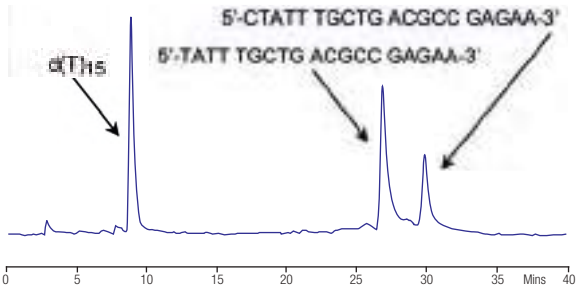



Column: PolyCAT A, 35 x 4.6mm (5µm, 1000Å)
Eluent: Gradient of increasing [NaCl]

Figure 4. Analysis of diabetic haemoglobin (C trait)

## PolyWAX LP™

PolyWAX LP™ is a hydrophilic weak anion-exchange (WAX) material prepared with linear polyethyleneimine (PEI), which confers greater selectivity and recovery than conventional branched polymer material. It was developed for the analysis of enzymes and other proteins. PolyWAX LP also offers excellent results for the analysis of larger oligonucleotides, their analogues and dsDNA fragments. The 3µm 1500Å version affords good resolution of oligonucleotides differing by just one base (see Figure 5).




Column: PolyWAX LP, 100 x 4.6mm (3µm, 1500Å)
Eluent: Gradient of increasing [NaCl] in 25mM Tris-Cl, pH 8 – $CH_3CN$ (70:30)
Flow rate: 0.5ml/min
Temperature: 60°C
Detection: UV, 250nm

Figure 5. Anion-exchange of oligonucleotides

## PolyGLYCOPLEX™

PolyGLYCOPLEX™ is a neutral material with a high capacity for retaining complex carbohydrates in the HILIC mode, frequently using just acetonitrile and water. Selectivity is good for both native glycans and derivatives such as those with the 2-aminopyridine fluorophore. Sialylated and asialoglycans can be resolved using the same operating conditions.

## PolySULFOETHYL A™

This strong cation-exchange (SCX) material was developed specifically for the HPLC of peptides, fractionating peptides by charge rather than polarity. Selectivity complements that of reversed-phase columns. Compared to other SCX materials based on sulphopropyl groups, PolySULFOETHYL A™ is unusually hydrophilic. This leads to higher recovery and higher capacity.

PolySULFOETHYL A is widely used in proteomics for the fractionation and identification of difficult proteins from complex tissue or cell extracts using 2D LC-MS/MS. By combining complementary HPLC techniques, an increased amount of information can be obtained. Different methods must be used for water-soluble and water-insoluble proteins, as shown in Figure 6. Some tryptic peptides from membrane proteins may be too hydrophobic for reversed-phase HPLC. A sequence of separations including PolySULFOETHYL A and/or PolyHYDROXYETHYL A materials provide a suitable alternative.

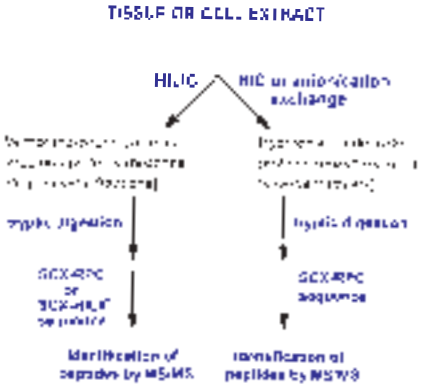



Figure 6

## Electrostatic Repulsion – Hydrophilic Interaction Chromatography (ERLIC)

The term ERLIC was devised by PolyLC, for HILIC separations where an ionic column surface chemistry is used to repel a common ionic polar group on an analyte or within a set of analytes. The column has the same charge as the sample solutes. The eluent contains enough organic solvent so that hydrophilic interaction keeps the solutes on the column despite the electrostatic repulsion. The pH of the eluent is selected to ensure that the solutes do have the same charge as the column. Some separations that would nomally require a gradient can be done isocratically using ERLIC.

### Applications of ERLIC include:

- Cation-exchange columns (PolySULFOETHYL A or PolyCAT A) are used for negatively charged solutes such as nucleotides and nucleic acids.
- Anion-exchange columns (PolyWAX LP) are used for amino acids, peptides and proteins. ERLIC of peptides on PolyWAX LP at pH 2 can be used to separate phosphopeptides from nonphosphorylated peptides.

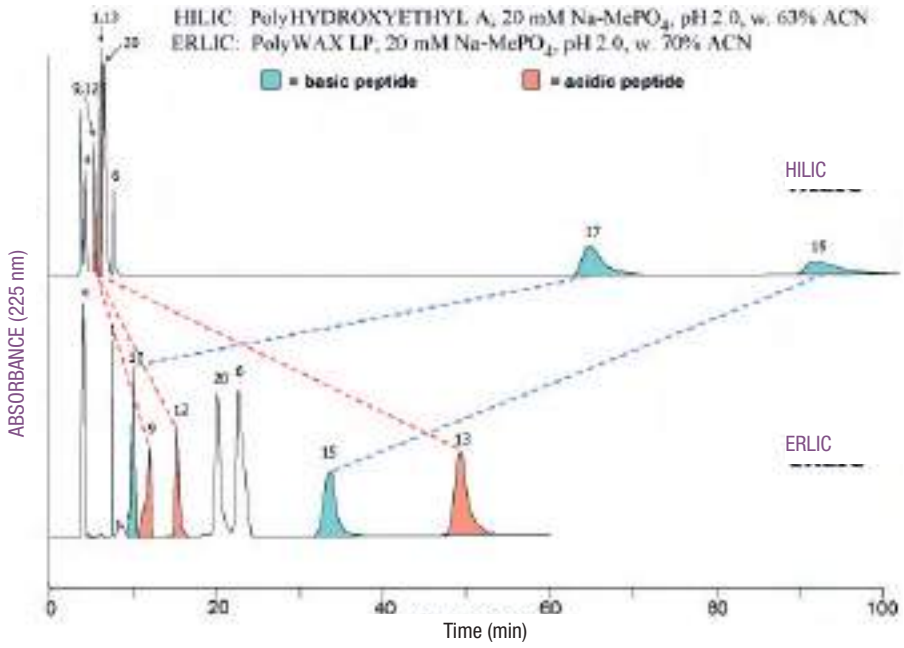



Figure 7. HILIC vs ERLIC separation of peptides

Figure 7 shows the elution of a mixture of acidic, basic and neutral peptides by HILIC on a PolyHYDROXYETHYL A column and ERLIC on a PolyWAX LP column. On the PolyHYDROXYETHYL A column, the basic peptides are much better retained than the neutral or acidic peptides, since basic solutes are the most polar of all. With the PolyWAX LP column, selective repulsion of the basic peptides puts them in the same elution time frame as the other peptides.

## PolyPROPYL A™, PolyETHYL A™ and PolyMETHYL A™

These materials separate proteins on the basis of hydrophobicity, using totally aqueous buffers and retaining tertiary structure and biological activity. Elution is typically with a decreasing salt gradient of sulphate or phosphate. The relative hydrophobic character of PolyPROPYL A™, PolyETHYL A™ and PolyMETHYL A™ is 100, 60 and 15 respectively.

### Sodium Dodecyl Sulphate (SDS) Removal

SDS is sometimes used to solubilise proteins. However, its presence interferes with subsequent bioanalysis. It can be removed by either:

1. Use of PolyHYDROXYETHYL A in HILIC mode
2. Use of specific PolyLC SPE cartridges in reversed-phase mode

### Ordering Information – PolyLC Phases

### Formulating Catalogue Numbers

Select column dimensions and phase from the table, then complete the catalogue number by adding a suffix to specify pore diameter as follows:

| Pore Diameter (Å) | 60 | 100 | 200 | 300 | 500 | 1000 | 1500 |
|---|---|---|---|---|---|---|---|
| Catalogue No. Suffix | -006 | -01 | -02 | -03 | -05 | -10 | -15 |

**Example:**
PolyCAT A column (200 x 4.6mm) with 300Å pores would be 204CT0503.

Please note that not all phases are available in all pore sizes.

For bulk material part numbers, specify pore diameter with the same suffixes as for column materials.

Example: Bulk material of PolyCAT A with 300Å pores and 5µm particle size would be BMCT0503.

### Columns

| PolyLC 5µm Phase[2] | Column Dimensions[1] (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 x 2.1 | 200 x 2.1 | 35 x 4.6 | 100 x 4.6 | 200 x 4.6 | 200 x 9.4 | 250 x 9.4 | 250 x 21.0 |
| PolyCAT A | 102CT05- - | 202CT05- - | 3.54CT05- - | 104CT05- - | 204CT05- - | 209CT05- - | 259CT05- - | 2521CT05- - |
| PolyPROPYL A | 102PR05- - | 202PR05- - | 3.54PR05- - | 104PR05- - | 204PR05- - | 209PR05- - | 259PR05- - | 2521PR05- - |
| PolyETHYL A | 102ET05- - | 202ET05- - | 3.54ET05- - | 104ET05- - | 204ET05- - | 209ET05- - | 259ET05- - | 2521ET05- - |
| PolyMETHYL A | 102ME05- - | 202ME05- - | 3.54ME05- - | 104ME05- - | 204ME05- - | 209ME05- - | 259ME05- - | 2521ME05- - |
| PolyWAX LP | 102WX05- - | 202WX05- - | 3.54WX05- - | 104WX05- - | 204WX05- - | 209WX05- - | 259WX05- - | 2521WX05- - |
| PolyHYDROXYETHYL A | 102HY05- - | 202HY05- - | 3.54HY05- - | 104HY05- - | 204HY05- - | 209HY05- - | 259HY05- - | 2521HY05- - |
| PolyGLYCOPLEX | 102GL0500 | 202GL0500 | 3.54GL0500 | 104GL0500 | 204GL0500 | 209GL0500 | 259GL0500 | 2521GL0500 |
| PolySULFOETHYL A | 102SE05- - | 202SE05- - | 3.54SE05- - | 104SE05- - | 204SE05- - | 209SE05- - | 259SE05- - | 2521SE05- - |

### Guard Cartridges[3], Solid Phase Extraction Cartridges and Bulk Material

| PolyLC 5µm Phase[2] | Guard Cartridge Dimensions[4,5] (mm) | | Solid Phase Extraction Cartridges (10/pk) | Bulk Material/g |
|---|---|---|---|---|
| | 10 x 2.1 | 10 x 4.0 | | |
| PolyCAT A | J22GCCT05- - | JGCCT05- - | SPECT1203 | BMCT05- - |
| PolyPROPYL A | J22GCPR05- - | JGCPR05- - | SPEPR1203 | BMPR05- - |
| PolyETHYL A | J22GCET05- - | JGCET05- - | SPEET1203 | BMET05- - |
| PolyMETHYL A | J22GCME05- - | JGCME05- - | SPEME1203 | BMME05- - |
| PolyWAX LP | J22GCWX05- - | JGCWX05- - | SPEWX1203 | BMWX05- - |
| SDS Removal | J2SDS | J4SDS | SPESDS1203 | BMSDS05- - |
| PolyHYDROXYETHYL A | J22GCHY05- - | JGCHY05- - | SPEHY1203 | BMHY05- - |
| PolyGLYCOPLEX | J22GCGL0500 | JGCGL0500 | SPEGL1200 | BMGL0500 |
| PolySULFOETHYL A | J22GCSE05- - | JGCSE05- - | SPESE1203 | BMSE05- - |

[1] Packed capillaries and 1mm i.d. columns also available
[2] 3 and 12µm particle size material also available
[3] Disposable Javelin design. No additional holder required
[4] 20 x 4.0 and 10 x 1.0mm cartridges also available
[5] Waters compatible cartridges available

# PRINCETON



Princeton Chromatography, Inc., New Jersey manufactures both HPLC and SFC columns. The company is particularly well known for the development of innovative SFC bonded phases and has been a pioneer of the technique for many years.

## Princeton SFC Columns

Supercritical Fluid Chromatography (SFC) applications typically require polar stationary phases such as silica, amino and diol. Although these phases are adequate for many applications, there is still a need for additional polar phases to meet the demands of difficult separations.

Princeton Chromatography has led the way in developing a series of amide, urea and pyridine phases that enhance the capability of the SFC technique by providing increased selectivity and loading capacity. Princeton Chromatography was the first company to develop and market the 2-Ethylpyridine phase which was launched in 2001 and later the 4-Ethylpyridine phase. PrincetonSFC™ 2-Ethylpyridine has become the column of choice for achiral SFC analysis of basic compounds. This phase generally requires no addition of amines to the eluent, producing excellent peak shape and reproducibility.

A diol phase is a popular and versatile stationary phase for SFC. Princeton Chromatography has also developed several different hydroxylated stationary phases, to give alternative selectivity and retention characteristics to the traditional diol. These phases are often a good starting point for the analysis of acidic compounds.

Figure 1 shows the structures of a selection of the Princeton SFC phases. The full range of available phases is listed on page 132.

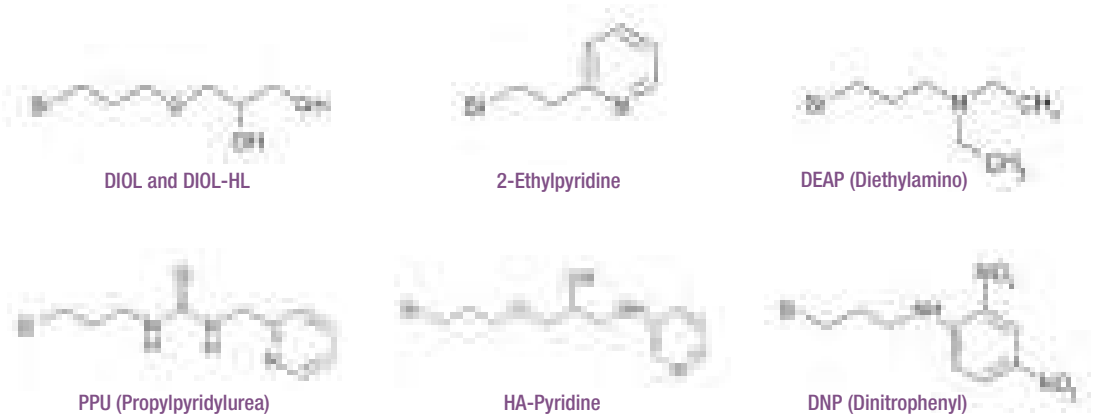



Figure 1. Structures of 6 of the many PrincetonSFC phases

PrincetonSFC analytical columns are available with lengths of 50 to 250mm and i.d.s of 2.0, 3.0, 4.0 or 4.6mm. All PrincetonSFC columns are individually quality assured by SFC. Particle sizes are 3, 5 and 10µm, with an additional 2.5µm particle phase column available for the PrincetonSPHER 2-Ethylpyridine phase.

### PrincetonSFC™ Semi-Preparative and Preparative Columns

All Princeton semi-preparative and preparative SFC columns are packed using the same high quality bonded phases as the corresponding analytical columns, making scale-up from analytical dimensions seamless and easy. Princeton preparative columns are available with i.d.s from 7.8mm to 50.0mm and in lengths from 50mm to 250mm. All columns are quality controlled by SFC and individual SFC documentation is included with each column.

The ordering information for 250 x 4.6mm, 250 x 10mm and 250 x 21.2mm i.d. columns is given on page 132. Please enquire for all other dimensions.

## PrincetonSFC™ Columns (continued)

The extensive range of PrincetonSFC™ phases is listed below. Many phases are available with pore sizes of 60Å or 100Å and with particle sizes of 3, 5 or 10µm. Semi-preparative and preparative columns (≥7.8mm i.d.) can be supplied packed with 5 and 10µm particles.

### PrincetonSFC Phases

| Phase | Pore Size (Å) | Particle Size (µm) | Ordering Information (5µm phase) | | |
|---|---|---|---|---|---|
| | | | 250 x 4.6mm[1] | 250 x 10.0mm[1] | 250 x 21.2mm[1] |
| 2-Ethylpyridine | 60<br>100 | 3, 5, 10<br>2.5, 3, 5, 10 | 250046-01577<br>250046-03577 | 250100-01577<br>250100-03577 | 250212-01577<br>250212-03577 |
| Silica | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01510<br>250046-03510 | 250100-01510<br>250100-03510 | 250212-01510<br>250212-03510 |
| Cyano | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01507<br>250046-03507 | 250100-01507<br>250100-03507 | 250212-01507<br>250212-03507 |
| DIOL | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01509<br>250046-03509 | 250100-01509<br>250100-03509 | 250212-01509<br>250212-03509 |
| Amino | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01508<br>250046-03508 | 250100-01508<br>250100-03508 | 250212-01508<br>250212-03508 |
| DEAP (Diethylamino) | 60 | 3, 5, 10 | 250046-01575 | 250100-01575 | 250212-01575 |
| DIOL-HL | 60 | 5, 10 | 250046-01579 | 250100-01579 | 250212-01579 |
| 2CN:DIOL | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01586<br>250046-03586 | 250100-01586<br>250100-03586 | 250212-01586<br>250212-03586 |
| Benzamide | 100 | 3, 5, 10 | 250046-03576 | 250100-03576 | 250212-03576 |
| PA (Propylacetamide) | 60 | 3, 5, 10 | 250046-01580 | 250100-01580 | 250212-01580 |
| PPU (Propylpyridiylurea) | 100 | 3, 5, 10 | 250046-03582 | 250100-03583 | 250212-03582 |
| Propylurea | 100 | 3, 5, 10 | 250046-03536 | 250100-03536 | 250212-03536 |
| DNP (Dinitrophenyl) | 100 | 3, 5, 10 | 250046-03593 | 250100-03593 | 250212-03593 |
| Pyridine Amide | 60 | 3, 5, 10 | 250046-01583 | 250100-01583 | 250212-01583 |
| 4-Ethylpyridine | 60<br>100 | 3, 5, 10<br>3, 5, 10 | 250046-01590<br>250046-03590 | 250100-01590<br>250100-03590 | 250212-01590<br>250212-03590 |
| Methane Sulfonamide (MeSAM) | 60 | 3, 5, 10 | 250046-01591 | 250100-01591 | 250212-01591 |
| Benzene Sulfonamide (BeSAM) | 100 | 3, 5, 10 | 250046-03592 | 250100-03592 | 250212-03592 |
| HA-Pyridine | 60 | 3, 5, 10 | 250046-01587 | 250100-01587 | 250212-01587 |
| HA-Dipyridyl | 100 | 3, 5, 10 | 250046-03588 | 250100-03588 | 250212-03588 |
| HA-DEA (Diethylamino) | 60 | 3, 5, 10 | 250046-01565 | 250100-01565 | 250212-01565 |
| HA-DHP (Dihydroxypropyl) | 100 | 3, 5, 10 | 250046-03569 | 250100-03569 | 250212-03569 |

[1] Other dimensions available – please enquire

Figures 2 and 3 show the SFC separation of β-blockers on PrincetonSFC Silica and a mixture of test compounds on PrincetonSFC Methane Sulfonamide respectively.

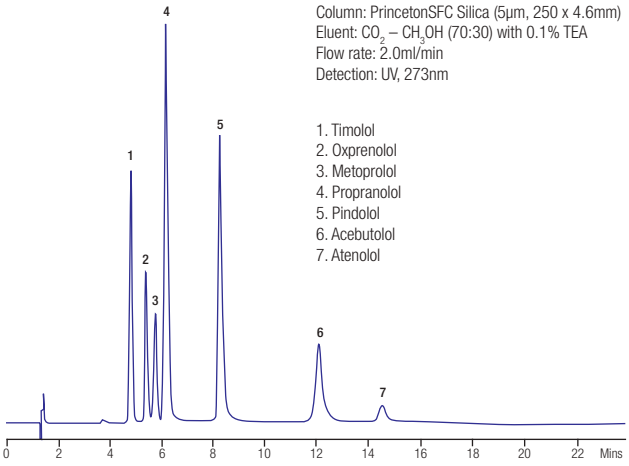



Figure 2. SFC separation of β-blockers

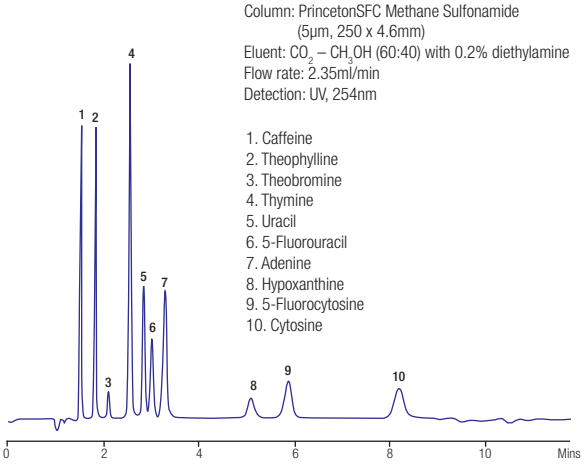



Figure 3. SFC separation of test compounds on PrincetonSFC Methane Sulfonamide

## PrincetonSFC™ Columns (continued)

The table below shows typical starting conditions for an analytical SFC assay. For basic analytes, a pyridine based column is a good starting point. For acidic compounds, a diol type column may be more retentive. Neutral compounds do not generally require an additive for elution.

### Typical Analytical Conditions for Achiral SFC

| | |
|---|---|
| **Stationary Phase** | Silica, Diol, 2-Ethylpyridine etc. (see Table on page 132) |
| **Column Dimensions** | |
| Length | 5, 10, 150, 250mm |
| i.d. | 2.0, 3.0, 4.0, 4.6mm |
| **Mobile Phase** | Flow rate: 1 - 5ml/min |
| $CO_2$ | |
| Modifier | Methanol (or ethanol) |
| Additive 1* | 5 - 20mM ammonium formate or acetate<br>Ammonium hydroxide or diethylamine (for basic compounds)<br>Formic acid or acetic acid (for acidic compounds) |
| **Gradient** | 5 - 50% modifier |
| **Pressure** | 100 - 200 bar |
| **Temperature** | 35 - 45°C |
| **Detection** | UV, MS, ELSD, CAD |

* 1 - 5% water may also be added if required

Figure 4 shows the separation of a mixture of 8 basic compounds on a 150 x 4.6mm PrincetonSFC™ Propylurea column. Figure 5 illustrates the effect on selectivity and retention times of changing the chemistry of the stationary phase. In each case methanol was used as the modifier without the addition of any additives.

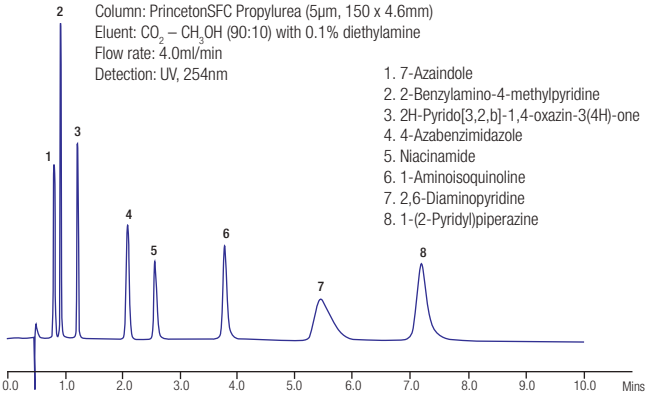
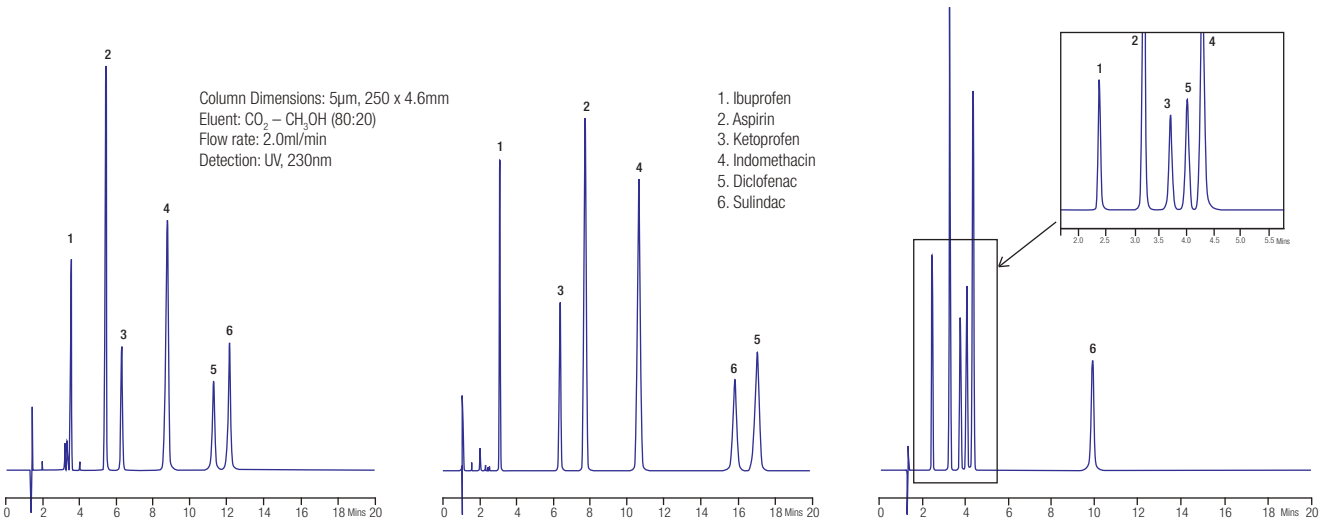



Figure 4. SFC separation of basic compounds




PrincetonSFC 2-Ethylpyridine | PrincetonSFC PPU | PrincetonSFC DIOL

Figure 5. Selectivity of PrincetonSFC 2-Ethylpyridine, PPU and DIOL

### Bulk Material

The majority of Princeton Chromatography SFC and HPLC phases are offered in bulk form, in a variety of particle sizes ranging from 10µm to ≥20µm. Please enquire for availability.

## PrincetonSPHER™ HPLC Columns

Princeton Chromatography also manufactures a range of porous silica bonded HPLC columns. An overview of a selection of these phases is given below.

### PrincetonSPHER™ HPLC Phases

| PrincetonSPHER Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | Endcapped | Phase Code |
|---|---|---|---|---|---|---|
| C18 | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 23, 19, 8 | Yes | 01 |
| C8 | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 15, 11, 5 | Yes | 02 |
| C6 | 3, 5, 10 | 60, 100 | 500, 325 | 10, 8 | Yes | 03 |
| C4 | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 8, 6, 3 | No | 04 |
| Phenyl | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 16, 12, 5 | Yes | 05 |
| PFP | 3, 5, 10 | 60, 100 | 500, 325 | 12, 9 | Yes | 06 |
| CN | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 8, 6, 3 | No | 07 |
| NH2 | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 6, 4, 3 | No | 08 |
| Diol | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | 6, 4, 2 | No | 09 |
| Silica | 3, 5, 10 | 60, 100, 300[1] | 500, 325, 100 | N/A | N/A | 10 |
| HTS (C12) | 3, 5, 10 | 60 | 500 | 16 | Yes | 70 |
| C27 | 3, 5, 10 | 100 | 325 | 19 | Yes | 71 |
| C30 | 3, 5, 10 | 200 | 200 | 19 | No | 74 |
| Cyclohexyl | 3, 5, 10 | 60 | 500 | 12 | Yes | 43 |
| Diphenyl | 5 | 60 | 500 | 10 | Yes | 51 |
| Fluoropropyl | 5, 10 | 100 | 325 | 5 | No | 41 |
| Fluorooctyl (FO) | 5, 10 | 100 | 325 | 8 | Yes | 42 |
| ULTIMA C18 | 3, 5, 10 | 100 | 325 | 16 | Yes | 21 |
| ULTIMA C8 | 3, 5, 10 | 100 | 325 | 13 | Yes | 22 |
| ULTIMA Phenyl | 3, 5, 10 | 100 | 325 | 12 | Yes | 23 |

[1] 300Å phases not available with 3µm particle size

### Princeton Chromatography HPLC Phases

**PrincetonSPHER-60 Series** phases (60Å pore size) are optimal for compounds under 1000MW. In most cases, the high surface area ($500m^2/g$) leads to longer retention times than with the corresponding 100Å materials. All PrincetonSPHER-60 columns are packed with high purity silica, bonded to ensure the highest standards of quality.

**PrincetonSPHER-100 Series** columns are Princeton Chromatography's most popular. With a pore size of 100Å and an average surface area of $325m^2/g$ these columns are extremely versatile and excellent for method development.

**PrincetonSPHER-300 Series** (300Å pore size) are the columns of choice for large molecules such as proteins and peptides. The low surface area of these packing materials is beneficial for separations requiring low percentages of organic modifiers.

**PrincetonSPHER-HTS** is a 60Å pore size C12 phase designed for **H**igh **T**hroughput **S**creening. The unique structure of this C12 ligand facilitates rapid equilibration between gradient runs.

**PrincetonSPHER C27** is a 100Å proprietary phase containing a well-defined and reproducible branched chain hydrocarbon ligand. The phase shows enhanced retention for most polar compounds and can be used with fully aqueous mobile phases.

**PrincetonSPHER C30** is a 200Å pore size material bonded with a distribution of long chain hydrocarbons that have an average length of C30. This material has been found to successfully separate many isomers in the carotenoid family of long chain molecules. It has also proved useful for the analysis of some larger molecules such as proteins and peptides.

**Princeton ULTIMA** bonded phases contain an amide polar embedded functionality, which deactivates neighbouring free silanol groups and enhances the wettability of the bonded ligands. The phases are compatible with the use of 100% aqueous eluents and are available as C18, C8 and Phenyl bonded phases.

Please enquire for ordering information for any PrincetonSPHER HPLC column or guard.

## PrincetonSPHER™ HPLC Columns (continued)

### PrincetonSPHER™ Column Dimensions

Analytical HPLC columns can be supplied with lengths of 50, 75, 100, 150 and 250mm and with i.d.s of 2.0, 3.0, 4.0 and 4.6mm.
Preparative HPLC columns can be supplied with lengths of 50, 100, 150 and 250mm and with i.d.s of 7.8, 10.0, 21.2, 30.0 and 50.0mm.
Guard cartridges for analytical and preparative dimension columns are available – please enquire.

### Creating a Part Number:

All Princeton Chromatography HPLC column part numbers are of the format XY-ABC

where X = column length in mm
Y = code for column i.d.
A = code for pore size
B = code for particle size
C = code for phase type (included in main phase specification table, page 134)

When creating a part number, please substitute the appropriate codes into this basic format.
e.g. For a 3µm 150 x 4.6mm i.d. PrincetonSPHER-100 C18 column, the part number is 150046-03301.

| **Column i.d. (mm)** | 2.0 | 3.0 | 4.0 | 4.6 | 7.8 | 10.0 | 21.2 | 30.0 | 50.0 |
|---|---|---|---|---|---|---|---|---|---|
| **Code** | 020 | 030 | 040 | 046 | 078 | 100 | 212 | 300 | 500 |

| **Pore Size (Å)** | 60 | 100 | 200 | 300 |
|---|---|---|---|---|
| **Code** | 01 | 03 | 07 | 08 |

| **Particle Size (µm)** | 3 | 5 | 10 |
|---|---|---|---|
| **Code** | 3 | 5 | 0 |

Figures 6 and 7 show the separation of diuretics and vanillins on PrincetonSPHER C18 and PrincetonSPHER Fluorooctyl (FO) respectively.

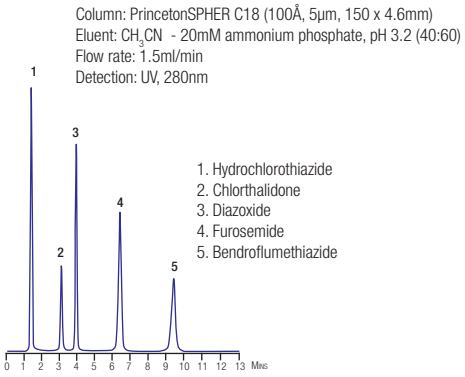



Figure 6. Diuretics on PrincetonSPHER C18

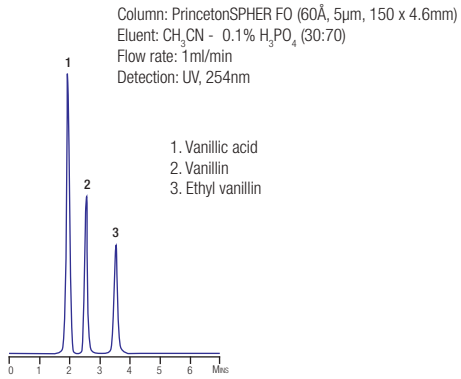



Figure 7. Vanillins on PrincetonSPHER FO

## PharmaBOND

PharmaBOND columns are packed with 125Å pore size 10µm (or 5µm for C18 phase) irregular silica particles. C18, Phenyl, CN, Amino and Silica phases are available and are suitable for legacy methods specifying this phase type. They are equivalent to Waters® µBondapak® and are supplied with internal diameters of 3.9 and 4.6mm.

### Ordering Information for PharmaBOND Columns

| PharmaBOND Phase | Column Dimensions (mm) | | | | | Guard Cartridges (5/pk, 10 x 4.0mm) |
|---|---|---|---|---|---|---|
| | 150 x 3.9 | 300 x 3.9 | 150 x 4.6 | 250 x 4.6 | 300 x 4.6 | |
| **5µm** | | | | | | |
| C18 | 150039-05501 | 300039-05501 | 150046-05501 | 250046-05501 | 300046-05501 | 14104-05501 |
| **10µm** | | | | | | |
| C18 | 150039-05001 | 300039-05001 | 150046-05001 | 250046-05001 | 300046-05001 | 14104-05001 |
| Phenyl | 150039-05005 | 300039-05005 | 150046-05005 | 250046-05005 | 300046-05005 | 14104-05005 |
| CN | 150039-05007 | 300039-05007 | 150046-05007 | 250046-05007 | 300046-05007 | 14104-05007 |
| Amino | 150039-05008 | 300039-05008 | 150046-05008 | 250046-05008 | 300046-05008 | 14104-05008 |
| Silica | 150039-05010 | 300039-05010 | 150046-05010 | 250046-05010 | 300046-05010 | 14104-05010 |

# REGIS® TECHNOLOGIES

Regis® Technologies, Inc. manufactures a wide range of chiral and speciality columns for analytical to preparative applications. They are well known for their range of Pirkle type chiral stationary phases, in particular the well established Whelk-0® columns, used for analytical and preparative applications. The company also manufactures a range of polysaccharide phases, a range of RAM and IAM columns and supplies the ChiroSil range of crown ether type chiral columns manufactured by RStech Corporation of Korea (see page 137).

## Pirkle Chiral Phases

Pirkle concept chiral stationary phases can be used in both normal-phase and reversed-phase modes. All columns show excellent durability due to covalent bonding and excellent chromatographic efficiency. They can be used with a wide variety of solvents and are applicable for both HPLC and SFC methods. For each phase listed in the table below, the optically active ligand is covalently bonded to 5µm, 100Å spherical silica.

### Regis Pirkle Chiral Phases

| Phase | Bonding | Class | Typical Applications |
|---|---|---|---|
| α-Burke 2 | N-3,5-dinitrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl phosphonate | π-electron acceptor | β-Blockers, amino alcohols |
| β-Gem 1 | N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)-propanoate | π-electron acceptor | Anilide derivatives of wide range of carboxylic acids |
| DACH-DNB | 3,5-Dinitrobenzoyl derivative of 1,2-diaminocyclohexane | π-electron acceptor/donor | Broad range |
| Leucine | 3,5-Dinitrobenzoylleucine | π-electron acceptor | Benzodiazepines |
| Phenylglycine | 3,5-Dinitrobenzoylphenylglycine | π-electron acceptor | Wide variety of compounds containing π-basic groups |
| Pirkle 1-J | 3-(3,5-Dinitrobenzamido)-4-phenyl-β-lactam | π-electron acceptor | Underivatised β-blockers, arylpropionic acids |
| ULMO | 3,5-Dinitrobenzoyl derivative of diphenylethylene-diamine | π-electron acceptor/donor | Wide range, particularly aryl carbinols |
| Whelk-01<br>Whelk-02 | 1-(3,5-dinitrobenzamido)-tetrahydrophenanthrene | π-electron acceptor/donor | Broad range of compounds |

Additional features of the Pirkle type phases include:

1. The ability to invert elution order by using the same type of chiral stationary phase (CSP) but with the opposite absolute configuration enables trace enantiomers to be eluted before the major. This is beneficial for enantiomeric purity determinations and for preparative separations.

2. All of Regis' Pirkle columns are available in both analytical and preparative sizes. The high loading factors offered by these phases make them particularly suited for scaling up.

**Whelk-0®1** is the most widely applicable of the Regis chiral stationary phases, due to the incorporation of both π -acceptor and π -donor characteristics. It was originally designed for the separation of underivatized non-steroidal anti-inflammatory drugs, but shows versatility in the analysis of a wide range of compounds, including amides, epoxides, esters, ureas, carbamates, ethers, aldehydes, ketones, carboxylic acids and alcohols. The newer Whelk-01 phase, based on 5µm Kromasil silica, shows higher efficiencies and greater resolving power in both HPLC and SFC modes than the original 5µm Whelk-01 phase based on Exsil silica and is now recommended for all new method development. Figures 1 and 2 show comparisons of the HPLC and SFC performances of these Whelk-01 phases for modafinil and chlormezanone respectively.

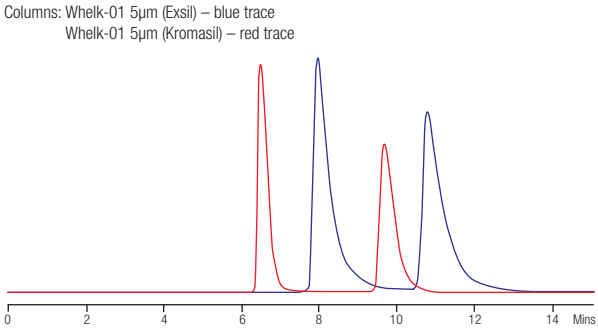



Figure 1. HPLC separation of modafinil

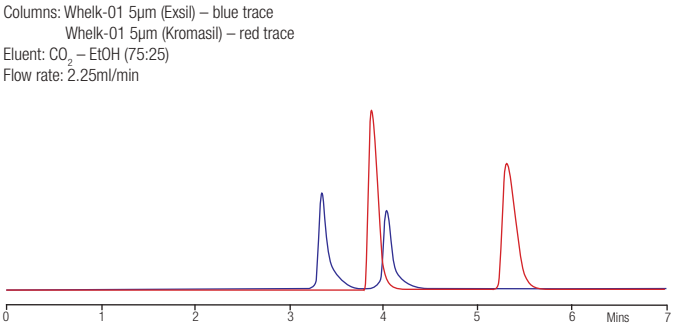



Figure 2. SFC separation of chlormezanone

**Whelk-0®2** is the covalent trifunctional version of the Whelk-01. It shows similar enantioselectivity as the Whelk-01, but demonstrates enhanced stability with strong organic modifiers.

Please enquire for ordering information on all Regis products.

## RegisCell®, RegisPack® and RegisPack CLA-1™

RegisCell® and RegisPack® chiral columns are polysaccharide based stationary phases. They are produced using a unique manufacturing process involving the coating of the polysaccharide cellulose and amylose chiral selectors respectively onto a high purity wide pore (1000Å) silica. Columns can be used in both HPLC and SFC modes. Figure 3 shows the fast SFC analysis of atenolol in less than one minute on a RegisCell column.

The newer RegisPack CLA-1™ is a coated chlorinated polysaccharide (amylose) phase. It shows complementary selectivity to the other Regis phases and can be a useful addition to a screening process. Figure 4 shows the separation of the four diastereomers of cyclandelate by HPLC on a RegisPack CLA-1 column.

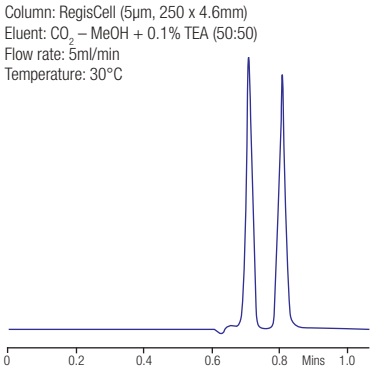



Figure 3. Fast SFC separation of atenolol

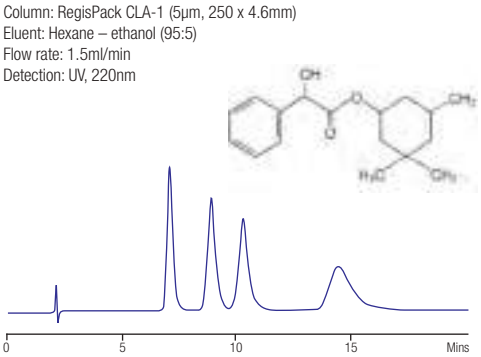



Figure 4. Cyclandelate on RegisPack CLA-1

## ChiroSil®

The ChiroSil® RCA(+) and SCA(-) crown ether chiral stationary phases are effective for the analysis of amino acids and primary amines. The high resolution capability of these phases enables applications to be scaled up from analytical to preparative dimensions. The availability of both enantiomeric forms of the phase enables the elution order to be inverted so that a trace enantiomer can be eluted first.

## Other Products

### Restricted Access Media (RAM)

RAM columns permit the direct HPLC analysis of drugs in a protein matrix without the use of multi-stage clean-up procedures. Two types of RAM direct injection technologies are available. Both consist of a distinct inner and outer surface. Large proteins (>12,000 MW) move through the column repelled by the outer hydrophilic surface. They are too large to access the internal surface of the pores and hence are eluted at the column's void volume. Analytes pass through the internal pores and are retained by the inner hydrophobic phase.

### Immobilised Artificial Membrane (IAM)

IAM chromatography phases prepared from analogues of phosphatidylcholine mimic the surface of a biological cell membrane. Their use provides a method for the prediction of drug membrane partitioning and large scale drug membrane screening. Columns are used primarily for membrane protein purification and in drug discovery for predicting drug membrane permeability.

### Ion Pairing Reagents

Ion pairing reagents can be used to selectively increase the retention of charged analytes on conventional hydrophobic HPLC phases. Regis manufactures both anionic sulphonate (S-Series) and cationic quaternary amine (Q-Series) ion pairing reagents with varying alkyl chain lengths. The S-Series reagents are available as 0.5M solutions of alkyl sulphonates (S5-S8, S12) or as bulk powder. The Q-Series consists of 0.5M solutions of quaternary alkyltriethylamines (Q5-Q8, Q12). Method development kits are also available.

### GC Derivatization Reagents

Regis manufactures a wide range of high purity derivatization reagents for GC. These include reagents for silylation, alkylation and acylation reactions. Please see page 171 for further details.

Please enquire for ordering information on all Regis products.

# SHODEX®

- Silica- and polymer-based phases
- Columns for all chromatographic modes
- Wide application range
- Capillary, microbore, analytical and preparative dimensions

Showa Denko K.K., Japan manufactures the Shodex® line of HPLC columns for the pharmaceutical and biotechnology industries. A wide range of products with both silica-based and polymer-based materials is available. An overview of the products is shown on the following pages: reversed-phase (page 138), ligand exchange (page 139), ion-exchange and ion chromatography (page 140), size exclusion (page 141), HIC (page 142) and affinity (page 142). Please enquire for additional details.

## Shodex Reversed-Phase Columns

The Shodex product range contains both polymer-based and silica-based phases. Please enquire for ordering details where not listed.

### Reversed-Phase (Polymer-based) Phases

The table below lists the polymer-based phases available.

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| RSpak | RP18, DS | Styrene-divinylbenzene | - | Analysis of proteins and peptides |
| RSpak | DE, GOLF, CARB | Polymethacrylate | - | DE series columns have similar selectivity to C18 columns |
| RSpak | DM-614 | Polyhydroxymethacrylate | - | Suitable for analysis of amino acids and polypeptides |
| RSpak | NN | Polyhydroxymethacrylate | Sulpho | Suitable for separation of mixtures of neutral and ionic compounds |
| RSpak | JJ-50 | Polyvinyl alcohol | Quaternary ammonium | |
| Asahipak | ODP | Polyvinyl alcohol | Octadecyl | Large pore size phases, suitable for high molecular weight compounds eg. proteins, as well as low molecular weight compounds. LC-MS applications |
| Asahipak | ODP2 HP | Polyhydroxymethacrylate | Octadecyl | |
| Asahipak | C8P-50 | Polyvinyl alcohol | Octyl | |
| Asahipak | C4P-50 | Polyvinyl alcohol | Butyl | |
| Asahipak | NH2P | Polyvinyl alcohol | Amino | Analysis of saccharides (NP) |

**Shodex ODP2 HP** is a macroporous polyhydroxymethacrylate reversed-phase material offering better efficiencies than many other resin-based phases and comparable to that of silica-based C18 columns. It has a recommended pH range of 3 to 12. The high polarity and small pore size (40Å) of this phase is designed to exclude proteins and can be used for the analysis of drugs in biological samples ie. as restricted access material (see Figure 1 for example).

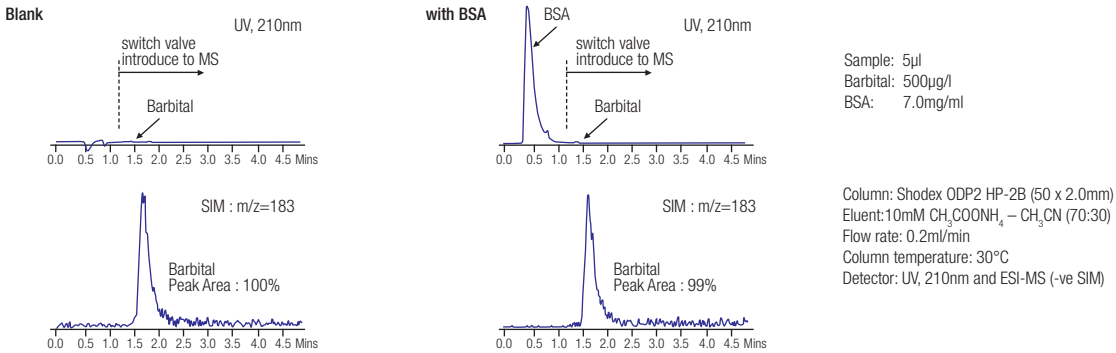



Figure 1. LC-MS of barbital in BSA

### Ordering Information - Shodex ODP2 HP

| Product | Particle Size (µm) | Column Dimensions (mm) | Catalogue No. |
|---|---|---|---|
| ODP2 HP-4B | 5 | 50 x 4.6 | F7622001 |
| ODP2 HP-4D | 5 | 150 x 4.6 | F7622002 |
| ODP2 HP-4E | 5 | 250 x 4.6 | F7622003 |
| ODP2 HPG-4A | 5 | 10 x 4.6 (guard column, 1/pk) | F6714010 |
| ODP2 HP-4GC | 5 | 10 x 4.0 (guard cartridge, 3/pk)[1] | F6714012 |
| ODP2 HP-2B | 5 | 50 x 2.0 | F7622004 |
| ODP2 HP-2D | 5 | 150 x 2.0 | F7622005 |
| ODP2 HPG-2A | 5 | 10 x 2.0 (guard column, 1/pk) | F6714011 |

[1] Use with guard cartridge holder HLD-4GC (Cat. No. F8700020).

### Reversed-Phase (Silica-based) Phases

Please enquire for details of reversed-phase silica-based columns, including Silica NPE (nitrophenylethyl) and Silica PYE (pyrenylethyl).

# Shodex® Columns for Saccharide Analysis

## Ligand Exchange Phases (Shodex SUGAR Series)

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| SUGAR | SH | Styrene-divinylbenzene | Sulpho ($H^+$) | Separation of saccharides by ligand exchange, size exclusion, ion exclusion and normal-phase. Suitable for separation of sugars and sugar alcohols |
| SUGAR | SC | Styrene-divinylbenzene | Sulpho ($Ca^{2+}$) | |
| SUGAR | SP | Styrene-divinylbenzene | Sulpho ($Pb^{2+}$) | |
| SUGAR | SZ | Styrene-divinylbenzene | Sulpho ($Zn^{2+}$) | |
| SUGAR | KS | Styrene-divinylbenzene | Sulpho ($Na^+$) | Separation of saccharides by size exclusion and ligand exchange |
| USPpak | MN | Styrene-divinylbenzene | Sulpho ($Ca^{2+}$) | Analysis of mannitol by USP method |

There are two series of Shodex® columns for saccharide analysis – the SUGAR series for operation in ligand exchange mode and Asahipak NH2P-50 polymeric amino columns for normal-phase and HILIC modes.

### A. Shodex SUGAR Series

The SUGAR series columns are based on polystyrene-divinylbenzene copolymer with strong cation-exchange resin incorporating functional sulpho groups coupled with metal counter ions ($H^+$, $Ca^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Na^+$).

### B. Shodex Asahipak NH2P

Shodex Asahipak NH2P is an amino bonded column in which polyamine is bonded to a hydrophilic polyvinyl alcohol polymer. The high separation power of conventional silica-based columns is maintained, whilst showing an increased usable pH range (2 – 13) and increased robustness. Figure 2 illustrates the reproducibility over time obtained from the polymeric Asahipak NH2P column.

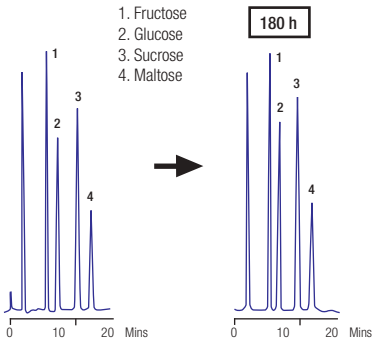



Figure 2. Reproducibility with Asahipak NH2P-50

## Ordering Information - Columns for Saccharide Analysis

| Product | Counter Ion | Separation Mode | Exclusion Limit (Da) | Particle Size (µm) | Column Dimensions[1] (mm) | Catalogue No. |
|---|---|---|---|---|---|---|
| SUGAR SH1011 | $H^+$ | SEC + IEX | 1,000 | 6 | 300 x 8.0 | F6378100 |
| SUGAR SH1821 | $H^+$ | SEC + IEX | 10,000 | 6 | 300 x 8.0 | F6378101 |
| SUGAR SH-G | $H^+$ | - | Guard column | 10 | 50 x 6.0 | F6700080 |
| SUGAR SC1011 | $Ca^{2+}$ | SEC + LEX | 1,000 | 6 | 300 x 8.0 | F6378102 |
| SUGAR EP SC1011-7F | $Ca^{2+}$ | SEC + LEX | 1,000 | 8 | 300 x 7.8 | F6379300 |
| SUGAR SC1821 | $Ca^{2+}$ | SEC + LEX | 10,000 | 6 | 300 x 8.0 | F6378103 |
| SUGAR SC-LG | $Ca^{2+}$ | - | Guard column | 10 | 50 x 6.0 | F6700090 |
| SUGAR SP0810 | $Pb^{2+}$ | SEC + LEX | 1,000 | 7 | 300 x 8.0 | F6378105 |
| SUGAR SP-G | $Pb^{2+}$ | - | Guard column | 10 | 50 x 6.0 | F6700081 |
| SUGAR SC1211 | $Ca^{2+}$ | NP + LEX | - | 6 | 250 x 6.0 | F7001400 |
| SUGAR SC-G | $Ca^{2+}$ | - | Guard column | 10 | 10 x 4.6 | F6700120 |
| SUGAR SZ5532 | $Zn^{2+}$ | NP + LEX | - | 6 | 150 x 6.0 | F7001300 |
| SUGAR SZ-G | $Zn^{2+}$ | - | Guard column | 6 | 10 x 4.6 | F6700110 |
| SUGAR KS-801 | $Na^+$ | SEC + LEX | 1,000 | 6 | 300 x 8.0 | F6378010 |
| SUGAR KS-802 | $Na^+$ | SEC + LEX | 10,000 | 6 | 300 x 8.0 | F6378020 |
| SUGAR KS-803 | $Na^+$ | SEC | 50,000 | 6 | 300 x 8.0 | F6378025 |
| SUGAR KS-804 | $Na^+$ | SEC | 400,000 | 7 | 300 x 8.0 | F6378035 |
| SUGAR KS-805 | $Na^+$ | SEC | 5,000,000 | 17 | 300 x 8.0 | F6378050 |
| SUGAR KS-806 | $Na^+$ | SEC | 50,000,000 | 17 | 300 x 8.0 | F6378060 |
| SUGAR KS-G | $Na^+$ | - | Guard column | 10 | 50 x 6.0 | F6700020 |
| SUGAR KS-807 | $Na^+$ | SEC | 200,000,000 | 30 | 300 x 8.0 | F6378070 |
| SUGAR KS-807G | $Na^+$ | - | Guard column | 30 | 50 x 8.0 | F6700021 |
| USPpak MN-431 | $Ca^{2+}$ | LEX | - | 8 | 250 x 4.0 | F6379230 |
| **Asahipak Phases** | | | | | | |
| NH2P-40 3E | - | NP/HILIC | - | 4 | 250 x 3.0 | F7630007 |
| NH2P-50G 3A | - | - | Guard column | 5 | 10 x 3.0 | F6710030 |
| NH2P-50 4D | - | NP/HILIC | - | 5 | 150 x 4.6 | F7630002 |
| NH2P-50 4E | - | NP/HILIC | - | 5 | 250 x 4.6 | F7630001 |
| NH2P-50G 4A | - | - | Guard column | 5 | 10 x 4.6 | F6710016 |
| NH2P-50 2D | - | NP/HILIC | - | 5 | 150 x 2.0 | F7630006 |
| NH2P-50G 2A | - | - | Guard column | 5 | 10 x 2.0 | F6713000 |
| NH2P-LF | - | - | Line filter | - | 75 x 8.0 | F6710100 |

[1] Preparative columns also available

# Shodex® Ion-Exchange Phases

## Ion-Exchange Phases

| Column Series | | Base Material | Functional Group | Comments/Applications | |
|---|---|---|---|---|---|
| IEC | QA | Polyhydroxymethacrylate | Quaternary ammonium | Strong anion-exchange | Suitable for analysis of proteins, peptides, DNA, RNA, oligonucleotides |
| IEC | DEAE | Polyhydroxymethacrylate | Diethylaminoethyl | Weak anion-exchange | |
| IEC | SP | Polyhydroxymethacrylate | Sulphopropyl | Strong cation-exchange | |
| IEC | CM | Polyhydroxymethacrylate | Carboxymethyl | Weak cation-exchange | |
| Asahipak | ES-502N | Polyvinyl alcohol | Diethylaminoethyl | Weak anion-exchange | |
| Asahipak | ES-502C | Polyvinyl alcohol | Carboxymethyl | Weak cation-exchange | |
| AXpak | WA | Polyhydroxymethacrylate | Diethylaminoethyl | Weak anion-exchange, suitable for analysis of nucleic acids | |
| CXpak | P | Styrene-divinylbenzene | Sulpho ($Na^+$) | Analysis of amino acids and amino sugars | |
| PIKESS | DEAE | Polyhydroxymethacrylate | Diethylaminoethyl | Weak anion-exchange | UHPLC compatible (2.5µm material) |
| PIKESS | SP | Polyhydroxymethacrylate | Sulphopropyl | Strong cation-exchange | |

The IEC series (QA and DEAE anion-exchange phases and SP and CM cation-exchange phases) is based on polyhydroxymethacrylate. Columns are suitable for the analysis of relatively high molecular weight compounds, such as proteins. The newer PIKESS materials are non-porous 2.5µm particles, suitable for rapid protein analyses using UHPLC systems (see Figure 3).

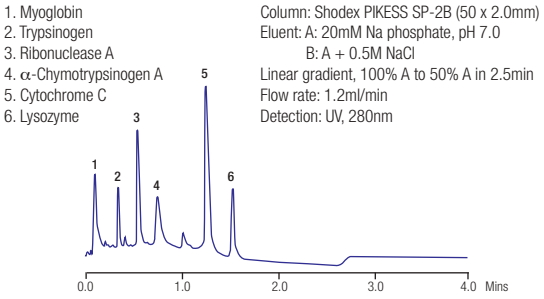



Figure 3. Rapid analysis of proteins using UHPLC and ion-exchange

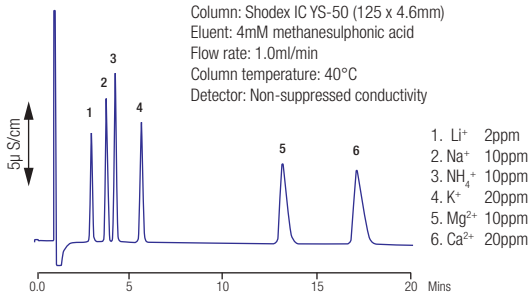



Figure 4. Separation of cations using ion chromatography

Please enquire for ordering details of ion-exchange columns.

# Shodex Ion Chromatography Phases

## Ion Chromatography Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| IC | NI, I | Polyhydroxymethacrylate | Quaternary ammonium | Anion analysis with non-suppressed detection |
| IC | SI | Polyvinyl alcohol | Quaternary ammonium | Anion analysis with suppressed detection |
| IC | Y, T, R | Styrene-divinylbenzene | Sulpho | Cations by non-suppressed detection. Y for alkylamines. T for transition metals and R for rare earth metals by post-column reaction |
| IC | YK | Silica | Carboxyl | Monovalent and divalent cations by non-suppressed detection |
| IC | YS | Silica | Carboxyl | Higher performance version of YK |

A range of Shodex columns are available for both non-suppressed and suppressed ion chromatography. Columns are also supplied for analysing transition metal ions and rare earth metal ions using post-column reactions. Figure 4 shows the separation of six common cations on a Shodex IC YS-50 column with non-suppressed conductivity detection.

## Ordering Information - Columns for Ion Chromatography

| Column | Particle Size (µm) | Column Dimensions (mm) | Catalogue No. | Guard Column | Catalogue No. |
|---|---|---|---|---|---|
| IC NI-424 | 5 | 100 x 4.6 | F6995243 | IC NI-G | F6709616 |
| IC I-524A | 12 | 100 x 4.6 | F6995240 | IC IA-G | F6700400 |
| IC Y-521 | 12 | 150 x 4.6 | F6995210 | IC Y-G | F6700230 |
| IC YK-421 | 5 | 125 x 4.6 | F7120012 | IC YK-G | F6709608 |
| IC YS-50 | 5 | 125 x 4.6 | F7122000 | IC YS-G | F6700530 |
| IC SI-90 4E[1] | 9 | 250 x 4.0 | F6995244 | IC SI-90G[1] | F6709620 |
| IC SI-50 4E[1] | 5 | 250 x 4.0 | F6995245 | IC SI-50G[1] | F6709625 |
| IC T-521[1] | 12 | 150 x 4.6 | F6995250 | IC T-G[1] | F6700412 |
| IC R-621 | 5 | 50 x 6.0 | F6998000 | IC R-G | F6709090 |

[1] PEEK hardware

# Shodex® GFC Phases

## Aqueous SEC (GFC) Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| PROTEIN | KW | Silica | Hydrophilic polymer | Analysis of high MW biological fluids. Suitable for separating proteins of MW few thousand to few million Da |
| OHpak | SB | Polyhydroxymethacrylate | - | Analysis of water-soluble samples and for molecular weight distribution |

PROTEIN KW-800 and KW400 are silica based series with high protein recovery rates. Whereas the KW-800 columns have the highest sample loading, the reduced particle size of KW400 columns enable 3-4 times greater sensitivity. Figure 5 illustrates the enhanced sensitivity achieved for a range of proteins with a KW400 column.

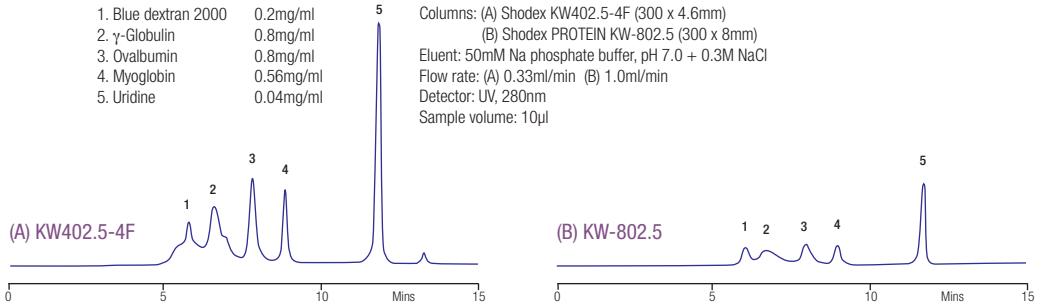



Figure 5. Comparison between KW400 and KW-800 columns

The OHpak SB-800 and SB400 series are based on polyhydroxymethacrylate and are used for the analysis of a wide range of molecular weight compounds.

## Ordering Information – GFC Columns

| PROTEIN Product | Particle Size (µm) | Pore Size (Å) | | Exclusion Limit (Da) | | Column Dimensions (mm) | Catalogue No. |
|---|---|---|---|---|---|---|---|
| | | Average | Maximum | Pullulan | Protein | | |
| KW-802.5 | 5 | 150 | 400 | 60,000 | 150,000 | 300 x 8.0 | F6989000 |
| KW-803 | 5 | 300 | 1,000 | 170,000 | 700,000 | 300 x 8.0 | F6989103 |
| KW-804 | 7 | 500 | 1,500 | 500,000 | 1,000,000 | 300 x 8.0 | F6989104 |
| KW-G | 7 | Guard column | | | | 50 x 6.0 | F6700131 |
| KW402.5-4F | 3 | 150 | 400 | 60,000 | 150,000 | 300 x 4.6 | F6989201 |
| KW403-4F | 3 | 250 | 800 | 150,000 | 600,000 | 300 x 4.6 | F6989202 |
| KW404-4F | 5 | 500 | 1,500 | 500,000 | 1,000,000 | 300 x 4.6 | F6989203 |
| KW405-4F | 5 | 700 | 2,000 | 1,300,000 | 20,000,000 | 300 x 4.6 | F6989204 |
| KW400G-4A | 5 | Guard column | | | | 10 x 4.6 | F6700132 |

| OHpak Product | Particle Size (µm) | Pore Size (Å) (Maximum) | Exclusion Limit (Da) Pullulan | Column Dimensions (mm) | Catalogue No. |
|---|---|---|---|---|---|
| SB-802 HQ | 8 | 100 | 4,000 | 300 x 8.0 | F6429100 |
| SB-802.5 HQ | 6 | 200 | 10,000 | 300 x 8.0 | F6429101 |
| SB-803 HQ | 6 | 800 | 100,000 | 300 x 8.0 | F6429102 |
| SB-804 HQ | 10 | 2,000 | 1,000,000 | 300 x 8.0 | F6429103 |
| SB-805 HQ | 13 | 7,000 | 4,000,000 | 300 x 8.0 | F6429104 |
| SB-806 HQ | 13 | 15,000 | 20,000,000 | 300 x 8.0 | F6429105 |
| SB-806M HQ | 13 | 15,000 | 20,000,000 | 300 x 8.0 | F6429106 |
| SB-G | 10 | Guard column | | 50 x 6.0 | F6709430 |
| SB-807 HQ | 35 | 30,000 | 500,000,000 | 300 x 8.0 | F6429108 |
| SB-807G | 35 | Guard column | | 50 x 8.0 | F6709431 |
| SB401-4E | 10 | 40 | 1,000 | 250 x 4.6 | F6429111 |
| SB402.5-4E | 6 | 200 | 10,000 | 250 x 4.6 | F6429112 |
| SB403-4E | 6 | 800 | 100,000 | 250 x 4.6 | F6429113 |
| SB404-4E | 7 | 2,000 | 1,000,000 | 250 x 4.6 | F6429114 |
| SB400G-4A | 7 | Guard column | | 10 x 4.6 | F6709432 |

# Other Shodex® Phases

## Aqueous/Organic SEC Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| Asahipak | GF | Polyvinyl alcohol | None | Both water and organic solvents can be used. Suitable for samples with both hydrophilic and hydrophobic moieties. |

The Asahipak GF Series columns are based on polyvinyl alcohol and can be used with both aqueous and organic solvents.
Please enquire for further details and ordering information for aqueous/organic SEC phases.

## Organic SEC (GPC) Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| GPC | KF, K, KD, HFIP, LF, HT, UT, AT | Styrene-divinylbenzene | None | KF-800 series shipped in THF. K-800 series shipped in $CHCl_3$. KD-800 series shipped in DMF – suitable for assay of polar polymers. HFIP suitable for engineering plastics eg. PET, polyamides. HT, UT and AT series for higher temperature GPC. |

Please enquire for further details and ordering information for organic SEC (GPC) phases.

## Hydrophobic Interaction Phase

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| HIC | PH | Polyhydroxymethacrylate | Phenyl | Analysis of proteins |

The HIC PH-814 column separates proteins without denaturation. It is applicable to samples after treatment of ammonium sulphate fractions.

## Organic Acids Phase

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| RSpak | KC | Styrene-divinylbenzene | Sulpho | Analysis of organic acids by ion exclusion and reversed-phase modes |

Shodex® RSpak KC columns are suitable for the analysis of organic acids by ion exclusion and reversed-phase modes.

## Affinity Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| AFpak | 6 kinds | Polyhydroxymethacrylate | 6 kinds of ligand | Purification of biological molecules depending on biochemical affinity |

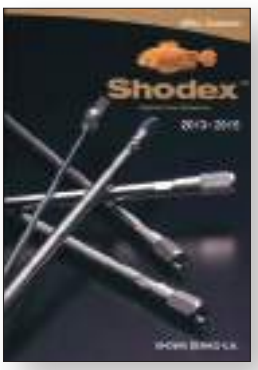
The Shodex AFpak range of affinity columns consists of 6 different ligand materials, designed for specific applications.

## Chiral Separation Phases

| Column Series | | Base Material | Functional Group | Comments/Applications |
|---|---|---|---|---|
| ORpak | CDA, CDB, CDC | Polyhydroxymethacrylate | Cyclodextrin derivative | Suitable for separation of optical isomers and structural isomers. CDA is α-CD, CDB is β-CD , CDC is γ-CD |
| ORpak | CDBS | Silica | Cyclodextrin derivative | CDBS is β-CD |
| ORpak | CRX | Polyhydroxymethacrylate | L-amino acid derivative | Separation of amino acids by ligand exchange |

Shodex ORpak CD phases consist of α-, β- or γ-cyclodextrin bonded to polyhydroxymethacrylate base material or silica (α-cyclodextrin). Shodex ORpak CRX columns have an L-amino acid derivative bonded to polyhydroxymethacrylate and are suitable for the separation of optical isomers of amino acids and hydroxy acids.

Please contact Hichrom for further information on any Shodex column and for ordering information for columns not listed.




# SIELC TECHNOLOGIES



- Novel silica-based mixed-mode phases
- Suitable for RP, NP, ion-exchange and ion-exclusion chromatography
- Unique adjustable selectivity
- Stable in 100% aqueous eluents
- LC-MS and preparative chromatography applications

Primesep® mixed-mode stationary phases have been developed by SIELC Technologies for separating a wide range of polar and non-polar compounds by different separation modes, based only on eluent selection. Ionizable compounds interact with the stationary phase by reversed-phase, ion-exchange or ion-exclusion mechanisms. In addition, Promix™ phases are available for biomolecule analysis. Obelisc™ mixed-mode phases are described on page 147 and the newer SHARC™ columns on page 148. Please see page 3 for Coresep™ (superficially porous) mixed-mode phases.

## Primesep® Phases

| Primesep Phase | Particle Size (µm) | Pore Size (Å) | Main Separation Modes | Typical Applications |
|---|---|---|---|---|
| A | 5, 10 | 100 | RP + cation-exchange + ion-exclusion | Neutral and weak basic compounds |
| 100 | 5, 10 | 100 | RP + cation-exchange + ion-exclusion | Neutral and basic compounds |
| 200 | 5, 10 | 100 | RP + cation-exchange + polar interaction | Neutral and strong basic compounds |
| 500 | 5, 10 | 100 | RP + cation-exchange + ion-exclusion | Neutral and basic compounds |
| C | 5, 10 | 100 | RP + cation-exchange + complex formation | Amines, sulphonium, phosphonium and metal ions |
| P | 5, 10 | 100 | RP + strong cation-exchange + π-π interaction | Neutral and basic compounds<br>Structural isomers of aromatic compounds |
| AB | 5, 10 | 100 | RP + cation-exchange + anion-exchange | Neutral, acidic and basic compounds |
| B | 5, 10 | 100 | RP + anion-exchange + ion-exclusion | Neutral and acidic compounds |
| B2 | 5, 10 | 100 | RP + anion-exchange + ion-exclusion | Neutral and acidic compounds |
| SB | 5, 10 | 100 | RP + anion-exchange + ion-exclusion | Neutral and acidic compounds |
| D | 5, 10 | 100 | RP + anion-exchange | Small hydrophobic and acidic compounds<br>Low MW plasma components and biofluids |

Table 1 summarises the retention of polar compounds on Primesep phases compared with typical C18 phases at various eluent pHs.

### Table 1. Retention of Polar Compounds

| Polar Compounds | Primesep | Typical C18 | |
|---|---|---|---|
| | | Acidic pH | Basic pH |
| Basic | Good | Poor | Good |
| Acidic | Good | Good | Poor |
| Zwitterionic | Good | Poor | Poor |

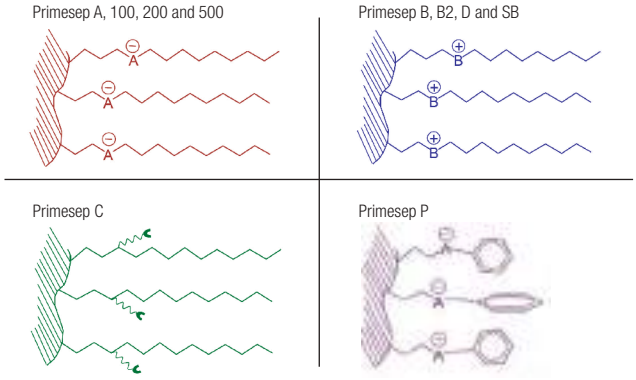



Figure 1. Primesep phases

Each Primesep column has a dual chemistry stationary phase containing a hydrophobic long alkyl chain and an ionisable cationic or anionic embedded group. Reversed-phase and ion-exchange interactions can be controlled independently; reversed-phase by organic concentration and ion-exchange by eluent ionic strength and pH. All columns offer the same hydrophobic retention properties but differ in their ion-exchange and other properties.

Primesep columns are suitable for analytical and preparative scale separations in isocratic and gradient modes, and are compatible with all common detection techniques.

These columns are resistant to dewetting in 100% aqueous eluent and are stable in pure organic and highly acidic conditions down to pH 1.5. They can efficiently separate organic and inorganic ions on the same column at the same time. This enables an organic pharmaceutical to be quantified simultaneously with its inorganic counter ion. Also, inorganic cations and anions can be run together without a specialised ion chromatography system.

The choice of buffer for use with Primesep columns depends on the detection technique. For UV detection, TFA, sulphuric acid, phosphoric acid and their salts are recommended. For LC-MS or ELSD detection, the best choices are TFA, ammonium formate, ammonium acetate, formic and acetic acids.

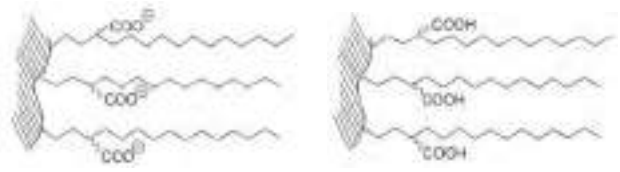
## Primesep® Embedded Acidic Phases

### Primesep A, 100, 200 and 500

Primesep® A, 100, 200 and 500 are reversed-phase columns with different strengths of embedded acidic (anionic) ion-pairing groups. Primesep A is the strongest acidic column, while Primesep 500 is the weakest acidic column. Differences in functional group acidity allow selection of the most appropriate column for a particular set of basic compounds that differ in their pka value.

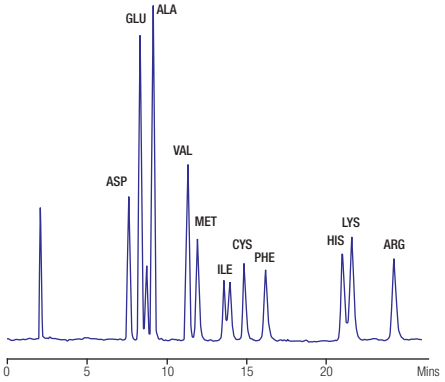
The embedded acidic functional group can be in an ionised form, or in a non-ionised form, depending on the pH of the eluent. When these two forms (see below) are in equilibrium, the phase is half ionised and half non-ionised. In order to achieve component retention by ion-exchange on these Primesep columns, eluent pH should be close to or above the transition value, as shown below.

| Primesep Phase | Transition pH |
|---|---|
| A | Ionized at all working pH |
| 100 | pH 1 |
| 200 | pH 2 |
| 500 | pH 5 |
| C | pH 3.5 |



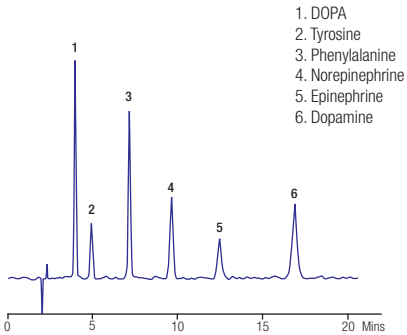

Ionised and non-ionised forms of Primesep embedded acidic phases

Primesep 100 and 200 are versatile columns for separation of a broad range of compounds. Figure 2 shows the separation of underivatized amino acids on Primesep 100. Figure 3 illustrates the application of Primesep 200 for catecholamines.




Column: Primesep 100 (5µm, 150 x 4.6mm)
Eluent: $CH_3CN$ - $H_2O$ (30:70)
Gradient of 0.05 to 0.3% TFA in 25mins
Flow rate: 1ml/min
Detection: ELSD

Figure 2. Analysis of amino acids on Primesep 100




Column: Primesep 200 (5µm, 150 x 4.6mm)
Eluent: $CH_3CN$ - 10mM ammonium formate, pH 2.9 (90:10)
Flow rate: 1ml/min
Detection: UV, 210nm

Figure 3. Analysis of catecholamines on Primesep 200

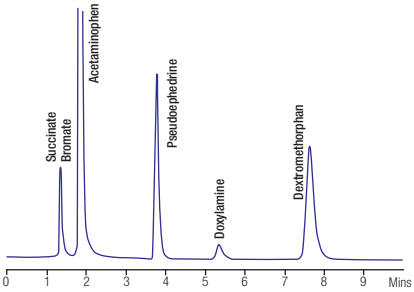
### Primesep C

Primesep C also contains an embedded anionic group (carboxylic acid) but with additional complex-formation properties. The latter facilitates retention of amines, sulphonium, phosphonium and metal ions. The degree of ion-exchange and complex formation can be effectively adjusted by the alteration of eluent pH, within the range 3 – 7. The unique complex forming properties of Primesep C columns lead to a reversal of elution order compared to ion-exchange, eg. $t_R$ $Li^+ > Na^+ > K^+$. Similarly primary amines are retained longer than secondary and tertiary amines on Primesep C columns.

Figure 4 shows the analysis of the active ingredients in a cough and cold medicine on Primesep C.

### Primesep P

The Primesep P phase provides three interactions with analytes – reversed-phase, π-π interaction and strong cation-exchange. It contains embedded acidic ion-pairing groups combined with an aromatic moiety and is useful for the separation of structural isomers of aromatic compounds. Enhanced π-π interaction can be achieved by adding THF to $CH_3CN$-$H_2O$ eluents.




Column: Primesep C (5µm, 150 x 4.6mm)
Eluent: $CH_3CN$ - 50mM TEA phosphate, pH 3.0 (60:40)
Flow rate: 1ml/min
Detection: UV, 205nm

Figure 4. Analysis of active ingredients in cough and cold drugs on Primesep C

## Primesep® Embedded Basic Phases

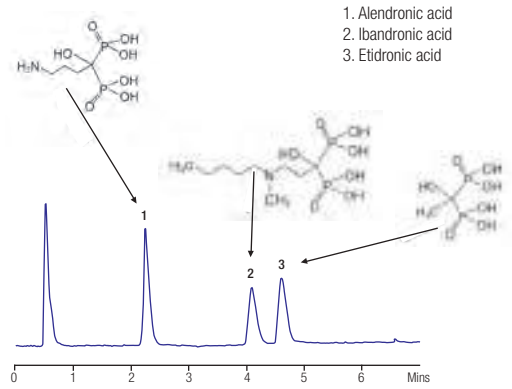
### Primesep B, B2 and SB

Primesep® B, B2 and SB contain embedded basic ion-pairing groups. In addition to improving the retention of acidic compounds by anion-exchange, the phases separate bases by an ion exclusion mechanism. Primesep B and SB are strong basic columns for operation in the pH range 1.5 to 4.5 and 1.5 to 5 respectively, created by the addition of TFA, phosphoric or perchloric acids to the eluent. Primesep B2 is a weak basic column that offers an extended pH range from 1.5 to 7, suitable for use with appropriate buffered solutions. For development of new methods, Primesep B2, SB or D are recommended, due to the extended pH stability range. Primesep B, B2, SB and D are fully ionised at all working pH values.

Figure 5 shows the simultaneous separation of dronic acids on Primesep SB.

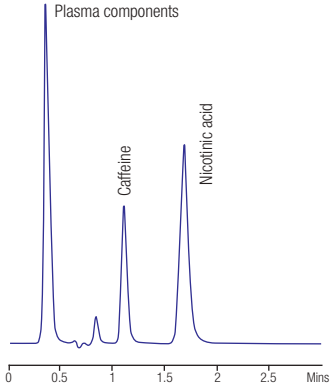
### Primesep D

Primesep D comprises an anion-exchange group embedded in a long alkyl chain. It is a weaker basic column than Primesep B2. In addition, Primesep D allows direct injection of plasma and other biofluids, enabling a broad range of small molecules to be analysed via a single column without any sample preparation. At pH 3.0, most proteins become positively charged and are excluded from the stationary phase, whilst small hydrophobic molecules are retained. Figure 6 illustrates a direct plasma injection on Primesep D.




Column: Primesep SB (5µm, 50 x 4.6mm)
Eluent: $CH_3CN$ - TFA (10:90)
TFA gradient 0.03 to 0.3% in 7mins
Flow rate: 1.0ml/min
Detection: ELSD

Figure 5. Analysis of dronic acids on Primesep SB




Column: Primesep D (5µm, 50 x 4.6mm)
Eluent: $CH_3CN$ - $H_2O$ - $HCO_2H$ (33:67:0.2)
Flow rate: 1ml/min
Detection: UV, 250 nm

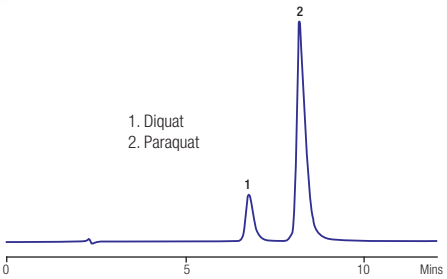
Figure 6. Direct plasma analysis on Primesep D

## Embedded Acidic and Basic Phase

### Primesep AB

Primesep AB is a zwitterionic reversed-phase column with embedded cation-exchange and anion-exchange functionalities, combining the properties of both in a single column. With basic analytes, Primesep AB behaves as if it has a negatively charged surface. Conversely, with strong acids Primesep AB behaves as if it has a positively charged surface. Anions and cations can be separated at the same time as neutrals. This is useful for complex mixtures which include polar ionisable compounds both of acidic and basic nature. The column is also capable of separating both the anion and cation of the same salt. This is important in analysis of pharmaceutical formulations, drug substances and other organic and inorganic salts.

Figure 7 shows the separation of a mixture of the quaternary compounds diquat and paraquat on Primesep AB.




Column: Primesep AB (5µm, 150 x 4.6mm)
Eluent: $CH_3CN$ - 0.3% TFA (10:90)
Flow rate: 1ml/min
Detection: UV, 250 nm

Figure 7. Separation of mixture of diquat and paraquat on Primesep AB

## Selectivity of Primesep® Phases

The cation-exchange Primesep phases (A, 100, 200 and P) show greater retention for basic compounds, whereas the anion-exchange Primesep phases (SB, B, D and B2) show the greatest retention for acidic compounds. Table 2 highlights the relative cationic and anionic strengths of the various Primesep phases.

### Table 2. Relative Strengths of Primesep Phases

| Cation-Exchange | | Anion-Exchange |
|---|---|---|
| Primesep A | Strong ↓ | Primesep SB |
| Primesep 100, P | | Primesep B |
| Primesep 200 | | Primesep D |
| Primesep C | Weak | Primesep B2 |

## Ordering Information – Primesep® (5µm, 100Å phases)[1]

When ordering please replace '**X**' with phase type ie. A, 100, 200, 500, C, B, B2, D, P, AB, SB

| Column i.d.[2] (mm) | Column Length[2] (mm) | | | | Guards[3] (2/pk) |
|---|---|---|---|---|---|
| | 50 | 100 | 150 | 250 | |
| 2.1 | **X**-21.050.0510 | **X**-21.100.0510 | **X**-21.150.0510 | **X**-21.250.0510 | **X**-21.G.0510 |
| 3.2 | **X**-32.050.0510 | **X**-32.100.0510 | **X**-32.150.0510 | **X**-32.250.0510 | **X**-32.G.0510 |
| 4.6 | **X**-46.050.0510 | **X**-46.100.0510 | **X**-46.150.0510 | **X**-46.250.0510 | **X**-46.G.0510 |
| 10 | **X**-100.050.0510 | **X**-100.100.0510 | **X**-100.150.0510 | **X**-100.250.0510 | - |
| 22 | **X**-220.050.0510 | **X**-220.100.0510 | **X**-220.150.0510 | **X**-220.250.0510 | - |
| 22 (10µm) | **X**-220.050.1010 | **X**-220.100.1010 | **X**-220.150.1010 | **X**-220.250.1010 | - |

[1] Other pore sizes and particle sizes available [2] Other dimensions available [3] Direct connect - no holder required

Primesep direct connect guard column

### Preparative Separations

Primesep® columns offer high capacity ion-exchange mechanisms for the retention of polar compounds. This enables these columns to be successfully used for scale-up separations. Conditions can be developed to be efficient and economical for preparative separations. For example, for ionisable compounds, conditions can be chosen where a high concentration of organic modifier is present, reducing the cost of solvent removal. In addition, the ability to reverse the elution order of differently charged components in the mixture, makes Primesep particularly effective for isolating specific components. Figure 8 demonstrates the high loading capacity of a Primesep 100 analytical column, which infers a high capacity ion-exchange mechanism on scaling up to preparative dimensions.

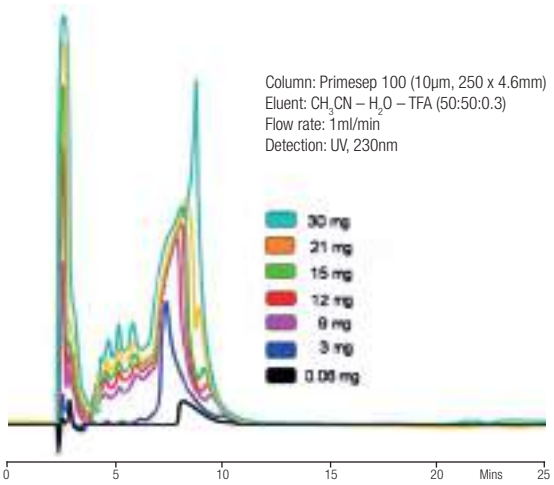



Figure 8. Loading study of HCl salt of polar compound

## PROMIX™

- Peptide and protein separations
- 2D HPLC with single column
- Alternative selectivity to reversed-phase 300Å columns
- Scalable from capillary to preparative

| Promix Phase | Particle Size (µm) | Pore Size (Å) |
|---|---|---|
| SP | 5, 10 | 100 |
| AP | 5, 10 | 100, 300 |
| MP | 5, 10 | 300, 800 |
| LP | 5, 10 | 800 |

The Promix™ range of columns, manufactured by SIELC, is designed for biomolecule analysis, in particular the analysis and purification of peptides and proteins, and for proteomics applications. Promix columns have the benefit of performing 2D HPLC with a single column, based on a combination of ionic and reversed-phase interactions.

Independent control of acid/buffer concentration and organic modifier offers almost unlimited control of retention and selectivity. Promix columns show enhanced selectivity compared to dedicated reversed-phase or ion-exchange columns for closely related peptides and proteins, being able to separate compounds differing in sequence by a single amino acid pair only. Methods on these columns are completely scalable from capillary to preparative. Figure 9 shows the separation of insulin analogues differing in sequence by a single amino acid pair.

Please contact Hichrom for ordering information for Promix columns.

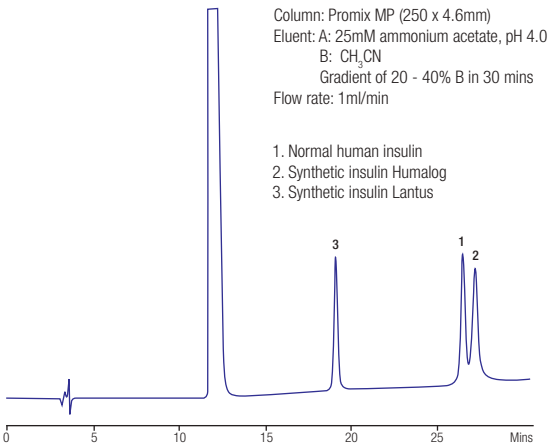



Figure 9. Separation of human and synthetic insulins on Promix

## Obelisc™

- Multiple separation modes
- Simple eluent selection
- MS, ELSD and low UV (<220nm) compatible
- Adjustable selectivity

Obelisc™ HPLC columns are based on a new generation of mixed-mode phases developed by SIELC Technologies. Two complementary columns, Obelisc R and Obelisc N, based on **Li**quid **S**eparation **C**ell (LiSC™) technology, offer a new approach to separating a variety of small polar and non-polar compounds, by multiple separation mechanisms. Like living cells, which exist in equilibrium with the outside environment, liquid separation cells exist in constant equilibrium with the eluent.

Obelisc R, with reversed-phase characteristics, and Obelisc N, with normal-phase characteristics, differ in the type and proximity of their charged groups and the hydrophobicity of their long linking chains. Obelisc R has cationic groups close to the 100Å silica surface separated from anionic groups by a hydrophobic chain. Obelisc N has anions close to the silica surface separated from cationic groups by a hydrophilic chain.

Typical eluents used with Obelisc columns are based on acetonitrile, water and MS compatible buffers ammonium formate (pH 3) and ammonium acetate (pH 5), or phosphate buffer for low UV detection.

### Obelisc R

Specially developed 'AQ' type columns are designed to work in low organic containing eluents, often 100% aqueous, for the analysis of polar compounds by reversed-phase. However, they can often offer insufficient improvement in retention of highly polar molecules. Obelisc R can be used in traditional RP type applications and it offers significant improvement in the degree of retention of both acidic and basic polar molecules, compared with typical 'AQ' type phases.

Obelisc R is recommended for the analysis of complex mixtures of polar and non-polar molecules, especially with MS, ELSD or CAD detection. Figure 10 shows the separation of a complex mixture of acids, bases, amino acids and neutral compounds on Obelisc R.

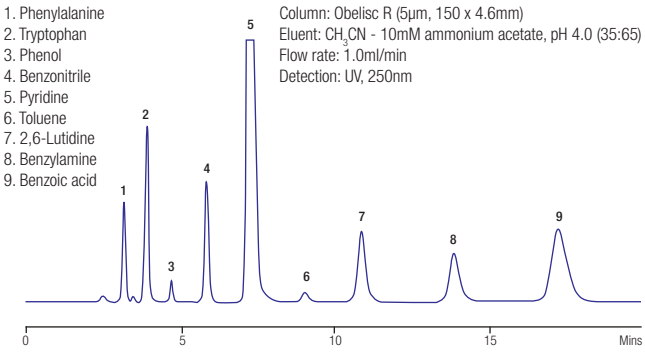



Figure 10. Separation of amino acids, bases, acids and neutrals on Obelisc R

### Obelisc N

Obelisc™ N has very polar characteristics and works well for polar and charged analytes. The positive and negative charges of the phase are well separated and independently accessible, which results in different selectivity compared to traditional HILIC and silica columns. Figure 11 shows the HILIC separation of sugars on Obelisc N. Eluent composition changes the conformation of the long hydrophilic chain, thus changing separation selectivity. Figure 12 shows the effect of buffer concentration on the resolution of isomeric aminobutyric acids.

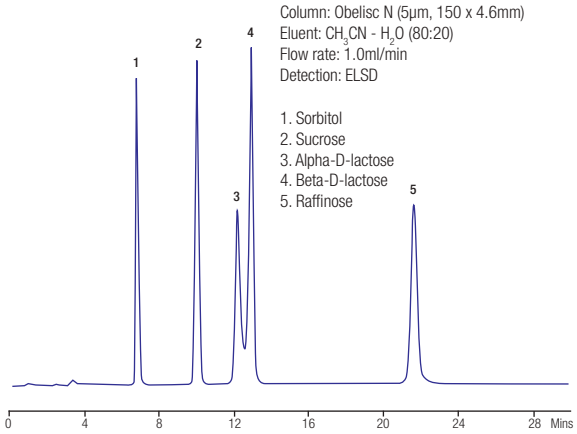



Figure 11. Separation of sugars in HILIC mode on Obelisc N

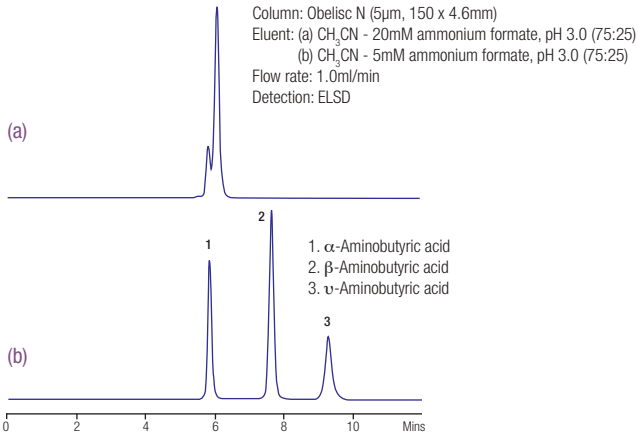



Figure 12. Separation of aminobutyric acids on Obelisc N

### Ordering Information (5µm, 100Å phases)[1]

When ordering please replace '**X**' with OR for Obelisc R or ON for Obelisc N

| Column i.d. (mm) | Column Length (mm) | | | | Guards[2] (2/pk) |
|---|---|---|---|---|---|
| | 50 | 100 | 150 | 250 | |
| 2.1 | X-21.050.0510 | X-21.100.0510 | X-21.150.0510 | X-21.250.0510 | X-21.G.0510 |
| 3.2 | X-32.050.0510 | X-32.100.0510 | X-32.150.0510 | X-32.250.0510 | X-32.G.0510 |
| 4.6 | X-46.050.0510 | X-46.100.0510 | X-46.150.0510 | X-46.250.0510 | X-46.G.0510 |
| 10 | X-100.050.0510 | X-100.100.0510 | X-100.150.0510 | X-100.250.0510 | - |
| 22 | X -220.050.0510 | X-220.100.0510 | X-220.150.0510 | X-220.250.0510 | - |

[1] 10µm phase also available [2] Direct connect – no holder required

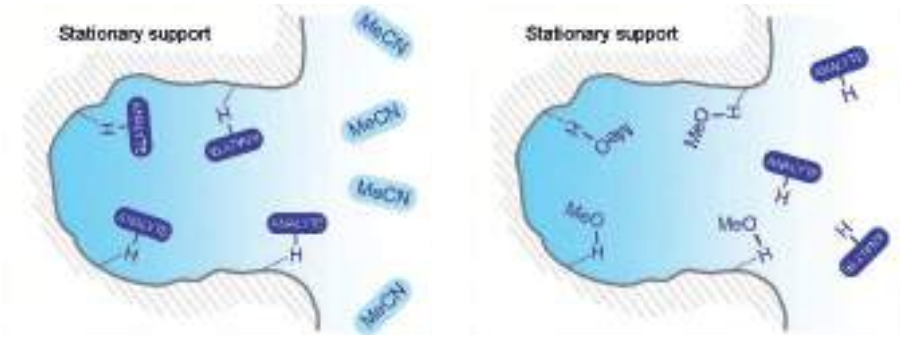
## SHARC™

SHARC™ (**S**pecific **H**ydrogen-bond **A**dsorption **R**esolution **C**hromatography) columns are the latest columns developed by SIELC Technologies. They are the first commercially available columns which provide separations based primarily on hydrogen bonding. SHARC 1 achieves separation based on the analyte's ability to act as a hydrogen atom donor or acceptor.

### Operating Conditions

Solvents used for SHARC separations are acetonitrile (MeCN) as the weak solvent and methanol (MeOH) as the strong solvent. Acetonitrile alone has a low level of hydrogen bonding with the SHARC stationary phase, whereas methanol interacts strongly, reducing retention of analytes capable of hydrogen interactions (Figure 13). By altering the ratio of acetonitrile/methanol, the optimum retention profile can be obtained for many types of molecules.

Analytes can retain on the stationary phase by more than one hydrogen bond and act as a donor or acceptor. The SHARC 1 column is a hydrogen atom acceptor type stationary phase, showing increased retention towards molecules with higher numbers of polar X-H bonds such as alcohols, amines, acids, amides, phenols etc.




(a) Interaction with H-bonding analytes in MeCN

(b) MeOH competes with analytes for stationary phase surface sites

Figure 13. SHARC technology

### Benefits of SHARC Columns

1) Acetonitrile-methanol mixtures have lower viscosity than aqueous solvent mixtures, enabling the use of smaller particles and faster flow rates, for fast analyses.
2) Methanol is one of the most universal solvents for organic compounds.
3) Acetonitrile-methanol mixtures have a low boiling point and are much easier to evaporate than water, a benefit for preparative separations.
4) Acetonitrile-methanol mixtures are MS friendly, enabling mass directed preparative strategies.
5) A wide range of compounds with functional groups containing oxygen and nitrogen can be retained and separated.

Figure 14 shows the separation of a mixture of four neurotransmitters, based on their ability to form hydrogen bonds with the SHARC 1 stationary phase. Elution order corresponds to the number and strength of interaction points.

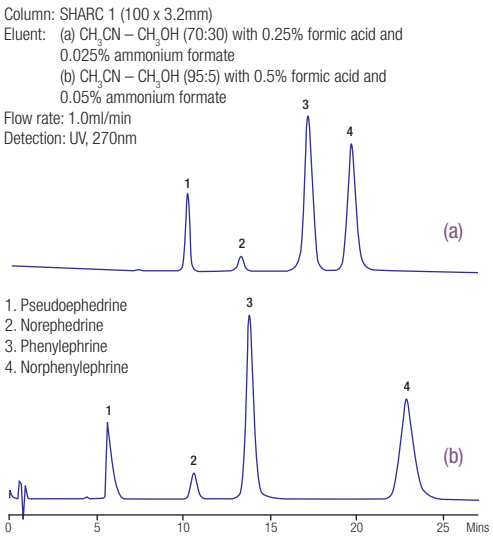



Figure 14. Effect of eluent composition on separation on SHARC 1

### Ordering Information – SHARC 1 (5µm, 100Å)

| Column i.d.[1] (mm) | Column Length[1] (mm) | | | | Guards[2] (2/pk) |
|---|---|---|---|---|---|
| | 50 | 100 | 150 | 250 | |
| 2.1 | SH1-21.050.0510 | SH1-21.100.0510 | SH1-21.150.0510 | SH1-21.250.0510 | SH1-21.G.0510 |
| 3.2 | SH1-32.050.0510 | SH1-32.100.0510 | SH1-32.150.0510 | SH1-32.250.0510 | SH1-32.G.0510 |
| 4.6 | SH1-46.050.0510 | SH1-46.100.0510 | SH1-46.150.0510 | SH1-46.250.0510 | SH1-46.G.0510 |
| 10 | SH1-100.050.0510 | SH1-100.100.0510 | SH1-100.150.0510 | SH1-100.250.0510 | - |
| 22 | SH1-220.050.0510 | SH1-220.100.0510 | SH1-220.150.0510 | SH1-220.250.0510 | - |
| 22 (10µm) | SH1-220.050.1010 | SH1-220.100.1010 | SH1-220.150.1010 | SH1-220.250.1010 | - |

[1] Other dimensions available, including capillary

[2] Direct connect - no holder required

In addition to the well-known classical Hypersil® and Hypersil BDS phases (see page 151), Thermo Scientific manufactures a wide range of premium phases, including Hypersil GOLD® (page 150) and Hypercarb® (page 150). Their newest development is the Accucore™ range, based on a core enhanced material. In addition, Acclaim™, ProSwift®, ProPac™ and PepSwift HPLC columns, developed by Dionex, are now sold under the Thermo Scientific brand (see pages 152-153).

## Accucore™

Using Core Enhanced Technology™, Accucore HPLC Columns offer higher speed, higher resolution separations compared to traditional larger fully porous particles. They are available in a wide range of selectivities and compatible with almost any instrument.

### Accucore HPLC Columns

These solid core particles have an overall diameter of 2.6µm with a porous layer thickness of 0.5µm and a very narrow particle size distribution. Accucore columns offer high speed, high resolution separations with significantly lower back pressures than those associated with UHPLC.

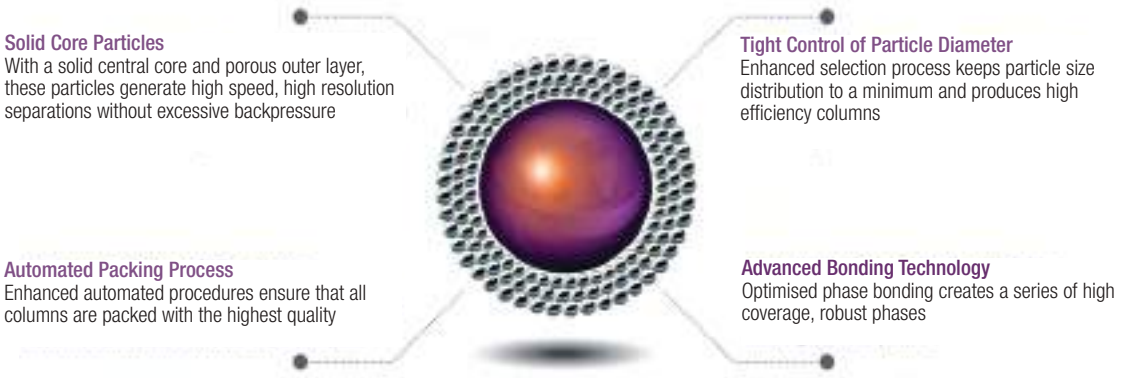
### Accucore HPLC Columns for Biomolecules

The range of Accucore columns packed with 150Å pore diameter particles (2.6µm) enables biomolecule separations to benefit from the high resolution and speed provided by Core Enhanced Technology.

### Accucore XL HPLC Columns

Using 4µm solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to obtain superior performance to that of columns packed with 5, 4 or 3µm fully porous particles.

### The key components of Core Enhanced Technology




### Accucore Phases

| Column | Phase | Bonding | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | pH Range |
|---|---|---|---|---|---|---|---|
| Accucore HPLC Columns | RP-MS | Proprietary | 2.6 | 80 | 130 | 7 | 2 - 9 |
| | C18 | C18 | 2.6 | 80 | 130 | 9 | 1 - 11 |
| | C8 | C8 | 2.6 | 80 | 130 | 5 | 2 - 9 |
| | aQ | C18 with polar endcapping | 2.6 | 80 | 130 | 9 | 2 - 9 |
| | Polar Premium | Amide embedded C18 | 2.6 | 150 | 80 | 8 | 1.5 - 10 |
| | Phenyl-Hexyl | Phenyl-Hexyl | 2.6 | 80 | 130 | 5 | 2 - 8 |
| | PFP | Pentafluorophenyl | 2.6 | 80 | 130 | 5 | 2 - 8 |
| | Phenyl-X | Proprietary | 2.6 | 80 | 130 | 6 | 2 - 8 |
| | C30 | C30 | 2.6 | 150 | 80 | 5 | 2 - 8 |
| | HILIC | - | 2.6 | 80 | 130 | - | 2 - 8 |
| | Urea-HILIC | Urea | 2.6 | 80 | 130 | - | 2 - 8 |
| Accucore Columns for Biomolecules | 150-C18 | C18 | 2.6 | 150 | 80 | 7 | 1 - 11 |
| | 150-C4 | C4 | 2.6 | 150 | 80 | 2 | 2 - 9 |
| | 150-Amide-HILIC | Amide | 2.6 | 150 | 80 | - | 2 - 8 |
| Accucore XL HPLC Columns | C18 | C18 | 4 | 80 | 90 | 7 | 1 - 11 |
| | C8 | C8 | 4 | 80 | 90 | 4 | 2 - 9 |

Please contact us for ordering information on all Accucore columns.

## Hypersil GOLD®

Hypersil GOLD® columns are based on high purity silica and a novel proprietary derivatization and endcapping procedure, which reduces unwanted secondary and tertiary interactions of an analyte with the silica. The range includes a number of different bonding chemistries. Columns can be supplied in a wide range of particle sizes (1.9 to 12µm) and in column dimensions from capillary to preparative. Hypersil GOLD, Hypersil GOLD aQ and Hypersil GOLD PFP are also available in nanobore formats for nanospray LC-MS applications, particularly proteomics.

### Hypersil GOLD Phases

| Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | pH Range |
|---|---|---|---|---|---|---|---|
| GOLD | Proprietary | Yes | 1.9, 3, 5, 8, 12 | 175 | 220 | 10 | 1 - 11 |
| GOLD C8 | Octyl | Yes | 1.9, 3, 5 | 175 | 220 | 8 | 2 - 9 |
| GOLD C4 | Butyl | Yes | 1.9, 3, 5 | 175 | 220 | 5 | 2 - 8 |
| GOLD aQ | Octadecyl | Yes | 1.9, 3, 5, 8, 12 | 175 | 220 | 12 | 2 - 9 |
| GOLD PFP | Perfluorophenyl | Yes | 1.9, 3, 5, 8, 12 | 175 | 220 | 8 | 2 - 8 |
| GOLD CN | Cyano | Yes | 1.9, 3, 5 | 175 | 220 | 4 | 2 - 8 |
| GOLD Phenyl | Phenyl | Yes | 1.9, 3, 5 | 175 | 220 | 8 | 2 - 8 |
| GOLD Amino | Amino | Yes | 1.9, 3, 5 | 175 | 220 | 2 | 2 - 8 |
| GOLD AX | Polymeric amine | No | 1.9, 3, 5 | 175 | 220 | 6 | 2 - 8 |
| GOLD SAX | Quaternary amine | Yes | 1.9, 3, 5 | 175 | 220 | 2.5 | 2 - 8 |
| GOLD Silica | - | - | 1.9, 3, 5 | 175 | 220 | - | 2 - 8 |
| GOLD HILIC | Polyethyleneimine | n/a | 1.9, 3, 5 | 175 | 220 | 6 | 2 - 8 |

### Hypersil GOLD Selectivities

In addition to the original **Hypersil GOLD** phase, eleven other phases are now available, offering a range of selectivity options to optimise separations and maximise productivity. The original Hypersil GOLD shows C18-like USP L1 retention and selectivity. The phase is claimed to be stable over the pH range 1 to 11.

**Hypersil GOLD C4** columns provide similar selectivity to C18 and C8 columns but with less retention. The shorter chain length and lower hydrophobic character make C4 a particularly useful phase for separation of hydrophobic polypeptides and small proteins.

**Hypersil GOLD aQ** is a polar endcapped C18 phase, which is compatible with highly aqueous eluents. It offers superior retention of polar compounds, due to the polar functional group providing additional interaction mechanisms with polar compounds.

**Hypersil GOLD PFP** columns offer alternative selectivity in reversed-phase HPLC by offering extra retention and selectivity for positional isomers of halogenated compounds. They are also well suited for the selective analysis of non-halogenated compounds, particularly polar compounds containing hydroxyl, carboxyl, nitro or other polar groups, especially when these groups are located on an aromatic or other rigid ring system.

**Hypersil GOLD Amino** can be used as a weak anion-exchange material for the analysis of anions and organic acids. It is also useful for carbohydrate analysis when used in reversed-phase or HILIC mode.

**Hypersil GOLD AX** columns utilise a novel polymeric amine ligand bonded to silica. It is a weak anion-exchange material, suitable for the analysis of smaller proteins and peptides and anionic species. They are particularly suited to the analysis of polar compounds in HILIC applications.

**Hypersil GOLD SAX** is a highly stable silica-based quaternary amine strong anion-exchange phase. Columns are suited to the analysis of smaller organic molecules such as nucleotides and organic acids.

Please contact us for ordering information on Hypersil GOLD columns.

## Hypercarb®

Hypercarb® is a unique **P**orous **G**raphitic **C**arbon (PGC) phase composed of flat sheets of hexagonally arranged carbon atoms, with a fully satisfied valence. The selectivity offered by Hypercarb is different to that of silica and polymeric phases. Hypercarb is ideally suited to the separation of highly polar compounds and compounds with closely related structures, including geometric isomers and diastereomers.

The physical characteristics of the material are different from conventional silica and polymer materials. Hypercarb is totally stable across the entire pH range. It can be used for both normal- and reversed-phase HPLC and is well suited for reversed-phase LC-MS assays as it is stable with any eluent and produces no phase bleed issues. Hypercarb is ideal for high temperature applications and the robust nature of the material ensures a long column lifetime.

| Particle Size (µm) | Pore Size (Å) | Pore Volume (cc/g) | Surface Area ($m^2/g$) |
|---|---|---|---|
| 3, 5, 7 | 250 | 0.7 | 120 |

Please contact us for ordering information on Hypercarb columns.

## Hypersil®

Hypersil® is a traditional 120Å pore size silica, manufactured by Thermo Scientific. The octyl-, cyano- and phenyl-bonded phases are available as both endcapped and non-endcapped materials.

### Hypersil Phases[1]

| Hypersil Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Silica | - | No | 3, 5 | 120 | 170 | - |
| SAS (C1) | Methyl | No | 5 | 120 | 170 | 2.5 |
| MOS (C8) | Octyl | No | 3, 5 | 120 | 170 | 6.5 |
| MOS-2 (C8) | Octyl | Yes | 5 | 120 | 170 | 6.5 |
| ODS (C18) | Octadecyl | Yes | 3, 5 | 120 | 170 | 10 |
| ODS-2 (C18) | Octadecyl | Yes | 3, 5 | 80 | 220 | 11 |
| Phenyl | Phenyl | No | 5 | 120 | 170 | 5 |
| Phenyl-2 | Phenyl | Yes | 5 | 120 | 170 | 5 |
| CPS (CN) | Cyano | No | 3, 5 | 120 | 170 | 4 |
| CPS-2 (CN) | Cyano | Yes | 5 | 120 | 170 | 4 |
| APS-2 (NH2) | Amino | No | 3, 5 | 120 | 170 | 2 |

[1] Please contact us for ordering information on Hypersil columns.

## Hypersil® BDS

Hypersil® BDS is base deactivated to remove the active silanols and present a homogeneous surface for bonding, resulting in improved peak shape and column performance. Hypersil BDS is available in C8, C18, phenyl and cyano phases.

### Hypersil BDS Phases[1]

| Hypersil BDS Phase | Particle Size (µm) | Pore Size (Å) | Pore Volume (cc/g) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|
| BDS C8 | 2.4, 3, 5 | 130 | 0.65 | 170 | 7 |
| BDS C18 | 2.4, 3, 5 | 130 | 0.65 | 170 | 11 |
| BDS Phenyl | 3, 5 | 130 | 0.65 | 170 | 5 |
| BDS Cyano (CPS) | 3, 5 | 130 | 0.65 | 170 | 4 |

[1] Please contact us for ordering information on Hypersil BDS columns.

## Syncronis™

Syncronis™ HPLC columns are based on high purity 100Å silica, with a surface area of 320$m^2/g$. Phases are available in three particle sizes: 1.7µm for rapid UHPLC separations and 3µm and 5µm for more traditional HPLC analyses. Syncronis reversed-phase columns are densely bonded and double endcapped to minimise the number of interacting silanols.

### Syncronis Phases[1]

| Syncronis Phase | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | pH Range |
|---|---|---|---|---|---|---|
| C18 | Yes | 1.7, 3, 5 | 100 | 320 | 16 | 2 - 9 |
| C8 | Yes | 1.7, 3, 5 | 100 | 320 | 10 | 2 - 8 |
| aQ | Polar | 1.7, 3, 5 | 100 | 320 | 19 | 2 - 8 |
| Phenyl | Yes | 1.7, 3, 5 | 100 | 320 | 11 | 2 - 8 |
| Amino | Yes | 1.7, 3, 5 | 100 | 320 | 4 | 2 - 8 |
| Silica | - | 1.7, 3, 5 | 100 | 320 | - | 2 - 8 |
| HILIC | - | 1.7, 3, 5 | 100 | 320 | 5 | 2 - 8 |

[1] Please contact us for ordering information on Syncronis columns.

## Fluophase®

Fluophase® RP (100Å) and WP (300Å) are straight chain perfluorohexyl materials, whereas Fluophase PFP is a perfluorophenyl phase. These fluorinated phases exhibit extra retention and selectivity for halogenated compounds compared with C18 phases. They also exhibit good shape selectivity for positional isomers of halogenated aromatic compounds. Polar compounds are often well retained on these materials. Fluophase phases are also excellent for the separation of difficult non-polar samples such as surfactants. They are frequently used in the analysis of complex taxane samples. Fluophase columns are stable under 100% aqueous conditions, so are ideal for 0 to 100% organic fast gradients.

Please contact us for ordering information on all Hypersil, Hypersil BDS, Syncronis and Fluophase columns.

## Acclaim™

Acclaim™ HPLC phases are based on high purity, porous silica particles (2.2, 3 and 5µm), with advanced and innovative column bonding technologies. This provides complementary selectivity, high column efficiencies and symmetrical peaks. Columns for reversed-phase, HILIC, mixed-mode and application specific analyses are available.

### Acclaim Reversed-Phase and HILIC Phases[1]

| Acclaim Phase | Chemistry | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | pH Range |
|---|---|---|---|---|---|---|---|
| 120 C18 | C18 | Yes | 2.2, 3, 5 | 120 | 300 | 18 | 2 - 8 |
| 300 C18 | C18 | Yes | 3 | 300 | 100 | 8 | 2.5 - 7.5 |
| C8 | C8 | Yes | 2.2, 3, 5 | 120 | 300 | 11 | 2 - 8 |
| C30 | C30 | Yes | 3, 5 | 200 | 200 | 13 | 2 - 8 |
| Phenyl-1 | Alkyl aromatic | Yes | 3 | 120 | 300 | 13 | 2 - 8 |
| PolarAdvantage | Embedded sulphonamide | Yes | 2.2, 3, 5 | 120 | 300 | 16 | 2 - 8 |
| PolarAdvantage II | Embedded amide | Yes | 2.2, 3, 5 | 120 | 300 | 16 | 1.5 - 10.5 |
| HILIC-10 | Proprietary | No | 3 | 120 | 300 | 8 | 2 - 8 |

[1] Please contact us for ordering information on Acclaim reversed-phase and HILIC columns.

**Acclaim PolarAdvantage (PA)** features a patented bonding chemistry that incorporates a polar sulphonamide group with an ether linkage near the silica surface. Acclaim PA columns are compatible with 100% aqueous eluents and offer unique selectivity and good peak shape for acidic, basic and neutral analytes.

**Acclaim PolarAdvantage II (PA2)** columns feature a patented surface chemistry that incorporates an amide-embedded polar group and multi-point attachment between the ligands and silica surface. This unique chemistry provides enhanced hydrolytic stability from pH 1.5 to 10.5 with 100% aqueous eluents. Acclaim PA2 is specifically designed to withstand high pH conditions, making it a good choice for the separation of both basic and acidic analytes.

## Acclaim™ Mixed-Mode Phases

Mixed-mode columns provide a unique, adjustable selectivity tool, using variation in pH, ionic strength or organic modifier to influence the separation selectivity of acids, bases, zwitterions and neutral molecules.

### Acclaim Mixed-Mode Phases[1]

| Acclaim Phase | Retention Mechanism | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | pH Range |
|---|---|---|---|---|---|
| Mixed-Mode WAX-1 | RP + WAX + cation exclusion + HILIC | 3, 5 | 120 | 300 | 2.5 - 7.5 |
| Mixed-Mode WCX-1 | RP + WCX + HILIC | 3, 5 | 120 | 300 | 2.5 - 7.5 |
| Mixed-Mode HILIC-1 | RP + HILIC | 3, 5 | 120 | 300 | 2.5 - 7.5 |
| Trinity P1 | SCX + WAX + RP | 3 | 300 | 100 | 2.5 - 7.0 |

[1] Please contact us for ordering information on Acclaim mixed-mode phases.

**Acclaim Mixed-Mode WAX-1** has a surface consisting of a hydrophobic alkyl chain with a tertiary amine group at the terminus. The hydrophobic moiety provides reversed-phase retention and the terminal amino group facilitates electrostatic interactions.

**Acclaim Mixed-Mode WCX-1** can separate compounds using multiple separation modes: reversed-phase, cation-exchange and HILIC, and is ideal for the separation of basic molecules. Selectivity can be adjusted by modifying ionic strength, pH or organic solvent composition.

**Acclaim Mixed-Mode HILIC-1** combines both reversed-phase and HILIC properties. The phase consists of a hydrophobic alkyl chain with a diol group at the terminus.

**Acclaim Trinity P1** is a trimodal phase consisting of high purity porous 3µm silica particles coated with charged nanopolymer beads. The phase retains both cations and anions at the same time, meaning that baseline separation can be achieved for both a drug and its counterions.

## Acclaim™ Application Specific Phases

Thermo Scientific Acclaim speciality phases are based on novel and unique chemistries and combine superior resolution with ease of use.

### Acclaim Application Specific Phases[1]

| Acclaim Phase | Particle Size (µm) | Pore Size (Å) | pH Range | Typical Applications |
|---|---|---|---|---|
| Organic Acid (OA) | 3, 5 | 120 | 2 - 8 | Hydrophilic, aliphatic and aromatic organic acids |
| Surfactant | 3, 5 | 120 | 2.5 - 7.5 | Anionic, cationic, non-ionic and amphoteric surfactants |
| Explosives E1 | 5 | 120 | 3 - 7 | Nitroaromatic and nitramine explosives |
| Explosives E2 | 2.2, 3, 5 | 120 | 2.5 - 8 | |
| Carbamate | 3, 5 | 120 | 2 - 8 | Carbamate pesticides |
| Carbonyl | 2.2 | 120 | 2.5 - 8 | DNPH derivatives of aldehydes and ketones |

[1] Please contact us for ordering information on Acclaim application specific columns.

## Thermo Scientific Columns for Biomolecules

Thermo Scientific manufacture a wide range of silica and polymeric columns specifically designed for analysis of proteins, peptides, oligonucleotides and other biomolecules by reversed-phase, ion-exchange, size exclusion, hydrophobic interaction and affinity chromatography.

## Columns for Proteins

### A) Reversed-Phase Columns

**BioBasic™** 300Å pore size reversed-phase columns are available in C18, C8 and C4 chemistries. The extra dense bonding produces a highly stable, reproducible surface, ideally suited for LC-MS separations.

**ProSwift® RP** phenyl bonded polystyrene-divinylbenzene monolith columns provide high resolution at exceptionally high flow rates for fast protein separations and analysis.

### B) Ion-Exchange Columns

**BioBasic™ AX and SCX** wide pore silica-based ion-exchange materials can be used across a wide range of pH and ionic strength conditions. BioBasic AX can also be used under high organic conditions in the HILIC mode.

**ProPac™ and MAbPac** phases are based on pellicular, non-porous core particles. They provide high resolution and efficiency for separations of protein variants, resolving isoforms that differ by a single charged residue. The ProPac series is based on non-porous polymer resin consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene coated with a hydrophilic layer.

**ProSwift® IEX** polymethacrylate monolithic columns provide the resolving power of non-porous analytical media combined with fast analysis performance. WCX, SCX, WAX and SAX chemistries are available.

### C) Size Exclusion Columns

**BioBasic™** SEC columns, based on silica with a proprietary polymeric coating, offer the mechanical stability of silica size exclusion columns, with higher efficiencies than that of polymer-based columns. Four pore sizes are available (60Å, 120Å, 300Å and 1000Å).

**MAbPac SEC-1** (300Å, 5µm diol-bonded silica) is a size exclusion column specifically designed for separation and characterisation of monoclonal antibodies and their aggregates, as well as the analysis of Fab and Fc fragments resulting from proteolysis.

### D) Hydrophobic Interaction Columns

The **ProPac HIC-10** column is a high resolution, high capacity, 300Å, 5µm silica based HIC column for the high resolution separations of proteins and protein variants.

### E) Affinity Columns

**ProPac IMAC-10** is a high resolution column for separation of proteins and peptides by immobilised metal affinity chromatography. It is packed with 10µm non-porous polymeric beads coated with a hydrophilic layer, then grafted with poly(iminodiacetic acid) chains.

**ProSwift® ConA-1S** affinity monolith column is designed for the efficient enrichment and purification of Concanavalin A binding glycans, glycopeptides and glycoproteins. It shows high capacity and high sample recovery.

## Columns for Oligonucleotides

Thermo Scientific offer DNAPac® pellicular anion-exchange resins and DNA Swift™ polymeric monolithic phase specifically for the analysis of oligonucleotides.

**DNAPac®** PA100 and PA200 are strong anion-exchange columns developed to provide high resolution analysis and purification of synthetic oligonucleotides. They are capable of resolving full length from n-1, n+1 and other failure sequences.

**DNASwift™ SAX-1S** is a strong porous anion-exchange monolithic column that provides exceptionally high purity and yield of oligonucleotides. It is compatible with high pH eluents and high temperatures and has a high sample capacity.

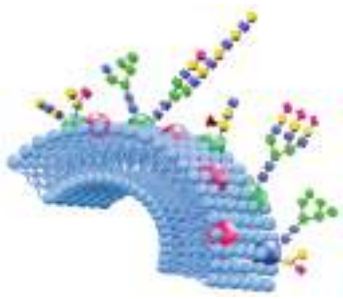
## Columns for Proteomics

**Acclaim™ PepMap** has become the standard for peptide separations in proteomics and can be used with all modern nano LC systems. The 2µm Acclaim PepMap RSLC phase has been developed for ultra-high resolution analyses of tryptic, natural and synthetic peptides. Acclaim PepMap Trap columns are typically applied for the desalting of peptides before LC separation with MS detection, thus allowing fast sample preconcentration.

**PepSwift and ProSwift** – PepSwift (100, 200 or 500µm i.d.) and ProSwift RP-10R (1.0mm i.d.) polystyrene-divinylbenzene monolithic columns show high sensitivity for LC-MS and are ideal for high speed peptide and protein separations.

## Columns for Glycans

**GlycanPac™ AXH-1** (1.9 and 3µm) is a silica-based column for simultaneous separation of glycans by charge, size and polarity. The column is based on innovative mixed-mode surface chemistry, specifically designed for qualitative, structural analysis and characterisation of uncharged and charged glycans present on proteins.



Please contact us for ordering information on all Thermo Scientific columns for biomolecules.

# TOSOH BIOSCIENCE

Tosoh Bioscience is a major supplier of liquid chromatography products in the pharmaceutical and biotechnology industries, for the analysis, isolation and purification of proteins, peptides, oligonucleotides and enzymes as well as low molecular weight compounds. TSKgel® is the most popular of several ranges of columns and bulk packing materials supplied by Tosoh Bioscience. An extensive range of products is offered for size exclusion, ion-exchange, reversed-phase, HILIC, HIC and affinity separation techniques.

## Size Exclusion Chromatography Phases

TSKgel® columns are available for both modes of size exclusion chromatography (SEC) – gel filtration chromatography (GFC) of water soluble polymers in aqueous eluents and gel permeation chromatography (GPC) of organic soluble polymers using non-aqueous eluents. The TSKgel SW and PW column lines are suitable for GFC, the H-type columns for GPC and the Alpha and SuperAW series can be used for both GFC and GPC.

### A. Silica-based Phases (SW)

**TSKgel SW and SW$_{XL}$** columns are widely used for aqueous separations of proteins, antibodies, enzymes, nucleic acids and other biological macromolecules. SEC phases with pore sizes of 125, 250 and 450Å enable the analysis of a wide range of molecular weight biomolecules. The smaller particle sizes of the SW$_{XL}$ columns result in reduced analysis times. All SW-type columns are available in stainless steel hardware; SW$_{XL}$ columns are also available in PEEK hardware. TSKgel G3000SW$_{XL}$ is the ideal scouting column for unknown molecular weight compounds.

The **SuperSW** materials (4µm) provide fast, high efficiency separations. The increased sensitivity compared with the corresponding SW$_{XL}$ or SW phase is especially useful for limited sample quantities.

### B. Polymer-based Phases (PW)

Polymeric **TSKgel PW and PW$_{XL}$** series columns are designed for GFC of water soluble organic polymers, polysaccharides, oligosaccharides, DNA and RNA. They are based on a hydrophilic polymethacrylate matrix, which is stable from pH 2 to 12 and in aqueous eluents with up to 50% polar organic solvent.

**TSKgel PW$_{XL}$-CP** columns are designed to facilitate the separation of water soluble cationic polymers by SEC.

**TSKgel SuperMultiporePW** columns are packed with monodisperse polymethacrylate particles, each containing a wide range of pore sizes to cover different molecular weight ranges (see Figure 1). Multi-pore particle technology is the best way to achieve near linear SEC calibration curves.

**TSKgel Alpha and SuperAW** series columns are based on a hydrophilic, highly cross-linked vinyl polymer resin. These materials are mechanically stable and exhibit minimal swelling and shrinkage. They are suitable for both GFC and GPC.

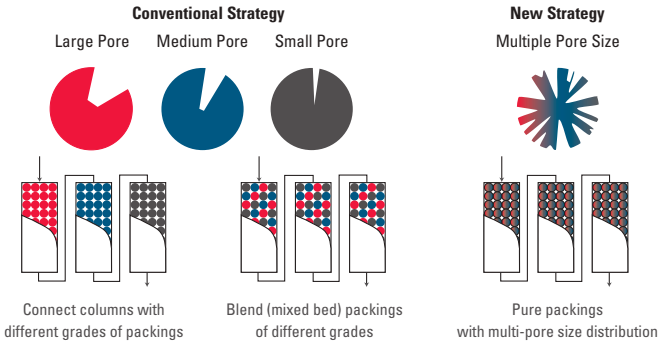



Figure 1. Strategy for wide range separation using SEC

**TSKgel H-type** columns are based on porous, highly cross-linked, spherical polystyrene-divinylbenzene resin. These materials are available in 8 pore sizes and various particle sizes, and are compatible with non-polar organic solvents.

## Reversed Phase Materials

Tosoh Bioscience offers a number of both silica- and polymer-based columns for reversed-phase applications.

### Silica-based Phases

**TSKgel ODS-140HTP** is a 2.3µm polymerically bonded C18 phase for high throughput applications.
**TSKgel ODS-100V** and **ODS-100Z** have a high surface area, with the ODS-100Z phase having higher density bonding. TSKgel ODS-100V is stable in 100% aqueous eluents.
**TSKgel Super Series** (2.3µm particle size) comprises Super-ODS, Super-Octyl and Super-Phenyl phases, which are polymerically bonded and exhaustively endcapped.
**TSKgel Protein C4-300** is a 300Å pore size phase, designed for optimal recovery and resolution of proteins such as recombinant proteins, antibody fragments or PEGylated proteins.

### Polymer-based Phases

The polymer-based phases are chemically stable from pH 2 – 12. TSKgel Octadecyl-4PW is suitable for the analysis of peptides and small proteins, whereas TSKgel Octadecyl-2PW is used for small pharmaceutical compounds at basic pH. TSKgel Phenyl-5PW has a high loading capacity and is ideal for the separation of high molecular weight proteins. Non-porous Octadecyl-NPR produces extremely fast kinetics and quantitative recovery of proteins.

## Hydrophobic Interaction (HIC) Phases

TSKgel® Phenyl-5PW and Ether-5PW are based on the porous G5000PW resin. The Ether-5PW phase is less hydrophobic than Phenyl-5PW and therefore less retentive. TSKgel Butyl-NPR is based on 2.5µm non-porous particles of the same chemical composition as G5000PW. It is more hydrophobic than Phenyl-5PW and also benefits from excellent mass recovery and is the preferred choice for process monitoring and quality control.

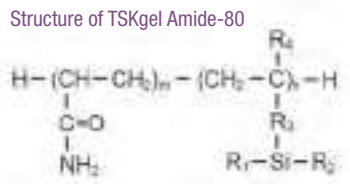
## Hydrophilic Interaction (HILIC) Phases

Tosoh Bioscience manufactures two phases for HILIC.

**TSKgel® Amide-80** consists of spherical silica particles (3 or 5µm) that are covalently bonded with non-ionic carbamoyl groups. Target applications include hydrophilic biomolecules, including saccharides, glycans, oligosaccharides, peptides, nucleic acids and small molecules for drug discovery.

**TSKgel NH2-100** (3µm) is an amino bonded phase with high ligand density and large surface area. It shows good retention for very polar compounds, including carbohydrates, peptides, vitamins and polar drugs.

Structure of TSKgel Amide-80



## Ion-Exchange Phases

Tosoh Bioscience offers a range of silica and methacrylate based high efficiency columns for analysis and isolation of biomolecules by anion and cation exchange chromatography. The BioAssist materials were created to provide higher capacity for larger proteins and have biocompatible PEEK hardware.

**Silica-based (SW)** materials have a smaller pore size (125 or 250Å) compared to the polymer-based phases, and are most suitable for analysing smaller molecular weight samples such as nucleotides, pharmaceuticals, catecholamines and small peptides.

**Polymer-based (PW)** phases are based on porous methacrylate resin (10µm, 1000Å) derived from G5000PW, derivatised with DEAE, SP or CM functionalities.

**TSKgel STAT** columns are based on monodisperse, non-porous polymethacrylate resin particles, the surface of which consists of an open access network of multi-layered anion- or cation-exchange groups. The innovative bonding chemistry, combined with a relatively large particle size, results in a high loading capacity and a low operating pressure, compared with traditional non-porous resins.

Four phases are available: TSKgel Q-STAT and DNA-STAT (triethylamino bonding), CM-STAT (carboxymethyl bonding) and SP-STAT (sulphopropyl bonding).

## Affinity Chromatography Phases

TSKgel affinity columns are based on G5000PW (5PW) porous resin and offer a high level of specificity and selectivity. Process scale media, based on TOYOPEARL HW-65 resin made by the same polymer chemistry, enable easy scale-up. Three affinity phases are available – Boronate-5PW, Chelate-5PW and Tresyl-5PW.

## Bulk Resins

Tosoh Bioscience offers a comprehensive range of media for all common modes of liquid chromatography.

**TOYOPEARL** resins are hydrophilic, macroporous methacrylate-based media for large-scale medium pressure liquid chromatographic applications. They exhibit high mechanical and chemical stability and are stable from pH 2 – 12 for normal operating conditions and pH 1 – 13 for cleaning conditions. The resins are available in average particle sizes of 35, 65, 75 and 100µm for ion-exchange, SEC, HIC and affinity applications.

**TOYOPEARL AF Protein A-650F** is an affinity resin designed for efficient and robust large-scale purification of monoclonal antibodies.

**TSKgel PW** resins are larger particle size versions (20 and 30µm) of the methacrylate packing materials used for ion-exchange and hydrophobic interaction chromatography columns.

## ToyoScreen® Process Development Columns

ToyoScreen columns are prepacked 1ml and 5ml cartridge design columns for short scouting runs and small scale purification. They are a convenient means of performing early resin screening for both target retention and recovery. They can be connected easily to AKTA®, FPLC and HPLC systems. All ToyoScreen columns are packed with TOYOPEARL materials which are also readily available as bulk polymeric media for scale-up and production.

# ULTRASPHERE®

- Acquired by Hichrom from Beckman Coulter
- High reproducibility
- Optimal surface coverage for long column lifetime
- Narrow particle size distribution for high efficiency and improved resolution
- Widely referenced silica

Hichrom acquired the Ultrasphere® HPLC column range from Beckman Coulter, and manufacture the complete range of Ultrasphere columns and media to the same exacting manufacturing protocols and to identical specifications previously used by Beckman Coulter. Part numbers also remain unaffected by this acquisition.

## Ultrasphere Phases

| Ultrasphere Phase | Particle Size (µm) | Pore Size (Å) | Endcapped |
|---|---|---|---|
| ODS (C18) | 3, 5 | 80 | Yes |
| Octyl (C8) | 3, 5 | 80 | Yes |
| Cyano (CN) | 3, 5 | 80 | No |
| Silica (Si) | 3, 5 | 80 | n/a |
| Ion Pair (IP) | 5 | 80 | Yes |

Ultrasphere columns are available in five phases with a 5µm particle size – ODS (C18), Octyl (C8), Cyano (CN), Ion Pair (IP) and unbonded silica (Si). Additionally, ODS, Octyl, Cyano and unbonded silica chemistries are available with a 3µm particle size.

Ultrasphere columns remain widely referenced within both industry and academia and are recognised to provide excellent chromatographic performance.

Columns are available in a wide range of dimensions, including microbore (2.0mm i.d.), analytical (4.6mm i.d.) and semi-preparative (10mm i.d.) options. Please contact Hichrom to request availability of any column dimension not listed.

## Ultrasphere-XL High Speed Cartridge Columns

In this cartridge design, the guard connects directly to the cartridge column with an integral holder. This integral design (see Figure 1) minimises the dead volume between guard and cartridge column, resulting in high efficiency columns. Please note that the cartridge and guard hardware detailed in Figure 1 are not compatible with the previous hardware design.

Figure 1. Ultrasphere-XL cartridge hardware

## Ordering Information – Ultrasphere®

### 5µm Analytical Columns

| Catalogue No. | Description |
|---|---|
| 235329 | Ultrasphere 5µm ODS column, 250 x 4.6mm |
| 235330 | Ultrasphere 5µm ODS column, 150 x 4.6mm |
| 243533 | Ultrasphere 5µm ODS pre-column, 45 x 4.6mm |
| 235332 | Ultrasphere 5µm Octyl column, 250 x 4.6mm |
| 235333 | Ultrasphere 5µm Octyl column, 150 x 4.6mm |
| 243532 | Ultrasphere 5µm Octyl pre-column, 45 x 4.6mm |
| 244071 | Ultrasphere 5µm Cyano column, 250 x 4.6mm |
| 244070 | Ultrasphere 5µm Cyano column, 150 x 4.6mm |
| 244072 | Ultrasphere 5µm Cyano pre-column, 45 x 4.6mm |
| 235341 | Ultrasphere 5µm Si column, 250 x 4.6mm |
| 235342 | Ultrasphere 5µm Si column, 150 x 4.6mm |
| 244011 | Ultrasphere 5µm Si pre-column, 45 x 4.6mm |
| 235335 | Ultrasphere 5µm IP column, 250 x 4.6mm |
| 235334 | Ultrasphere 5µm IP column, 150 x 4.6mm |
| 243534 | Ultrasphere 5µm IP pre-column, 45 x 4.6mm |
| 240002 | Ultrasphere 5µm ODS-DABS column, 250 x 4.6mm |

### 5µm Micro Columns

| Catalogue No. | Description |
|---|---|
| 244434 | Ultrasphere 5µm ODS column, 250 x 2.0mm |
| 237390 | Ultrasphere 5µm ODS column, 150 x 2.0mm |
| 237396 | Ultrasphere 5µm Octyl column, 250 x 2.0mm |
| 237395 | Ultrasphere 5µm Octyl column, 150 x 2.0mm |
| 237394 | Ultrasphere 5µm Cyano column, 250 x 2.0mm |
| 237393 | Ultrasphere 5µm Cyano column, 150 x 2.0mm |
| 237392 | Ultrasphere 5µm Si column, 250 x 2.0mm |
| 237391 | Ultrasphere 5µm Si column, 150 x 2.0mm |

### 5µm Semi-Prep Columns

| Catalogue No. | Description |
|---|---|
| 235328 | Ultrasphere 5µm ODS column, 250 x 10mm |
| 244046 | Ultrasphere 5µm ODS column, 150 x 10mm |
| 235331 | Ultrasphere 5µm Octyl column, 250 x 10mm |
| 244048 | Ultrasphere 5µm Octyl column, 150 x 10mm |
| 244073 | Ultrasphere 5µm Cyano column, 250 x 10mm |
| 235340 | Ultrasphere 5µm Si column, 250 x 10mm |

### 3µm Analytical Columns

| Catalogue No. | Description |
|---|---|
| 244254 | Ultrasphere 3µm ODS High Speed Column, 75 x 4.6mm |
| 244228 | Ultrasphere 3µm Octyl High Speed Column, 75 x 4.6mm |
| 237573 | Ultrasphere 3µm Cyano High Speed Column, 75 x 4.6mm |
| 237574 | Ultrasphere 3µm Si High Speed Column, 75 x 4.6mm |

### 3µm XL Cartridge Columns

| Catalogue No. | Description |
|---|---|
| 238370 | Ultrasphere-XL 3µm ODS Starter Set; contains<br>1 x Cartridge Column (70 x 4.6mm), 2 x guard cartridges, 1 x holder |
| 237500 | Ultrasphere-XL 3µm ODS Cartridge Column, 70 x 4.6mm |
| 237520 | Ultrasphere-XL 3µm ODS Guard Cartridge (5mm length, 2/pk) |
| 238371 | Ultrasphere-XL 3µm Octyl Starter Set; contains<br>1 x Cartridge Column (70 x 4.6mm), 2 x guard cartridges, 1 x holder |
| 237501 | Ultrasphere-XL 3µm Octyl Cartridge Column, 70 x 4.6mm |
| 237521 | Ultrasphere-XL 3µm Octyl Guard Cartridge (5mm length, 2/pk) |
| 237060 | Ultrasphere-XL Column Holder Assembly |

### Accessories

| Catalogue No. | Description |
|---|---|
| 250001 | Column – Pre-column Connector Kit (contains pre-cut 1/16″ SS tubing and 2 PEEK fingertight fittings) |
| 250002 | PEEK Fingertight Fitting (10/pk) – suitable for connection of all Ultrasphere columns and pre-columns to all 1/16″ tubing types, slip free to >6000psi |

# ULTRON CHIRAL COLUMNS

The Ultron ES series of chiral columns is manufactured by Shinwa Chemical Industries Ltd., Japan. Columns packed with protein immobilised silica (Ultron ES-OVM and Ultron ES-Pepsin) and chemically bonded cyclodextrin (Ultron ES-CD and ES-PhCD) are available.

## Ultron Phases

| Ultron Phase | Description | Particle Size (µm) | Pore Size (Å) | pH Range |
|---|---|---|---|---|
| ES-OVM | Ovomucoid protein | 5, 10 | 120 | 3 - 7.5 |
| ES-Pepsin | Pepsin | 5 | 120 | 3 - 6 |
| ES-CD | β-Cyclodextrin | 5 | 120 | 3 - 7.5 |
| ES-PhCD | Phenylcarbamated β-Cyclodextrin | 5 | 120 | 3 - 7.5 |

## Ultron ES-OVM and ES-OVM-C

- USP L57 designation
- Wide range of chiral recognition
- Reversed-phase eluents
- No derivatization required

Ultron ES-OVM is a chiral separation column immobilised with ovomucoid protein. Applications include pharmaceutical compounds, pesticides and organic compounds. Figure 1 shows the separation of the widely prescribed drug clopidogrel bisulphate and related substances according to the USP method, which specifies an L57 column. Ultron ES-OVM-C (5µm, 150 x 4.6mm) is a newer version of the Ultron ES-OVM column, specifically tested for clopidogrel bisulphate under the recommended USP conditions.

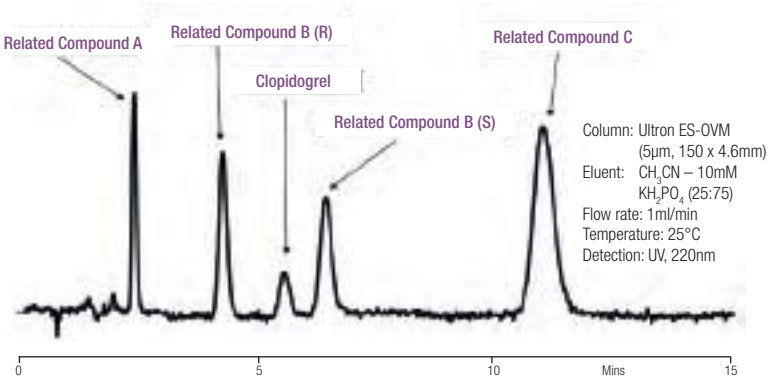



Figure 1. USP assay for clopidogrel bisulphate and related substances

## Ultron ES-Pepsin

- Reversed-phase eluents
- No derivatization required

Ultron ES-Pepsin is a chiral separation column immobilised with pepsin. It is particularly effective for the enantiomer separation of basic compounds and is complementary to the more universally applicable Ultron ES-OVM.

## Ultron ES-CD and ES-PhCD

- USP L45 designation
- Reversed-phase and normal-phase
- Excellent stability and durability

Ultron ES-CD and ES-PhCD are chiral separation columns chemically bonded with β-cyclodextrin and phenylcarbamated β-cyclodextrin respectively. They are particularly effective for the enantiomeric separation of hydrophobic cyclic compounds.

## Ordering Information

| Description | Dimensions (mm) | Particle Size (µm) | ES-OVM[3] | ES-Pepsin | ES-CD | ES-PhCD |
|---|---|---|---|---|---|---|
| Microbore | 150 x 2.0 | 5 | ESOVM-1502 | - | ESCD-1502 | ESPCD-1502 |
| Analytical | 150 x 4.6 | 5 | ESOVM-1546 | ESPEP-1546 | - | - |
| Analytical | 150 x 6.0 | 5 | ESOVM-1506 | - | ESCD-1506 | ESPCD-1506 |
| Guard column | 10 x 4.0 | 5 | ESOVM-GA | ESPEP-GA | ESCD-GA | ESPCD-GA |
| Microbore guard cartridge (2/pk) | 5 x 2.0 | 5 | ESOVM-GD52[1] | - | ESCD-GD52[1] | ESPCD-GD52[1] |
| Analytical guard cartridge (2/pk) | 10 x 4.6 | 5 | ESOVM-GD1046[2] | - | ESCD-GD1046[2] | ESPCD-GD1046[2] |
| Analytical | 250 x 4.6 | 10 | ESOVM-10-2546 | - | - | - |
| Preparative | 250 x 20.0 | 10 | ESOVM-10-2520 | - | - | - |
| Preparative guard | 15 x 8.0 | 10 | ESOVM-10-GP | - | - | - |

[1] Use with holder ULT-H52 (includes adaptor)
[2] Use with holder ULT-H1046 (includes adaptor)
[3] Ultron ES-OVM-C available as 150 x 4.6mm column. Please specify part number ESOVM-C-1546 when ordering

# WAKOPAK® FLUOFIX®



- Unique selectivity
- High recognition capability of halogenated compounds
- Separation of geometric and positional isomers
- High durability

Fluofix® is a fluorinated silica based material for selective reversed-phase HPLC. Initially manufactured by Neos, it is now manufactured by Wako Chemicals, Japan. The newer Wakopak Fluofix-II 120E is now the recommended Fluofix phase for method development.

| Wakopak Phase | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Endcapped | Application |
|---|---|---|---|---|---|
| Fluofix-II 120E | 5 | 120 | 300 | Yes | General analyses |
| Fluofix 120E | 5 | 120 | 300 | Yes | General analyses |
| Fluofix 120N | 5 | 120 | 300 | No | Acidic compounds |

Wakopak Fluofix phases are bonded with branched chain perfluorohexyl groups on 120Å pore size silica (see Figure 1). Both endcapped and non-endcapped versions are available. They exhibit increased retention and selectivity of compounds containing fluorine and chlorine substituents when compared to C18 phases. This is due to the more polar nature of the carbon-fluorine bond in Fluofix compared to the carbon-hydrogen bond of C18 phases.

Wakopak Fluofix-II 120E has improved endcapping efficiency, in order to minimise non-specific adsorption compared with Fluofix 120E. The retention capacity of Fluofix-II 120E is comparable to that of a C4 bonded phase.

Figure 2 shows a comparison of the separation of fluorinated positional isomers using Wakopak Fluofix-II 120E, Wakopak Fluofix 120E and ODS (C18) columns. The high shape selectivity observed with Fluofix-II 120E and Fluofix 120E for halogenated isomers, is due to the extra rigidity imposed by the perfluorinated bonded phase.

Wakopak Fluofix phases are also highly selective for the analysis of non-halogenated polar compounds containing hydroxyl, carboxyl, nitro and other polar groups. This is most apparent when the functional groups are located on an aromatic or other rigid ring system.

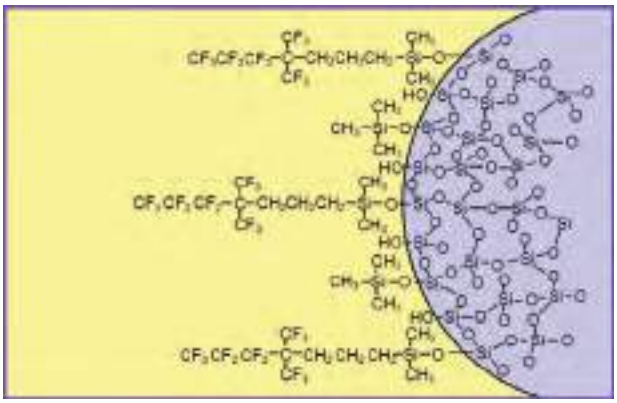


Figure 1. Schematic surface model of Fluofix

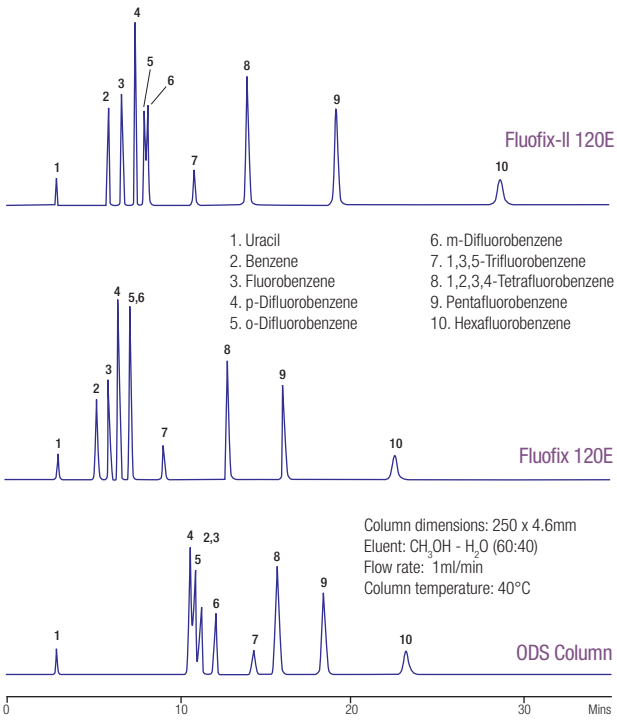



Figure 2. Fluorinated positional isomers*

*The comparative separations presented here may not be representative for all applications.

## Ordering Information

| Wakopak Phase | Column Dimensions[1,2] (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 50 x 2.0 | 150 x 2.0 | 250 x 2.0 | 30 x 4.6 | 50 x 4.6 | 150 x 4.6 | 250 x 4.6 |
| Fluofix-II 120E | 233-63393 | 236-63403 | 233-63413 | 237-63433 | 230-63423 | 239-63373 | 236-63383 |
| Fluofix 120E | 236-61943 | 233-61953 | - | 237-61973 | 234-61983 | 231-61993 | 238-62003 |
| Fluofix 120N | 230-61843 | 237-61853 | - | 231-61873 | 238-61883 | 235-61893 | 238-61903 |

[1] 10mm i.d. columns also available

[2] Part numbers listed correspond to columns with Waters type endfittings. Columns with DuPont type endfittings also available – please enquire

Wakopak Wakosil columns also available – please enquire for details.

# WATERS

## Waters Spherisorb®

- Spherical porous silica
- 3, 5 and 10µm particle sizes
- Widely referenced HPLC columns
- Hichrom high efficiency

Spherisorb® is a classical 80Å pore size silica manufactured by the Waters Corporation. Waters Spherisorb HPLC columns are widely referenced in the scientific literature.

### Spherisorb Phases

| Spherisorb Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Silica | - | - | 3, 5, 10 | 80 | 220 | - |
| C1 | Methyl | No | 3, 5 | 80 | 220 | 2.2 |
| C6 | Hexyl | Yes | 3, 5 | 80 | 220 | 4.7 |
| C8 | Octyl | Yes | 3, 5, 10 | 80 | 220 | 5.8 |
| ODS1 | Octadecyl | No | 3, 5, 10 | 80 | 220 | 6.2 |
| ODS2 | Octadecyl | Yes | 3, 5, 10 | 80 | 220 | 11.5 |
| ODSB | Octadecyl | Yes | 5 | 80 | 220 | 11.5 |
| Phenyl | Phenyl | No | 3, 5 | 80 | 220 | 2.5 |
| NH2 | Amino | No | 3, 5, 10 | 80 | 220 | 1.9 |
| CN | Cyano | No | 3, 5 | 80 | 220 | 3.1 |
| SAX | Tetramethyl Ammonium | No | 5 | 80 | 220 | 4 |
| SCX | Sulphonic Acid | No | 5,10 | 80 | 220 | 4 |

### Ordering Information – Waters Spherisorb - Hichrom Manufactured Columns

#### Microbore (1.0mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm i.d. Waters Spherisorb columns

#### Microbore (2.1mm i.d.) Columns

| Spherisorb Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| **3µm** | | | | | |
| Silica | S3W-50AM | S3W-100AM | S3W-150AM | S3W-250AM | S3W-10CM5 |
| C1 | S3C1-50AM | S3C1-100AM | S3C1-150AM | S3C1-250AM | S3C1-10CM5 |
| C6 | S3C6-50AM | S3C6-100AM | S3C6-150AM | S3C6-250AM | S3C6-10CM5 |
| C8 | S3C8-50AM | S3C8-100AM | S3C8-150AM | S3C8-250AM | S3C8-10CM5 |
| ODS1 | S3ODS1-50AM | S3ODS1-100AM | S3ODS1-150AM | S3ODS1-250AM | S3ODS1-10CM5 |
| ODS2 | S3ODS2-50AM | S3ODS2-100AM | S3ODS2-150AM | S3ODS2-250AM | S3ODS2-10CM5 |
| Phenyl | S3P-50AM | S3P-100AM | S3P-150AM | S3P-250AM | S3P-10CM5 |
| NH2 | S3NH-50AM | S3NH-100AM | S3NH-150AM | S3NH-250AM | S3NH-10CM5 |
| CN | S3CN-50AM | S3CN-100AM | S3CN-150AM | S3CN-250AM | S3CN-10CM5 |
| **5µm** | | | | | |
| Silica | S5W-50AM | S5W-100AM | S5W-150AM | S5W-250AM | S5W-10CM5 |
| C1 | S5C1-50AM | S5C1-100AM | S5C1-150AM | S5C1-250AM | S5C1-10CM5 |
| C6 | S5C6-50AM | S5C6-100AM | S5C6-150AM | S5C6-250AM | S5C6-10CM5 |
| C8 | S5C8-50AM | S5C8-100AM | S5C8-150AM | S5C8-250AM | S5C8-10CM5 |
| ODS1 | S5ODS1-50AM | S5ODS1-100AM | S5ODS1-150AM | S5ODS1-250AM | S5ODS1-10CM5 |
| ODS2 | S5ODS2-50AM | S5ODS2-100AM | S5ODS2-150AM | S5ODS2-250AM | S5ODS2-10CM5 |
| Phenyl | S5P-50AM | S5P-100AM | S5P-150AM | S5P-250AM | S5P-10CM5 |
| NH2 | S5NH-50AM | S5NH-100AM | S5NH-150AM | S5NH-250AM | S5NH-10CM5 |
| CN | S5CN-50AM | S5CN-100AM | S5CN-150AM | S5CN-250AM | S5CN-10CM5 |
| ODSB | S5ODSB-50AM | S5ODSB-100AM | S5ODSB-150AM | S5ODSB-250AM | S5ODSB-10CM5 |
| SAX | S5SAX-50AM | S5SAX-100AM | S5SAX-150AM | S5SAX-250AM | S5SAX-10CM5 |
| SCX | S5SCX-50AM | S5SCX-100AM | S5SCX-150AM | S5SCX-250AM | S5SCX-10CM5 |

[1] Other column dimensions available – please enquire [2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] 5/pk – Starter kits also available – see p. 21

## Ordering Information – Waters Spherisorb® - Hichrom Manufactured Columns (continued)

### Medium Bore (3.2mm i.d.) Columns

| Spherisorb Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| **3µm** | | | | | |
| Silica | S3W-50AS | S3W-100AS | S3W-150AS | S3W-250AS | S3W-10C5 |
| C1 | S3C1-50AS | S3C1-100AS | S3C1-150AS | S3C1-250AS | S3C1-10C5 |
| C6 | S3C6-50AS | S3C6-100AS | S3C6-150AS | S3C6-250AS | S3C6-10C5 |
| C8 | S3C8-50AS | S3C8-100AS | S3C8-150AS | S3C8-250AS | S3C8-10C5 |
| ODS1 | S3ODS1-50AS | S3ODS1-100AS | S3ODS1-150AS | S3ODS1-250AS | S3ODS1-10C5 |
| ODS2 | S3ODS2-50AS | S3ODS2-100AS | S3ODS2-150AS | S3ODS2-250AS | S3ODS2-10C5 |
| Phenyl | S3P-50AS | S3P-100AS | S3P-150AS | S3P-250AS | S3P-10C5 |
| NH2 | S3NH-50AS | S3NH-100AS | S3NH-150AS | S3NH-250AS | S3NH-10C5 |
| CN | S3CN-50AS | S3CN-100AS | S3CN-150AS | S3CN-250AS | S3CN-10C5 |
| **5µm** | | | | | |
| Silica | S5W-50AS | S5W-100AS | S5W-150AS | S5W-250AS | S5W-10C5 |
| C1 | S5C1-50AS | S5C1-100AS | S5C1-150AS | S5C1-250AS | S5C1-10C5 |
| C6 | S5C6-50AS | S5C6-100AS | S5C6-150AS | S5C6-250AS | S5C6-10C5 |
| C8 | S5C8-50AS | S5C8-100AS | S5C8-150AS | S5C8-250AS | S5C8-10C5 |
| ODS1 | S5ODS1-50AS | S5ODS1-100AS | S5ODS1-150AS | S5ODS1-250AS | S5ODS1-10C5 |
| ODS2 | S5ODS2-50AS | S5ODS2-100AS | S5ODS2-150AS | S5ODS2-250AS | S5ODS2-10C5 |
| Phenyl | S5P-50AS | S5P-100AS | S5P-150AS | S5P-250AS | S5P-10C5 |
| NH2 | S5NH-50AS | S5NH-100AS | S5NH-150AS | S5NH-250AS | S5NH-10C5 |
| CN | S5CN-50AS | S5CN-100AS | S5CN-150AS | S5CN-250AS | S5CN-10C5 |
| ODSB | S5ODSB-50AS | S5ODSB-100AS | S5ODSB-150AS | S5ODSB-250AS | S5ODSB-10C5 |
| SAX | S5SAX-50AS | S5SAX-100AS | S5SAX-150AS | S5SAX-250AS | S5SAX-10C5 |
| SCX | S5SCX-50AS | S5SCX-100AS | S5SCX-150AS | S5SCX-250AS | S5SCX-10C5 |

### Analytical (4.6mm i.d.) Columns (please enquire for 4.0mm i.d. columns)

| Spherisorb Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| **3µm** | | | | | |
| Silica | S3W-50A | S3W-100A | S3W-150A | S3W-250A | S3W-10C5 |
| C1 | S3C1-50A | S3C1-100A | S3C1-150A | S3C1-250A | S3C1-10C5 |
| C6 | S3C6-50A | S3C6-100A | S3C6-150A | S3C6-250A | S3C6-10C5 |
| C8 | S3C8-50A | S3C8-100A | S3C8-150A | S3C8-250A | S3C8-10C5 |
| ODS1 | S3ODS1-50A | S3ODS1-100A | S3ODS1-150A | S3ODS1-250A | S3ODS1-10C5 |
| ODS2 | S3ODS2-50A | S3ODS2-100A | S3ODS2-150A | S3ODS2-250A | S3ODS2-10C5 |
| Phenyl | S3P-50A | S3P-100A | S3P-150A | S3P-250A | S3P-10C5 |
| NH2 | S3NH-50A | S3NH-100A | S3NH-150A | S3NH-250A | S3NH-10C5 |
| CN | S3CN-50A | S3CN-100A | S3CN-150A | S3CN-250A | S3CN-10C5 |
| **5µm** | | | | | |
| Silica | S5W-50A | S5W-100A | S5W-150A | S5W-250A | S5W-10C5 |
| C1 | S5C1-50A | S5C1-100A | S5C1-150A | S5C1-250A | S5C1-10C5 |
| C6 | S5C6-50A | S5C6-100A | S5C6-150A | S5C6-250A | S5C6-10C5 |
| C8 | S5C8-50A | S5C8-100A | S5C8-150A | S5C8-250A | S5C8-10C5 |
| ODS1 | S5ODS1-50A | S5ODS1-100A | S5ODS1-150A | S5ODS1-250A | S5ODS1-10C5 |
| ODS2 | S5ODS2-50A | S5ODS2-100A | S5ODS2-150A | S5ODS2-250A | S5ODS2-10C5 |
| Phenyl | S5P-50A | S5P-100A | S5P-150A | S5P-250A | S5P-10C5 |
| NH2 | S5NH-50A | S5NH-100A | S5NH-150A | S5NH-250A | S5NH-10C5 |
| CN | S5CN-50A | S5CN-100A | S5CN-150A | S5CN-250A | S5CN-10C5 |
| ODSB | S5ODSB-50A | S5ODSB-100A | S5ODSB-150A | S5ODSB-250A | S5ODSB-10C5 |
| SAX | S5SAX-50A | S5SAX-100A | S5SAX-150A | S5SAX-250A | S5SAX-10C5 |
| SCX | S5SCX-50A | S5SCX-100A | S5SCX-150A | S5SCX-250A | S5SCX-10C5 |
| **10µm** | | | | | |
| Silica | S10W-50A | S10W-100A | S10W-150A | S10W-250A | S10W-10C5 |
| C8 | S10C8-50A | S10C8-100A | S10C8-150A | S10C8-250A | S10C8-10C5 |
| ODS1 | S10ODS1-50A | S10ODS1-100A | S10ODS1-150A | S10ODS1-250A | S10ODS1-10C5 |
| ODS2 | S10ODS2-50A | S10ODS2-100A | S10ODS2-150A | S10ODS2-250A | S10ODS2-10C5 |
| NH2 | S10NH-50A | S10NH-100A | S10NH-150A | S10NH-250A | S10NH-10C5 |
| SCX | S10SCX-50A | S10SCX-100A | S10SCX-150A | S10SCX-250A | S10SCX-10C5 |

[1] Other column dimensions available – please enquire [2] Use with free-standing holder HI-161 and column coupler HI-081 – see p. 20 [3] 5/pk – Starter kits also available – see p. 21

### Semi-Preparative and Preparative (7.75 – 21.2mm i.d.) Columns

Please contact Hichrom for further details of 7.75 – 21.2mm i.d. Waters Spherisorb columns

**Please contact us for ordering details on all Waters Spherisorb columns not listed**

# ZIRCHROM®

- Porous zirconia based materials
- High pH and temperature stability
- High efficiency
- Fast solvent equilibration
- Long column lifetimes

Zirconia bonded phases, manufactured by ZirChrom Separations, Inc. offer unique selectivity relative to conventional silica based columns, combined with extreme chemical and thermal stability. Unlike polymeric phases, zirconia does not shrink or swell as a function of eluent organic content or ionic strength.

## ZirChrom® Phases

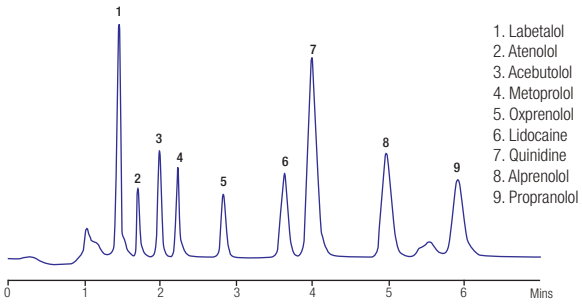
| ZirChrom Phase | Mode | Particle Size[1] (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) | pH Range | Temperature Limit (°C) |
|---|---|---|---|---|---|---|---|
| PBD | RP | 1.9, 3, 5 | 300 | 30 | 2.5 | 1 - 14 | 150 |
| PS | RP | 3, 5 | 300 | 30 | 1.0 | 1 - 13 | 150 |
| EZ | RP | 3, 5 | 300 | 30 | 2.7 | 1 - 10 | 50 |
| MS | RP | 1.9, 3, 5 | 300 | 30 | 3.1 | 1 - 10 | 50 |
| CARB | RP | 1.9, 3, 5 | 300 | 30 | - | 1 - 14 | 150 |
| DiamondBond-C18 | RP | 3, 5 | 300 | 30 | 3.5 | 1 - 14 | 200 |
| PHASE | NP + SEC | 1.9, 3, 5 | 300 | 30 | - | 1 - 14 | 150 |
| SAX | Strong Anion-exchange | 1.9, 3, 5 | 300 | 30 | 1.2 | 1 - 12 | 80 |
| SHAX | Strong Anion-exchange | 3, 5 | 300 | 30 | 0.9 | 1 - 12 | 80 |
| WAX | Weak Anion-exchange | 3, 5 | 300 | 30 | 1.2 | 3 - 9 | 50 |
| WCX | Weak Cation-exchange | 3, 5 | 300 | 30 | - | 1 - 10 | 50 |
| PEZ | Cation-exchange | 3, 5 | 300 | 30 | - | 1 - 10 | 50 |

[1] Additional particle sizes available

## Reversed-Phase Materials

**ZirChrom-PBD** is produced by coating ultra-stable zirconia particles with an extremely thin layer of crosslinked polybutadiene. The chemical selectivity and efficiency of ZirChrom-PBD is similar to that of traditional C8 or C18 silica based columns for non-ionic analytes. However ZirChrom-PBD has greater stability than silica phases and higher efficiencies than polymer phases. Selectivity can be modified by the addition of a strong Lewis base, such as fluoride, phosphate or hydroxide, to the eluent. Figure 1 shows the high pH separation of β-blockers in conjunction with UV detection. ZirChrom-PBD corresponds to the USP L49 designation.

**DiamondBond-C18** uses a proprietary covalent bonding technology to graft C18 groups to a carbon-clad zirconia, giving it outstanding stability for LC-MS applications. DiamondBond-C18 has selectivity that is intermediate between ODS silica and unbonded carbon supports for non-electrolytes.




Column: ZirChrom-PBD (3µm, 150 x 4.6mm)
Eluent: $CH_3CN$ - 20mM potassium phosphate, pH 12.0 (35 : 65)
Flow rate: 1ml/min Temperature: 30°C Detection: UV, 254nm

Figure 1. Separation of ß-blockers on ZirChrom-PBD

**ZirChrom-CARB** is produced by coating zirconia particles with a thin layer of elemental carbon. It is an alternative to porous graphitic carbon (PGC) supports for the separation of geometrical isomers, diastereomers and other closely related molecules. It offers very different selectivity to ODS or polymeric materials, making it an excellent choice for orthogonal screening in drug discovery.

**ZirChrom-EZ** is a highly efficient phase created by first coating zirconia particles with an extremely thin layer of crosslinked polybutadiene and then deactivating the Lewis acid sites by applying a strong metal chelator. This enables Lewis base analytes such as carboxylates, sulphates and phosphates to be analysed throughout the pH range of 1 – 10 using conventional LC-MS buffers such as acetate or formate.

**ZirChrom-MS** is designed specifically for LC-MS, particularly applications involving basic pharmaceuticals. It has the same deactivation chemistry as ZirChrom-EZ but with a covalent attachment for low bleed and is approximately 2.5 times more retentive for simple compounds. The phase exhibits mixed mode retention characteristics – reversed-phase and cation-exchange.

**ZirChrom-PS** consists of zirconia coated with an extremely thin layer of polystyrene. The phase offers an alternative selectivity and less retention than ZirChrom-PBD making it ideal for non-polar analytes, or where highly aqueous eluents are necessary.

## ZirChrom® Ion-Exchange Materials

**ZirChrom®-SAX** is a highly efficient strong anion-exchanger, created by coating zirconia particles with a thin layer of crosslinked polyethyleneimine. It is useful for the separation of inorganic anions, organic anions and biomolecules such as nucleotides, nucleosides, amino acids and peptides.

**ZirChrom-WAX** is a highly efficient weak anion-exchanger, produced by coating zirconia particles with a thin layer of crosslinked polyethyleneimine. In addition to separating inorganic anions, organic ions and biomolecules, it is an extremely stable amino phase for the separation of carbohydrates.

**ZirChrom-SHAX** consists of quaternised polyethyleneimine-coated zirconia and is used for strong hydrophilic anion-exchange. It is more hydrophilic than ZirChrom-SAX, making it useful for the anion-exchange of proteins.

**ZirChrom-WCX** is a phosphate-coated zirconia phase for weak cation-exchange. It is useful for protein chromatography.

**ZirChrom-PEZ** is an EDTPA-coated zirconia phase for the cation-exchange chromatography of proteins and the separation of monoclonal antibodies.

## Sub 2 micron ZirChrom Phases

A selection of ZirChrom phases is now also available with a particle size of 1.9µm (see Table on page 162). Please contact Hichrom for further details.

## ZirChrom-Chiral Columns

Currently, ZirChrom offers 5 different chiral columns in the ZirChrom-Chiral range, in addition to ZirChrom CelluloZe, a cellulose derivative. Please contact Hichrom for ordering information for ZirChrom chiral columns.

| ZirChrom Phase | Chiral Selector | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) |
|---|---|---|---|---|
| Chiral(S)LEU | (S)-3,5-dinitrobenzoylleucine | 3, 5 | 300 | 30 |
| Chiral(R)NESA | (R)-N-[1-(1-naphthyl)ethyl]succinamic acid | 3, 5 | 300 | 30 |
| Chiral(S)NESA | (S)-N-[1-(1-naphthyl)ethyl]succinamic acid | 3, 5 | 300 | 30 |
| Chiral(R)PG | (R)-3,5-dinitrobenzoylphenylglycine | 3, 5 | 300 | 30 |
| Chiral(S)PG | (S)-3,5-dinitrobenzoylphenylglycine | 3, 5 | 300 | 30 |
| CelluloZe | 3,5-dimethylphenylcarbamoyl cellulose | 3, 5 | 300 | 30 |

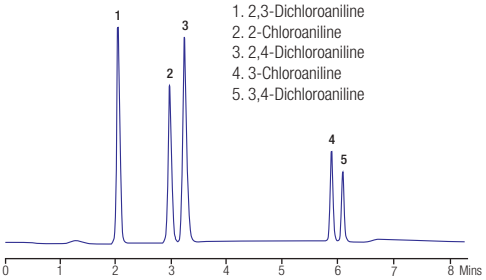
## Sachtopore® Columns

- Highly stable titania based particles
- Reversed-phase and normal-phase
- Ideal for preparative scale-up

### Sachtopore Phases

| | RP | NP |
|---|---|---|
| **Bonded Phase** | Polyethylene coated $TiO_2$ | Unbonded $TiO_2$ |
| **Particle Size (µm)** | 3, 5 | 3, 5 |
| **Pore Size[1] (Å)** | 300 | 300 |
| **pH Range** | 1 - 12 | 1 - 14 |
| **Maximum Temperature** | 100°C | 100°C |

[1] Other pore sizes available




Column: Sachtopore-NP (100Å, 5µm, 150 x 4.6mm)
Eluent: n-Heptane - 2-propanol
Gradient of 99:1 to 19:81 in 4 mins
Flow rate: 1.5ml/min Temperature: 43°C

Figure 2. Separation of chloroaniline isomers on Sachtopore-NP

Sachtopore® phases from ZirChrom Separations are based on porous, spherical titania ($TiO_2$) particles. Sachtopore-NP (normal-phase) is bare titania, whereas Sachtopore-RP (reversed-phase) is polyethylene coated titania. This phase shows similar selectivity to ZirChrom-PBD. Both Sachtopore phases are very chemically and thermally stable and offer unique chromatographic selectivity.

Whereas reversed-phase titania is useful for the separation of basic pharmaceuticals at high pH, Sachtopore-NP can enable the separation of isomeric compounds (see Figure 2).

Sachtopore can also be used in the selective enrichment and purification of phosphorylated peptides from complex mixtures.

All Sachtopore materials are available in microbore, analytical, semi-preparative and preparative formats and a wide range of particle sizes (3, 5, 10, 20, 40 and 80µm) and pore sizes (60, 100, 300, 1000 and 2000Å).

Please contact Hichrom for further information.

## Ordering Information – ZirChrom® and Sachtopore® Phases

| Phase | Column Dimensions[1] (mm) | | | | | | Guard Column | |
|---|---|---|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | For 2.1mm i.d. Columns[2] | For 4.6mm i.d. Columns[3] |
| **3µm** | | | | | | | | |
| PBD | ZR03-0521 | ZR03-1021 | ZR03-1521 | ZR03-0546 | ZR03-1046 | ZR03-1546 | ZR03-G20 | ZR03-G40 |
| PS | ZR09-0521 | ZR09-1021 | ZR09-1521 | ZR09-0546 | ZR09-1046 | ZR09-1546 | ZR09-G20 | ZR09-G40 |
| EZ | EZ01-0521 | EZ01-1021 | EZ01-1521 | EZ01-0546 | EZ01-1046 | EZ01-1546 | EZ01-G20 | EZ01-G40 |
| MS | MS01-0521 | MS01-1021 | MS01-1521 | MS01-0546 | MS01-1046 | MS01-1546 | MS01-G20 | MS01-G40 |
| PHASE | ZR02-0521 | ZR02-1021 | ZR02-1521 | ZR02-0546 | ZR02-1046 | ZR02-1546 | ZR02-G20 | ZR02-G40 |
| SAX | ZR06-0521 | ZR06-1021 | ZR06-1521 | ZR06-0546 | ZR06-1046 | ZR06-1546 | ZR06-G20 | ZR06-G40 |
| SHAX | ZR07-0521 | ZR07-1021 | ZR07-1521 | ZR07-0546 | ZR07-1046 | ZR07-1546 | ZR07-G20 | ZR07-G40 |
| WAX | ZR05-0521 | ZR05-1021 | ZR05-1521 | ZR05-0546 | ZR05-1046 | ZR05-1546 | ZR05-G20 | ZR05-G40 |
| WCX | ZR04-0521 | ZR04-1021 | ZR04-1521 | ZR04-0546 | ZR04-1046 | ZR04-1546 | ZR04-G20 | ZR04-G40 |
| PEZ | ZR08-0521 | ZR08-1021 | ZR08-1521 | ZR08-0546 | ZR08-1046 | ZR08-1546 | ZR08-G20 | ZR08-G40 |
| Sachtopore-RP | TI01-0521 | TI01-1021 | TI01-1521 | TI01-0546 | TI01-1046 | TI01-1546 | TI01-G20 | TI01-G40 |
| Sachtopore-NP | TI02-0521 | TI02-1021 | TI02-1521 | TI02-0546 | TI02-1046 | TI02-1546 | TI02-G20 | TI02-G40 |
| CARB | ZR01-0521 | ZR01-1021 | ZR01-1521 | ZR01-0546 | ZR01-1046 | ZR01-1546 | ZR01-G20 | ZR01-G40 |
| DiamondBond-C18 | DB01-0521 | DB01-1021 | DB01-1521 | DB01-0546 | DB01-1046 | DB01-1546 | DB01-G20 | DB01-G40 |
| **5µm** | | | | | | | | |
| PBD | ZR03-0521-5 | ZR03-1021-5 | ZR03-1521-5 | ZR03-0546-5 | ZR03-1046-5 | ZR03-1546-5 | ZR03-G20 | ZR03-G40 |
| PS | ZR09-0521-5 | ZR09-1021-5 | ZR09-1521-5 | ZR09-0546-5 | ZR09-1046-5 | ZR09-1546-5 | ZR09-G20 | ZR09-G40 |
| EZ | EZ01-0521-5 | EZ01-1021-5 | EZ01-1521-5 | EZ01-0546-5 | EZ01-1046-5 | EZ01-1546-5 | EZ01-G20 | EZ01-G40 |
| MS | MS01-0521-5 | MS01-1021-5 | MS01-1521-5 | MS01-0546-5 | MS01-1046-5 | MS01-1546-5 | MS01-G20 | MS01-G40 |
| PHASE | ZR02-0521-5 | ZR02-1021-5 | ZR02-1521-5 | ZR02-0546-5 | ZR02-1046-5 | ZR02-1546-5 | ZR02-G20 | ZR02-G40 |
| SAX | ZR06-0521-5 | ZR06-1021-5 | ZR06-1521-5 | ZR06-0546-5 | ZR06-1046-5 | ZR06-1546-5 | ZR06-G20 | ZR06-G40 |
| SHAX | ZR07-0521-5 | ZR07-1021-5 | ZR07-1521-5 | ZR07-0546-5 | ZR07-1046-5 | ZR07-1546-5 | ZR07-G20 | ZR07-G40 |
| WAX | ZR05-0521-5 | ZR05-1021-5 | ZR05-1521-5 | ZR05-0546-5 | ZR05-1046-5 | ZR05-1546-5 | ZR05-G20 | ZR05-G40 |
| WCX | ZR04-0521-5 | ZR04-1021-5 | ZR04-1521-5 | ZR04-0546-5 | ZR04-1046-5 | ZR04-1546-5 | ZR04-G20 | ZR04-G40 |
| PEZ | ZR08-0521-5 | ZR08-1021-5 | ZR08-1521-5 | ZR08-0546-5 | ZR08-1046-5 | ZR08-1546-5 | ZR08-G20 | ZR08-G40 |
| Sachtopore-RP | TI01-0521-5 | TI01-1021-5 | TI01-1521-5 | TI01-0546-5 | TI01-1046-5 | TI01-1546-5 | TI01-G20 | TI01-G40 |
| Sachtopore-NP | TI02-0521-5 | TI02-1021-5 | TI02-1521-5 | TI02-0546-5 | TI02-1046-5 | TI02-1546-5 | TI02-G20 | TI02-G40 |
| CARB | ZR01-0521-5 | ZR01-1021-5 | ZR01-1521-5 | ZR01-0546-5 | ZR01-1046-5 | ZR01-1546-5 | ZR01-G20 | ZR01-G40 |
| DiamondBond-C18 | DB01-0521-5 | DB01-1021-5 | DB01-1521-5 | DB01-0546-5 | DB01-1046-5 | DB01-1546-5 | DB01-G20 | DB01-G40 |

[1] Other dimension columns available on request [2] 3/pk – Use with guard holder Z-852-00 [3] 3/pk – Use with guard holder Z-850-00

Reversed-phase and ion-exchange method development kits are also available.
Please contact Hichrom for ordering information for ZirChrom 1.9µm particle size columns and for ZirChrom chiral columns.

## ProTain® In-Line Protein Removal

ProTain® is a system for the effective in-line removal of matrix proteins from samples. It is based on a 3µm polymer-coated zirconia material which acts as a protein sponge, allowing small molecules of interest to pass through the analytical column. ProTain can be used in-line with any type of silica, polymer or zirconia-based analytical column.

### Ordering Information

| Description | Dimensions | |
|---|---|---|
| | 20 x 2.1 | 20 x 4.6 |
| ProTain Media Insert[1] (3/pk) | PT01-0221 | PT01-0246 |
| Holder | Z-852-00-2 | Z-850-00-2 |

[1] Holder required for use – includes capillary tubing, nuts and ferrules

# ZORBAX®



- Spherical porous silica
- Narrow particle and pore size distribution
- Hichrom high efficiency

ZORBAX® spherical particles are manufactured from small uniform colloidal silica microbeads which are agglutinated in a Du Pont patented organic polymerisation process. The organic polymer is removed by a controlled sintering process to yield mechanically stable silica particles. ZORBAX is manufactured by Agilent Technologies Inc. Their original ZORBAX phases (listed below) are based on Type A silica.

### ZORBAX Phases

| ZORBAX Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size (Å) | Surface Area ($m^2/g$) | Carbon Load (%) |
|---|---|---|---|---|---|---|
| Silica | - | - | 5 | 70 | 330 | - |
| TMS | Methyl | Yes | 5 | 70 | 330 | 4 |
| C8 | Octyl | Yes | 5 | 70 | 330 | 12 |
| ODS | Octadecyl | Yes | 5 | 70 | 330 | 20 |
| Phenyl | Phenyl | Yes | 5 | 70 | 330 | 12 |
| NH2 | Amino | Yes | 5 | 70 | 330 | 4 |
| CN | Cyano | Yes | 5 | 70 | 330 | 7 |

## Ordering Information – Hichrom Manufactured Columns

### Microbore (1.0mm i.d.) Columns

Please contact Hichrom for further details of 1.0mm i.d. ZORBAX columns.

### Microbore (2.1mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 2.1mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | |
| Silica | ZSIL-50AM | ZSIL-100AM | ZSIL-150AM | ZSIL-250AM | ZSIL-10CM5 |
| TMS | ZTMS-50AM | ZTMS-100AM | ZTMS-150AM | ZTMS-250AM | ZTMS-10CM5 |
| C8 | ZC8-50AM | ZC8-100AM | ZC8-150AM | ZC8-250AM | ZC8-10CM5 |
| ODS | ZODS-50AM | ZODS-100AM | ZODS-150AM | ZODS-250AM | ZODS-10CM5 |
| Phenyl | ZPH-50AM | ZPH-100AM | ZPH-150AM | ZPH-250AM | ZPH-10CM5 |
| NH2 | ZNH-50AM | ZNH-100AM | ZNH-150AM | ZNH-250AM | ZNH-10CM5 |
| CN | ZCN-50AM | ZCN-100AM | ZCN-150AM | ZCN-250AM | ZCN-10CM5 |

### Medium Bore (3.2mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 3.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 3.2 | 100 x 3.2 | 150 x 3.2 | 250 x 3.2 | |
| Silica | ZSIL-50AS | ZSIL-100AS | ZSIL-150AS | ZSIL-250AS | ZSIL-10C5 |
| TMS | ZTMS-50AS | ZTMS-100AS | ZTMS-150AS | ZTMS-250AS | ZTMS-10C5 |
| C8 | ZC8-50AS | ZC8-100AS | ZC8-150AS | ZC8-250AS | ZC8-10C5 |
| ODS | ZODS-50AS | ZODS-100AS | ZODS-150AS | ZODS-250AS | ZODS-10C5 |
| Phenyl | ZPH-50AS | ZPH-100AS | ZPH-150AS | ZPH-250AS | ZPH-10C5 |
| NH2 | ZNH-50AS | ZNH-100AS | ZNH-150AS | ZNH-250AS | ZNH-10C5 |
| CN | ZCN-50AS | ZCN-100AS | ZCN-150AS | ZCN-250AS | ZCN-10C5 |

[1] Other column dimensions available – please enquire

[2] Use with free-standing holder HI-161 and column coupler HI-081 – see p.20

[3] 5/pk – Starter kits also available – see p.21

### Analytical (4.0mm i.d.) Columns

Please contact Hichrom for further details of 4.0mm i.d. ZORBAX columns.

## ZORBAX® – Hichrom Manufactured Columns (continued)

### Analytical (4.6mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[2,3] (For 4.6mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 4.6 | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | |
| Silica | ZSIL-50A | ZSIL-100A | ZSIL-150A | ZSIL-250A | ZSIL-10C5 |
| TMS | ZTMS-50A | ZTMS-100A | ZTMS-150A | ZTMS-250A | ZTMS-10C5 |
| C8 | ZC8-50A | ZC8-100A | ZC8-150A | ZC8-250A | ZC8-10C5 |
| ODS | ZODS-50A | ZODS-100A | ZODS-150A | ZODS-250A | ZODS-10C5 |
| Phenyl | ZPH-50A | ZPH-100A | ZPH-150A | ZPH-250A | ZPH-10C5 |
| NH2 | ZNH-50A | ZNH-100A | ZNH-150A | ZNH-250A | ZNH-10C5 |
| CN | ZCN-50A | ZCN-100A | ZCN-150A | ZCN-250A | ZCN-10C5 |

### Semi-Preparative (7.75mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 7.75mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 7.75 | 100 x 7.75 | 150 x 7.75 | 250 x 7.75 | |
| Silica | ZSIL-50SP | ZSIL-100SP | ZSIL-150SP | ZSIL-250SP | ZSIL-10CP3 |
| TMS | ZTMS-50SP | ZTMS-100SP | ZTMS-150SP | ZTMS-250SP | ZTMS-10CP3 |
| C8 | ZC8-50SP | ZC8-100SP | ZC8-150SP | ZC8-250SP | ZC8-10CP3 |
| ODS | ZODS-50SP | ZODS-100SP | ZODS-150SP | ZODS-250SP | ZODS-10CP3 |
| Phenyl | ZPH-50SP | ZPH-100SP | ZPH-150SP | ZPH-250SP | ZPH-10CP3 |
| NH2 | ZNH-50SP | ZNH-100SP | ZNH-150SP | ZNH-250SP | ZNH-10CP3 |
| CN | ZCN-50SP | ZCN-100SP | ZCN-150SP | ZCN-250SP | ZCN-10CP3 |

### Semi-Preparative (10.0mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 10.0mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 10.0 | 100 x 10.0 | 150 x 10.0 | 250 x 10.0 | |
| Silica | ZSIL-50SP1 | ZSIL-100SP1 | ZSIL-150SP1 | ZSIL-250SP1 | ZSIL-10CP3 |
| TMS | ZTMS-50SP1 | ZTMS-100SP1 | ZTMS-150SP1 | ZTMS-250SP1 | ZTMS-10CP3 |
| C8 | ZC8-50SP1 | ZC8-100SP1 | ZC8-150SP1 | ZC8-250SP1 | ZC8-10CP3 |
| ODS | ZODS-50SP1 | ZODS-100SP1 | ZODS-150SP1 | ZODS-250SP1 | ZODS-10CP3 |
| Phenyl | ZPH-50SP1 | ZPH-100SP1 | ZPH-150SP1 | ZPH-250SP1 | ZPH-10CP3 |
| NH2 | ZNH-50SP1 | ZNH-100SP1 | ZNH-150SP1 | ZNH-250SP1 | ZNH-10CP3 |
| CN | ZCN-50SP1 | ZCN-100SP1 | ZCN-150SP1 | ZCN-250SP1 | ZCN-10CP3 |

### Preparative (21.2mm i.d.) Columns

| 5µm ZORBAX Phase | Column Dimensions[1] (mm) | | | | Guard Cartridges[4] (For 21.2mm i.d. Columns) |
|---|---|---|---|---|---|
| | 50 x 21.2 | 100 x 21.2 | 150 x 21.2 | 250 x 21.2 | |
| Silica | ZSIL-50P | ZSIL-100P | ZSIL-150P | ZSIL-250P | ZSIL-10CP3 |
| TMS | ZTMS-50P | ZTMS-100P | ZTMS-150P | ZTMS-250P | ZTMS-10CP3 |
| C8 | ZC8-50P | ZC8-100P | ZC8-150P | ZC8-250P | ZC8-10CP3 |
| ODS | ZODS-50P | ZODS-100P | ZODS-150P | ZODS-250P | ZODS-10CP3 |
| Phenyl | ZPH-50P | ZPH-100P | ZPH-150P | ZPH-250P | ZPH-10CP3 |
| NH2 | ZNH-50P | ZNH-100P | ZNH-150P | ZNH-250P | ZNH-10CP3 |
| CN | ZCN-50P | ZCN-100P | ZCN-150P | ZCN-250P | ZCN-10CP3 |

[1] Other column dimensions available – please enquire
[2] Use with free-standing holder HI-161 and column coupler HI-081 – see p.20
[3] 5/pk – Starter kits also available – see p.21
[4] 3/pk – Use with free-standing holder HI-150 and column coupler HI-081

**Please contact us for ordering details on all ZORBAX columns not listed**

# GC COLUMN SELECTION BY USP SPECIFICATIONS



The USP specifications for GC column selection are given below with some matching capillary columns. In some cases, a particular column may fit into more than one category.

| Method | Phase Composition | Recommendations |
|---|---|---|
| G1 | Dimethylpolysiloxane oil | HiCap 1MS, TG-1MS, TR-1MS, DB-1ms, HP-1ms, OPTIMA 1 MS |
| G2 | Dimethylpolysiloxane gum | HiCap 1MS, TG-1MS, TR-1MS, DB-1ms, HP-1ms, OPTIMA 1 MS |
| G3 | 50% Phenyl-50% methylpolysiloxane | HiCap 17MS, TG-17MS, TR-50MS, DB-17ms, OPTIMA 17 MS |
| G5 | 3-Cyanopropylpolysiloxane | TR-FAME, DB-23 |
| G6 | Trifluoropropylmethylpolysiloxane | HiCap 210, TG-200MS, DB-210, OPTIMA 210 |
| G7 | 50% 3-Cyanopropyl-50% phenylmethylsilicone | HiCap 225, TG-225MS, TR-225, DB-225ms, OPTIMA 225 |
| G9 | Methylvinylpolysiloxane | DB-1ms, DB-1ht |
| G14 | Polyethylene glycol (ave. mol. wt. of 950 to 1050) | DB-WAX, DB-WAXetr, HP-INNOWAX |
| G15 | Polyethylene glycol (ave. mol. wt. of 3000 to 3700) | DB-WAX, DB-WAXetr, HP-INNOWAX |
| G16 | Polyethylene glycol (ave. mol. wt. ~15,000) with diepoxide linker | HiCap WAX, HiCap WAX ETR, HiCap WAX2, OPTIMA WAX, TR-Wax, TG-WaxMS, |
| G17 | 75% Phenyl-25% methylpolysiloxane | DB-17ms, HP-50+ |
| G19 | 25% Phenyl-25% cyanopropyl-50% methylsilicone | HiCap 225, TG-225MS, DB-225ms, OPTIMA 225 |
| G20 | Polyethylene glycol (ave. mol. wt. of 380 to 420) | HiCap WAX, HiCap WAX2, HiCap WAX ETR, TR-Wax, TG-WaxMS, DB-WAX |
| G25 | Polyethylene glycol TPA (Carbowax 20M terephthalic acid) | TR-FFAP, DB-FFAP, HP-FFAP, OPTIMA FFAP |
| G27 | 5% Phenyl-95% methylpolysiloxane | HiCap 5MS, TG-5MS, TR-5MS, DB-5ms, HP-5ms, OPTIMA 5 MS |
| G28 | 25% Phenyl-75% methylpolysiloxane | HiCap 25, DB-35, HP-35, OPTIMA 35 MS |
| G32 | 20% Phenylmethyl-80% dimethylpolysiloxane | DB-35ms, HP-35 |
| G35 | Polyethylene glycol and diepoxide esterified with nitroterephthalic acid | HiCap FFAP, DB-FFAP, HP-FFAP, OPTIMA FFAP |
| G36 | 1% Vinyl-5% phenylmethylpolysiloxane | TR-5MS, DB-5ms, HP-5ms, OPTIMA 5 MS |
| G38 | Phase G1 plus a tailing inhibitor | TR-1MS, HP-1ms, DB-1ms, OPTIMA 1 MS |
| G39 | Polyethylene glycol (ave. mol. wt. about 1500) | HiCap WAX, HiCap WAX2, HiCap WAX ETR, DB-Wax, HP-INNOWAX |
| G41 | Phenylmethyldimethylsilicone (10% phenyl-substituted) | HiCap 5MS2, DB-5, DB-5ms, HP-5, HP-5ms |
| G42 | 35% Phenyl-65% dimethylpolysiloxane | HiCap 35, TG-35MS, TR-35MS, DB-35ms, OPTIMA 35 MS |
| G43 | 6% Cyanopropylphenyl-94% dimethylpolysiloxane | HiCap 624, HiCap 1301, TG-624, TG-17MS, TG-1301MS, TR-V1, DB-1301, DB-624, OPTIMA 1301, OPTIMA 624 |
| G45 | Divinylbenzene-ethyleneglycol-dimethacrylate | TG-Bond U |
| G46 | 14% Cyanopropylphenyl-86% methylpolysiloxane | HiCap 1701, TG-1701MS, TR-1701, DB-1701, OPTIMA 1701 |
| G47 | Polyethylene glycol (ave. mol. wt. of about 8000) | DB-Wax, HP-INNOWAX |
| G48 | 90% Biscyanopropyl 10% cyanopropylphenylpolysiloxane | TG-POLAR, HP-88 |
| G49 | Proprietary derivatized phenyl groups on a polysiloxane backbone | OPTIMA δ-3 |

## Packed GC Columns

Hichrom also supply a range of packed GC columns. Please enquire regarding ordering information and availability of packed GC columns.

# HICAP CAPILLARY GC COLUMNS



- Excellent reproducibility
- High separation efficiency
- Low GC-MS bleed
- Wide range of phases

Hichrom's HiCap capillary GC columns are manufactured to high specifications and demonstrate both excellent reproducibility and high separation efficiency. A wide range of different polarity phases are available for both general purpose and application specific analyses. HiCap 1MS, 5MS, 5MS2 and 17MS are high performance, low bleed phases designed specifically for GC-MS analyses. In addition, less column conditioning is required between analyses.

## HiCap Range of Phases

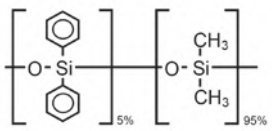
| Phase | Phase Composition | Polarity | Max. Temp. (°C)* | Applications |
|---|---|---|---|---|
| HiCap 1MS | 100% Dimethylpolysiloxane | Low | 325/350 | General purpose, hydrocarbons, PCBs, high volatility solvents, phenols |
| HiCap 1 | 100% Dimethylpolysiloxane | Low | 325/350 | General purpose, hydrocarbons, PCBs, high volatility solvents, phenols |
| HiCap 5MS | 5% Phenyl-95% methylpolysiloxane | Slight | 325/350 | General purpose, phenols, pesticides, halogenated compounds, FAMEs |
| HiCap 5MS2 | 5% Phenyl-95% methylpolysilarylene | Slight | 325/350 | General purpose, phenols, pesticides, halogenated compounds, FAMEs |
| HiCap 5 | 5% Phenyl-95% methylpolysiloxane | Slight | 325/350 | General purpose, phenols, pesticides, halogenated compounds, FAMEs |
| HiCap PE | 5% Phenyl-95% methylpolysiloxane | Slight | 325/350 | Pesticides |
| HiCap 1301 | 6% Cyanopropylphenyl-94% methylpolysiloxane | Medium | 280/300 | Pesticides, PCBs, alcohols, VOCs |
| HiCap 624 | 6% Cyanopropylphenyl-94% methylpolysiloxane | Medium | 260/260 | VOCs, alcohols |
| HiCap 25 | 25% Phenyl-75% methylpolysiloxane | Medium | 280/300 | Pesticides, PCBs, alcohols, VOCs |
| HiCap VOC | 25% Phenyl-75% methylpolysiloxane | Medium | 200/220 | VOCs, organic solvents |
| HiCap SO | | | 260/260 | |
| HiCap 35 | 35% Phenyl-65% methylpolysiloxane | Medium | 280/300 | Pesticides, amines, drugs of abuse, PCBs |
| HiCap 1701 | 14% Cyanopropylphenyl-86% methylpolysiloxane | Medium | 280/300 | Sugars, TMS derivatives, drugs, alcohols, steroids |
| HiCap 17MS | 50% Phenyl-50% methylpolysiloxane | Medium | 320/340 | Drugs, pesticides, steroids |
| HiCap 17 | 50% Phenyl-50% methylpolysiloxane | Medium | 320/340 | Drugs, pesticides, steroids |
| HiCap 210 | 50% Trifluoropropyl-50% methylpolysiloxane | Medium | 240/260 | Organophosphorus pesticides |
| HiCap 225 | 50% Cyanopropylmethyl-50% phenylmethylpolysiloxane | Medium | 220/240 | FAMEs, geometrical isomers, food and flavour components |
| HiCap WAX | Polyethylene glycol | High | 250/260 | Esters, perfumes, flavours, alcohols, aromatic hydrocarbons, FAMEs |
| HiCap WAX2 | Polyethylene glycol | High | 260/260 | Esters, perfumes, flavours, alcohols, aromatic hydrocarbons, FAMEs |
| HiCap WAX ETR | Polyethylene glycol | High | 270/280 | Esters, perfumes, flavours, alcohols, aromatic hydrocarbons, FAMEs |
| HiCap FFAP | Nitroterephthalic acid modified polyethylene glycol | High | 240/250 | Free fatty acids, aldehydes, ketones, alcohols, organic acids |
| HiCap AM | Proprietary | - | 265/300 | Amines |

* Two temperature limits are listed, the lower one for isothermal conditions and the higher one for temperature programmed operations.
For columns with 0.53mm i.d. or with thicker films, temperature limits are generally lower.

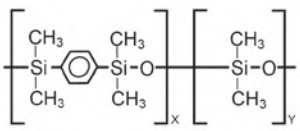
## HiCap 5MS vs HiCap 5MS2

The HiCap 5MS and HiCap 5MS2 phases show virtually identical selectivity. However, HiCap 5MS2 incorporates arylene bonding into the siloxane polymer. This strengthens the polymer backbone, thereby reducing stationary phase degradation and bleed.

Please see page 170 for ordering information.



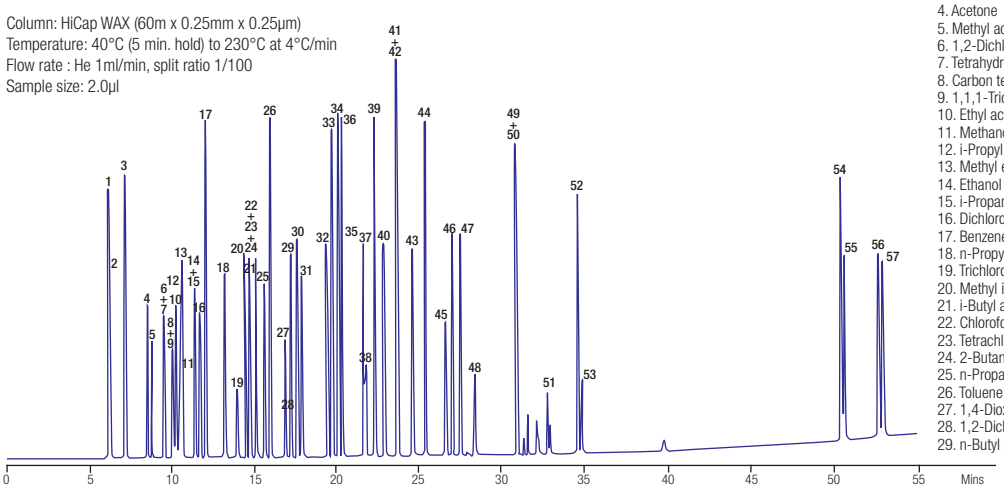

HiCap 5 and HiCap 5MS




HiCap 5MS2

## Application Examples

Column: HiCap WAX (60m x 0.25mm x 0.25µm)
Temperature: 40°C (5 min. hold) to 230°C at 4°C/min
Flow rate : He 1ml/min, split ratio 1/100
Sample size: 2.0µl




1. Hexane
2. Ethyl ether
3. Carbon disulphide
4. Acetone
5. Methyl acetate
6. 1,2-Dichloroethylene
7. Tetrahydrofuran
8. Carbon tetrachloride
9. 1,1,1-Trichloroethane
10. Ethyl acetate
11. Methanol
12. i-Propyl acetate
13. Methyl ethyl ketone
14. Ethanol
15. i-Propanol
16. Dichloromethane
17. Benzene
18. n-Propyl acetate
19. Trichloroethylene
20. Methyl i-butyl ketone
21. i-Butyl acetate
22. Chloroform
23. Tetrachloroethylene
24. 2-Butanol
25. n-Propanol
26. Toluene
27. 1,4-Dioxane
28. 1,2-Dichloroethane
29. n-Butyl acetate
30. Methyl n-butyl ketone
31. i-Butanol
32. i-Amyl acetate
33. Ethylbenzene
34. p-Xylene
35. n-Butanol
36. m-Xylene
37. n-Amyl acetate
38. Methyl cellosolve
39. o-Xylene
40. i-Amyl alcohol
41. Chlorobenzene
42. Ethyl cellosolve
43. n-Amyl alcohol
44. Styrene
45. Cellosolve acetate
46. Cyclohexanone
47. Methylcyclohexanone
48. N,N-Dimethylformamide
49. Cyclohexanol
50. Butyl cellosolve
51. Methylcyclohexanol
52. o-Dichlorobenzene
53. 1,1,2,2-Tetrachloroethane
54. o-Cresol
55. Phenol
56. m-Cresol
57. p-Cresol

Figure 1. Organic solvents

Column: HiCap 35 (30m x 0.25mm x 0.25µm)
Temperature: 60°C to 290°C at 10°C/min (7 min. hold)
Flow rate: He 35cm/sec, split ratio 1/30
Sample size: 2.0µl
Detection: MS

1. α-BHC
2. γ-BHC
3. β-BHC
4. Heptachlor
5. Aldrin
6. p,p'-DDE
7. Dieldrin
8. o,p'-DDD
9. Endrin
10. p,p'-DDD
11. o,p'-DDT
12. p,p'-DDT

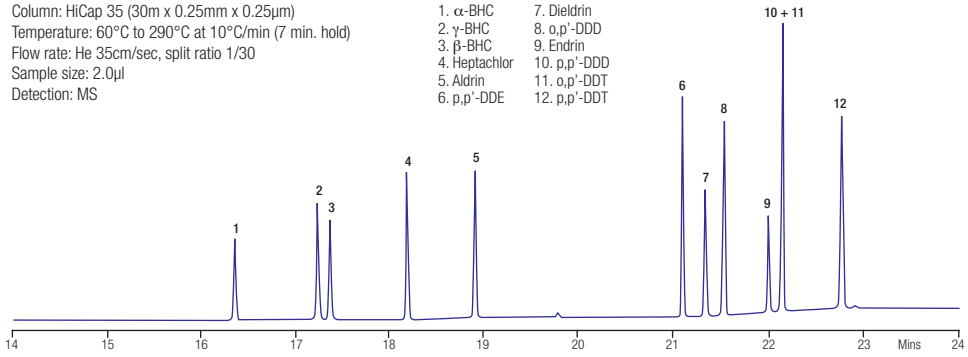



Figure 2. Pesticides

Column: HiCap 225 (30m x 0.25mm x 0.25µm)
Temperature: 40°C (1 min. hold) to 220°C at 10°C/min (30 min. hold)
Injection: split 1:50
Sample size: 0.4µl
Detection: FID

1. Methyl butanoate
2. Methyl hexanoate
3. Methyl octanoate
4. Methyl decanoate
5. Methyl dodecanoate
6. Methyl tetradecanoate
7. Methyl myristoleate
8. Methyl hexadecanoate
9. Methyl palmitelaidate
10. Methyl octadecanoate
11. Methyl oleate
12. Methyl eicosanoate
13. Methyl cis-11-eicosenoate
14. Methyl docosanoate

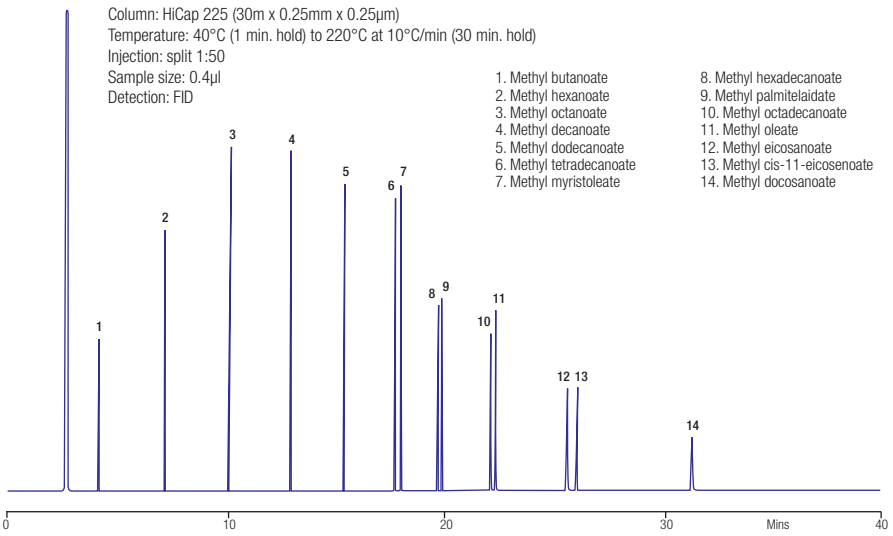



Figure 3. Fatty Acid Methyl Esters (FAMEs)

## Ordering Information for HiCap Capillary GC Columns

The following ordering information relates to a selection of dimensions for some of the more popular phases. Other phases (see page 168) and dimensions are available. Please enquire for details.

| Column Dimensions | | | HiCap 1MS | HiCap 5MS | HiCap 5MS2 | HiCap 17MS |
|---|---|---|---|---|---|---|
| I.D. (mm) | Length (m) | Film (µm) | | | | |
| 0.25 | 15 | 0.25 | HI1MS-0.25-15-0.25 | HI5MS-0.25-15-0.25 | HI5MS2-0.25-15-0.25 | HI17MS-0.25-15-0.25 |
| | 30 | 0.10 | HI1MS-0.25-30-0.10 | HI5MS-0.25-30-0.10 | HI5MS2-0.25-30-0.10 | - |
| | | 0.25 | HI1MS-0.25-30-0.25 | HI5MS-0.25-30-0.25 | HI5MS2-0.25-30-0.25 | HI17MS-0.25-30-0.25 |
| | 60 | 0.25 | HI1MS-0.25-60-0.25 | HI5MS-0.25-60-0.25 | HI5MS2-0.25-60-0.25 | HI17MS-0.25-60-0.25 |
| 0.32 | 30 | 0.25 | HI1MS-0.32-30-0.25 | HI5MS-0.32-30-0.25 | HI5MS2-0.32-30-0.25 | HI17MS-0.32-30-0.25 |
| | 60 | 0.25 | HI1MS-0.32-60-0.25 | HI5MS-0.32-60-0.25 | HI5MS2-0.32-60-0.25 | HI17MS-0.32-60-0.25 |

| Column Dimensions | | | HiCap 1 | HiCap 5 | HiCap 25 | HiCap 35 | HiCap 1701 |
|---|---|---|---|---|---|---|---|
| I.D. (mm) | Length (m) | Film (µm) | | | | | |
| 0.25 | 15 | 0.25 | HI1-0.25-15-0.25 | HI5-0.25-15-0.25 | HI25-0.25-15-0.25 | HI35-0.25-15-0.25 | HI1701-0.25-15-0.25 |
| | | 0.50 | HI1-0.25-15-0.50 | HI5-0.25-15-0.50 | HI25-0.25-15-0.50 | HI35-0.25-15-0.50 | HI1701-0.25-15-0.50 |
| | 30 | 0.10 | HI1-0.25-30-0.10 | HI5-0.25-30-0.10 | - | - | - |
| | | 0.25 | HI1-0.25-30-0.25 | HI5-0.25-30-0.25 | HI25-0.25-30-0.25 | HI35-0.25-30-0.25 | HI1701-0.25-30-0.25 |
| | | 0.50 | HI1-0.25-30-0.50 | HI5-0.25-30-0.50 | HI25-0.25-30-0.50 | HI35-0.25-30-0.50 | HI1701-0.25-30-0.50 |
| | | 1.00 | HI1-0.25-30-1.00 | HI5-0.25-30-1.00 | HI25-0.25-30-1.00 | HI35-0.25-30-1.00 | HI1701-0.25-30-1.00 |
| | 60 | 0.25 | HI1-0.25-60-0.25 | HI5-0.25-60-0.25 | HI25-0.25-60-0.25 | HI35-0.25-60-0.25 | HI1701-0.25-60-0.25 |
| | | 0.50 | HI1-0.25-60-0.50 | HI5-0.25-60-0.50 | HI25-0.25-60-0.50 | HI35-0.25-60-0.50 | HI1701-0.25-60-0.50 |
| | | 1.00 | HI1-0.25-60-1.00 | HI5-0.25-60-1.00 | HI25-0.25-60-1.00 | HI35-0.25-60-1.00 | HI1701-0.25-60-1.00 |
| 0.32 | 15 | 0.25 | HI1-0.32-15-0.25 | HI5-0.32-15-0.25 | HI25-0.32-15-0.25 | HI35-0.32-15-0.25 | HI1701-0.32-15-0.25 |
| | | 0.50 | - | - | HI25-0.32-15-0.50 | HI35-0.32-15-0.50 | HI1701-0.32-15-0.50 |
| | | 1.00 | - | - | HI25-0.32-15-1.00 | HI35-0.32-15-1.00 | HI1701-0.32-15-1.00 |
| | 30 | 0.25 | HI1-0.32-30-0.25 | HI5-0.32-30-0.25 | HI25-0.32-30-0.25 | HI35-0.32-30-0.25 | HI1701-0.32-30-0.25 |
| | | 0.50 | HI1-0.32-30-0.50 | HI5-0.32-30-0.50 | HI25-0.32-30-0.50 | HI35-0.32-30-0.50 | HI1701-0.32-30-0.50 |
| | | 1.00 | HI1-0.32-30-1.00 | HI5-0.32-30-1.00 | HI25-0.32-30-1.00 | HI35-0.32-30-1.00 | HI1701-0.32-30-1.00 |
| | | 5.00 | HI1-0.32-30-5.00 | - | - | - | - |
| | 60 | 0.25 | HI1-0.32-60-0.25 | HI5-0.32-60-0.25 | HI25-0.32-60-0.25 | HI35-0.32-60-0.25 | HI1701-0.32-60-0.25 |
| | | 0.50 | HI1-0.32-60-0.50 | HI5-0.32-60-0.50 | HI25-0.32-60-0.50 | HI35-0.32-60-0.50 | HI1701-0.32-60-0.50 |
| | | 1.00 | HI1-0.32-60-1.00 | - | HI25-0.32-60-1.00 | HI35-0.32-60-1.00 | HI1701-0.32-60-1.00 |
| 0.53 | 15 | 1.00 | HI1-0.53-15-1.00 | HI5-0.53-15-1.00 | HI25-0.53-15-1.00 | HI35-0.53-60-1.00 | HI1701-0.53-60-1.00 |
| | | 2.00 | HI1-0.53-15-2.00 | HI5-0.53-15-2.00 | - | - | - |
| | | 3.00 | HI1-0.53-15-3.00 | HI5-0.53-15-3.00 | - | - | - |
| | 30 | 1.00 | HI1-0.53-30-1.00 | HI5-0.53-30-1.00 | HI25-0.53-30-1.00 | HI35-0.53-30-1.00 | HI1701-0.53-30-1.00 |
| | | 1.50 | HI1-0.53-30-1.50 | HI5-0.53-30.1.50 | - | - | - |
| | | 2.00 | HI1-0.53-30-2.00 | HI5-0.53-30-2.00 | - | - | - |
| | | 3.00 | HI1-0.53-30-3.00 | HI5-0.53-30-3.00 | - | - | - |
| | | 5.00 | HI1-0.53-30-5.00 | HI5-0.53-30-5.00 | - | - | - |
| | 60 | 5.00 | HI1-0.53-60-5.00 | - | - | - | - |

| Column Dimensions | | | HiCap WAX | HiCap WAX2 | HiCap WAX ETR | HiCap FFAP |
|---|---|---|---|---|---|---|
| I.D. (mm) | Length (m) | Film (µm) | | | | |
| 0.25 | 15 | 0.25 | HIWAX-0.25-15-0.25 | - | HIWAXETR-0.25-15-0.25 | HIFFAP-0.25-15-0.25 |
| | 30 | 0.25 | HIWAX-0.25-30-0.25 | HIWAX2-0.25-30-0.25 | HIWAXETR-0.25-30-0.25 | HIFFAP-0.25-30-0.25 |
| | 60 | 0.25 | HIWAX-0.25-60-0.25 | HIWAX2-0.25-60-0.25 | HIWAXETR-0.25-60-0.25 | HIFFAP-0.25-60-0.25 |
| | | 0.50 | HIWAX-0.25-60-0.50 | HIWAX2-0.25-60-0.50 | HIWAXETR-0.25-60-0.50 | HIFFAP-0.25-60-0.50 |
| 0.32 | 30 | 0.25 | HIWAX-0.32-30-0.25 | HIWAX2-0.32-30-0.25 | HIWAXETR-0.32-30-0.25 | HIFFAP-0.32-30-0.25 |
| | 60 | 0.25 | HIWAX-0.32-60-0.25 | HIWAX2-0.32-60-0.25 | HIWAXETR-0.32-60-0.25 | HIFFAP-0.32-60-0.25 |
| 0.53 | 15 | 1.00 | HIWAX-0.53-15-1.00 | HIWAX2-0.53-15-1.00 | HIWAXETR-0.53-15-1.00 | HIFFAP-0.53-15-1.00 |
| | 30 | 1.00 | HIWAX-0.53-30-1.00 | HIWAX2-0.53-30-1.00 | HIWAXETR-0.53-30-1.00 | HIFFAP-0.53-30-1.00 |

| Column Dimensions | | | HiCap 624 | HiCap VOC | HiCap SO |
|---|---|---|---|---|---|
| I.D. (mm) | Length (m) | Film (µm) | | | |
| 0.25 | 30 | 1.40 | HI624-0.25-30-1.40 | - | HISO-0.25-30-1.40 |
| | 60 | 1.00 | - | HIVOC-0.25-60-1.00 | - |
| | | 1.40 | HI624-0.25-60-1.40 | - | HISO-0.25-60-1.40 |
| 0.32 | 30 | 1.80 | HI624-0.32-30-1.80 | - | HISO-0.32-30-1.80 |
| | 60 | 1.40 | - | HIVOC-0.32-60-1.40 | - |
| | | 1.80 | HI624-0.32-60-1.80 | - | HISO-0.32-60-1.80 |
| 0.53 | 30 | 3.00 | HI624-0.53-30-3.00 | - | HISO-0.53-30-3.00 |
| | 75 | 3.00 | HI624-0.53-75-3.00 | - | HISO-0.53-75-3.00 |

# GC DERIVATIZATION REAGENTS



- Increase sample volatility
- Improve selectivity and efficiency
- Enhance detectability

GC derivatization is frequently used to simplify complex separation problems. For GC analysis, this has a number of benefits including those detailed above. Hichrom offers a range of GC derivatization reagents as detailed below. Please enquire for details of any product not listed.

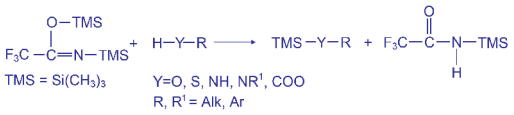
## Derivatization Reagents

A wide range of reagents is available for silylation, acylation or alkylation of reactive functional groups. A selection of these reagents is discussed below.

### BSTFA +TMCS (1%, 10%)

[N,O-Bis(trimethylsilyl)trifluoroacetamide]

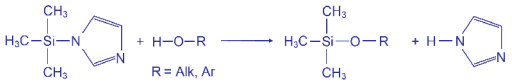
- Highly volatile and stable products
- Excellent solubility and solvency
- Addition of TMCS catalyzes reactions of hindered functional groups and other difficult functionalities




### TMSI

[Trimethylsilylimidazole]

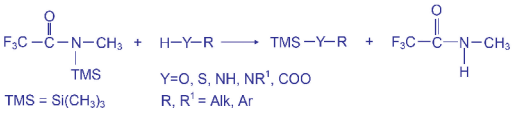
- Potent, selective TMS donor that reacts with hydroxyls but not amines or amides
- Derivatizes wet sugar samples, hindered hydroxyl groups in steroids, and amino acids in fluorinated acylation reagents




### MSTFA

[N-Methyltrimethylsilyltrifluoroacetamide]

- Most volatile of the TMS-acetamides
- Useful in analysis of volatile trace materials where derivatives elute near reagent or byproduct peak




### PFPA

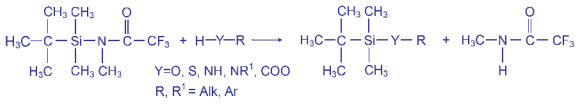
[Pentafluoropropionic anhydride]

- Most commonly used for ECD
- Reacts with alcohols, amines and phenols
- Frequently used for drugs of abuse confirmation

### MTBSTFA/MTBSTFA + 1% t-BDMCS

[N-Methyl-N-(t-butyldimethylsilyl)trifluoroacetamide]

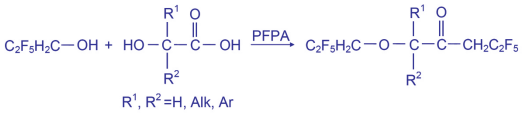
- Derivatives are $10^4$ times more stable to hydrolysis than corresponding TMS derivatives
- Produce easily interpreted mass spectra for GC-MS
- Addition of t-BDMCS catalyzes reactions of hindered alcohols and amines




### PFPOH

[2,2,3,3,3-Pentafluoropropanol]

- Used in combination with PFPA to make ECD derivatives of the most common functional groups, especially polyfunctional bio-organic compounds




## Ordering Information - Regis GC Derivatization Reagents[1]

| Reagent | Package Size[2] | | | |
|---|---|---|---|---|
| | 10 x 1g (ampoule) | 5g | 4 x 5g | 25g |
| BSTFA | 270111 | - | 270112 | 270113 |
| BSTFA + 1% TMCS | 270121 | - | 270122 | 270123 |
| BSTFA + 10% TMCS | 270131 | - | 270132 | 270133 |
| MSTFA | 270590 | - | - | 270593 |
| MTBSTFA | - | - | - | 270243 |
| MTBSTFA + t-BDMCS | 270144 | - | - | 270143 |
| TMSI | 270401 | 270402 | - | 270403 |
| PFPA | 640110 | - | - | 640113 |
| PFPOH | - | 270815 | - | 270816 |

[1] Other reagents available – please enquire [2] Other package sizes available – please enquire

Regis also offers a wide range of additional derivatization and other reagents. Please contact Hichrom for information on reagents not listed here.

# GC INLET SUPPLIES



## Inlet Liners

Choosing the optimum inlet liner and injection parameters for a specific application can increase peak areas and reduce detection limits. Characteristics including liner volume, liner treatments and deactivation and liner design are all important. Deactivation procedures are used to produce inert liners with long lifetimes. These are particularly useful for splitless injections or for analysis of polar compounds. Many liner designs use deactivated glass wool packing, placed near the centre of the liner. A small selection of some of the more common designs is illustrated in Figure 1.

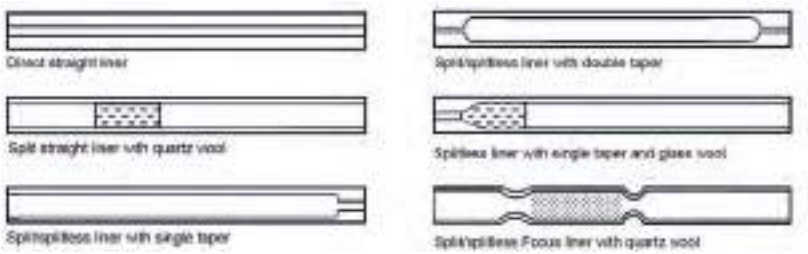



Figure 1. Selection of GC inlet liners

## Septa

Septa are available for a variety of different applications and different temperature ranges. Lower temperature septa are generally softer, seal better and can withstand more injections than their higher temperature counterparts. General purpose silicone rubber septa are cost-effective choices, providing low bleed, long lifetime and easy penetration. BTO (Bleed and Temperature Optimized) septa have an extended upper temperature range of 400°C and are ideal for use with low-bleed 'MS' type capillary columns. Various other septa types including PTFE coated silicone, PTFE multi-layer silicone and pre-pierced septa are available. Please enquire for further details.

## Ferrules

A comprehensive selection of ferrules is available, made of different materials and configurations for a leak-free connection between the column and the injector. Four main types of ferrules are used with capillary GC columns – graphite, vespel/graphite composites, vespel and stainless steel.

**1) Graphite** ferrules can be used at temperatures up to 450°C without producing bleed or decomposition products. They are very soft and porous to oxygen, making them suitable for most applications except GC-MS connections.

**2) Vespel/graphite** (85%/15%) ferrules are mechanically robust, have a long lifetime and are compatible with GC-MS. As they form a strong grip with the column, they cannot be re-used.

**3) Vespel** is a high temperature polyimide material which is very hard. It has low oxygen permeability making it an excellent sealing material. Vespel ferrules are reusable but are suitable for isothermal operation only.

**4) SilTite™ stainless steel** ferrules form strong permanent airtight seals with capillary column and MS interface. Unlike other ferrules they do not need re-tightening after installation.

100% Graphite ferrules

SilTite metal ferrules

The properties of these materials are summarised in Table 1.

### Table 1

| Material | Upper Temp. Limit | Suitable for GC-MS | Re-usable |
|---|---|---|---|
| Graphite (100%) | 450°C | No | Yes |
| Vespel/Graphite (85%/15%) | 350°C | Yes | No |
| Vespel | 280°C | Yes | Yes |
| Stainless steel (SilTite™) | 500°C | Yes | No |

Please contact Hichrom for ordering information for inlet liners, septa and ferrules and for information on any additional GC accessories.

# DIGITAL FLOW METER

- Handy and compact
- Wide flow rate range
- Easy to operate

Accurate gas flow measurement is a fundamental requirement for consistent and reliable gas chromatographic results. The GLF-1000 flow meter, manufactured by GL Sciences, measures target gases accurately and safely. The GLF-1000 is based on differential pressure (DP) measurement. Target gas flows up to 1000ml/min can be easily measured. The GLF-1000 operates off a simple 9V battery.

## Specifications:

| | |
|---|---|
| **Gases** | Helium, nitrogen, hydrogen, air |
| **Range** | 1 – 1000ml/min |
| **Accuracy (He)** | ± 10% of flow reading or<br>± 0.8ml/min whichever is greater (for 5 – 50ml/min)<br>± 5% of flow reading (for 50-900ml/min) |
| **Temperature Range** | 5 - 40°C |
| **Battery** | 9 volt square battery (1 pc) |
| **Dimensions** | 80 (W) x 145 (D) x 38.5 (H) mm |
| **Weight** | 180g excluding battery |
| **Others** | Auto power off function<br>Battery monitor function<br>CE certified |


## Ordering Information

| Description | Catalogue No. |
|---|---|
| Digital flow meter GLF-1000 | 2709-11000 |
| Capillary column connecting adaptor | 2709-55015 |

# GAS LEAK DETECTOR

- Super compact
- Easy to use
- High sensitivity
- USB port recharge

GL Sciences' LD239 gas leak detector utilises thermal conductivity measurements between target and reference gases. The LD239 is small, light and very sensitive. It detects up to 0.0005ml/min of helium gas, and can easily be recharged anywhere using the USB port connection.

## Specifications:

| | |
|---|---|
| **Detection Method** | Thermal conductivity |
| **Target Gases** | Helium, $CO_2$, Ar, Ne etc |
| **Sensitivity (He)** | 0.005ml/min at minimum (standard range)<br>0.0005ml/min at minimum (high range) |
| **Display** | LCD |
| **Temperature Range** | 10 - 40°C |
| **Battery** | Lithium ion battery installed, rechargeable through a USB port, 2.5 hours for full recharge. 5 hour battery life with continuous use. |
| **Dimensions** | 50 (W) x 20 (D) x 111 (H) mm |
| **Weight** | 95g |
| **Accessories included** | USB cable (1m), sample gas filter (probe built-in), referential gas filter (built-in), instruction manual, warranty certificate |

## Ordering Information

| Description | Catalogue No. |
|---|---|
| Gas leak detector LD239 | 2702-19340 |
| **Replacement Accessories** | |
| Battery for LD239 | 2702-19341 |
| USB cable | 2702-19331 |
| Sample filter | 2702-19333 |
| Reference filter | 2702-19334 |

# CE CAPILLARIES



## FunCap®-CE Series

- High reproducibility of migration time
- Fast analysis
- Compatible with wide pH range

GL Sciences manufacture the FunCap®-CE series of chemically modified fused silica capillaries. General purpose FunCap-CE capillaries are 1m in length, but with the detection window, have an effective length of 0.75m. For CE-MS, capillaries are 1.5m, with no detection window. Four types of chemical modifications are available. In addition, the FunCap-CE-V series capillaries, with high quality and performance, are available with an inspection report on the reproducibility of migration times.

**FunCap-CE Type A** The internal surface of the fused silica tubing is chemically modified by cationic groups. The electroosmotic flow (EOF) runs from anode to cathode, independent of pH, enabling usage in the pH range below 7.0. Type A capillary columns are suited for the analysis of cationic compounds.

**FunCap-CE Type S** The internal surface of the fused silica tubing is chemically modified by anionic groups. The EOF runs from cathode to anode, independent of pH, enabling usage in a wide pH range below 9.0.

**FunCap-CE Type D** This capillary is chemically modified by polar functional groups, resulting in less adsorption of proteins and therefore reduced deterioration of the capillary column surface.

**FunCap-CE Type C** In addition to lower protein adsorption, Type C capillary columns provide fast electroosmotic flow for protein analyses. These capillaries are suited for the analyses of acidic and neutral proteins.

Bare fused silica and deactivated fused silica capillaries are also available – please enquire for details.

### Ordering Information

| Description[1] | Capillary Length (m) | Type A | Type S | Type D | Type C |
|---|---|---|---|---|---|
| For CE (with detection window) | 1.0 | 1010-33012 | 1010-33010 | 1010-33013 | 1010-33011 |
| For CE-MS (without detection window) | 1.5 | 1010-33022 | 1010-33020 | 1010-33023 | 1010-33021 |
| | | **Type A-V** | **Type S-V** | **Type D-V** | **Type C-V** |
| For CE-MS (without detection window) | 1.0 | 1010-33032 | 1010-33030 | 1010-33033 | 1010-33031 |
| | 1.5 | 1010-33042 | 1010-33040 | 1010-33043 | 1010-33041 |

[1] All capillaries are 0.05mm i.d. and 0.375mm o.d.

## MicroSolv CE Capillaries

MicroSolv Technology Corporation manufactures several ranges of capillaries for CE.

**Simplus Capillaries™** These bare fused silica capillaries are 365µm (0.375mm) o.d. and available in 1m, 10m or 25m lengths.

**Zero™ EOF Capillaries** These are neutral coated capillaries designed to eliminate EOF and minimize wall interaction. They are suited to analysis of proteins, peptides or basic molecules.

**Celerity CE™ Capillaries** These have etched internal walls which increase surface area. Bonded phases include C18, C8, Diol, Butylphenyl and Cholesterol. Applications include hard to separate proteins, peptides or small molecules.

**Controlled™ EOF Capillaries** These capillaries are pH independent, so that the EOF does not change when the pH of the buffer is changed, encouraging reproducible CE and long capillary lifetime.

Please contact Hichrom for further technical details and ordering information on MicroSolv CE capillaries.

### Detection Window Maker™

The detection Window Maker™ from MicroSolv is easy to use for all capillaries for CE. It can be used to remove 1-2mm of the polyimide coating from 365µm o.d. capillaries for detection by UV and LIF. The Window Maker is shipped with a standard 2mm module for burning the capillary.


| Description | Catalogue No. |
|---|---|
| Window Maker, 220/240 VAC, EU with 2mm module[1] | 07300-S |
| Replacement heating module, 2mm window | 07201-S |

[1] Other size heating modules available – please enquire

# SOLID PHASE EXTRACTION



- High recovery and concentration of analytes
- Highly purified extracts
- Fast sample preparation
- Ease of automation
- Reduction in organic solvent consumption

Effective sample preparation procedures improve analytical results irrespective of the final analytical technique used.

Solid Phase Extraction (SPE) is a powerful technique for sample preparation. It is used in a broad range of application areas, including environmental analyses, pharmaceutical and biochemical analyses, organic chemistry and food analyses.

SPE utilises a liquid-solid extraction separation principle in which a large particle sized sorbent is sealed into a small chromatographic column. The required analytes are then selectively removed from the column either before or after removal of interfering compounds.

The main objectives of SPE are removal of interfering matrix components and selective concentration and isolation of the analytes of interest. This enables improved qualitative or quantitative analyses by GC, HPLC or other chromatographic techniques. Enrichment can increase detection sensitivity by a factor of 100 to 5000, which is particularly beneficial for trace analyses. Figures 1 and 2 below illustrate the two general separation procedures.

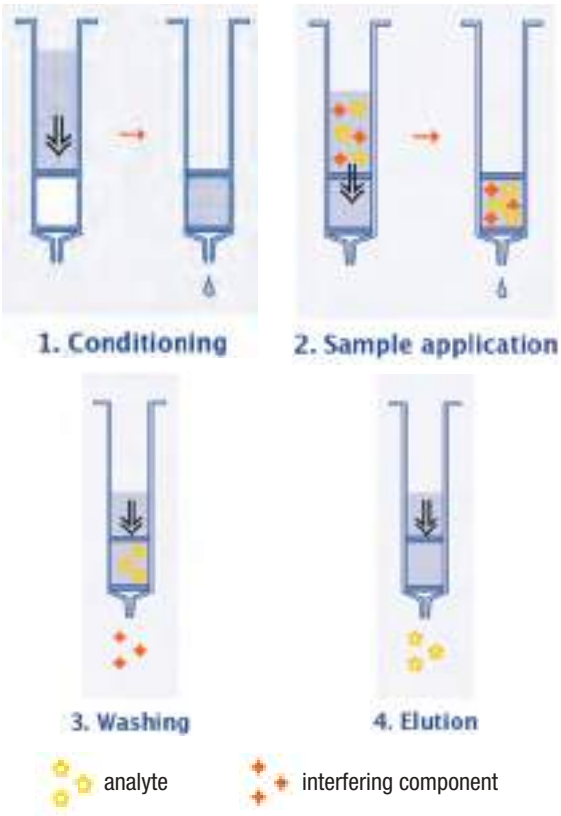



Figure 1. Retention of the analyte

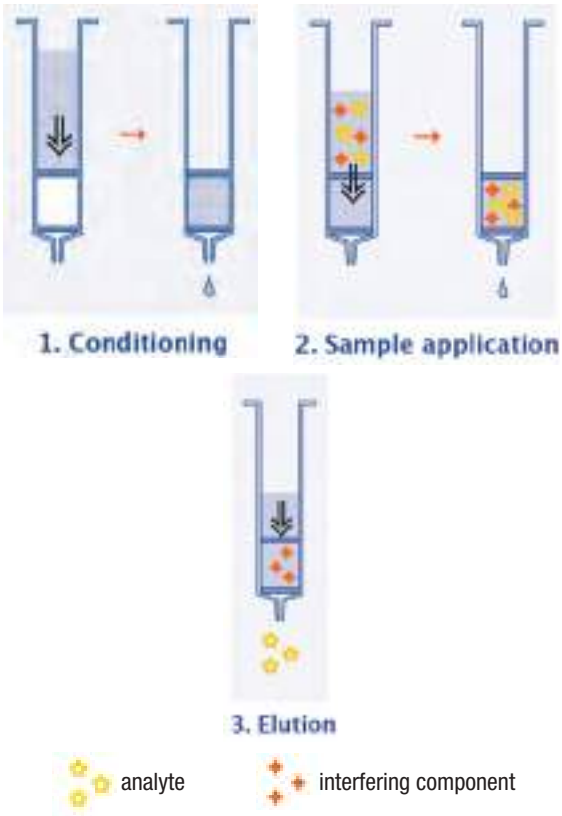



Figure 2. Retention of the interfering components

## Retention of the analyte

- Analyte molecules are enriched on the adsorbent
- Interfering components and solvent molecules (matrix) are not retained
- Remaining interfering components are washed from the adsorbent
- The analyte is removed from the adsorbent by elution with a suitable solvent

## Retention of interfering components

- Analyte molecules show no interaction with the adsorbent
- Interfering components and solvent molecules (matrix) are retained
- Analyte molecules are 'washed' from the adsorbent
- The solid phase is simply used to 'filter' the sample

**Hichrom offers several ranges of SPE products – please contact us to discuss your application requirements**

# SMART BAG AIR SAMPLING BAGS

GL Sciences offer the Smart Bag series of sampling bags, based on a range of materials and dimensions, suitable for different target compounds. Sampling bags are widely used as a convenient sampling method for automobile interior materials, automobile emissions, work environments, building materials and textiles. Gas samples collected can be directly injected into a gas chromatograph. These bags are an ideal accessory for applications involving MonoTrap sampling.

## Sampling Bags

| Description | Material | Temperature Limit (°C) | Film Thickness (µm) | Properties | Applications |
|---|---|---|---|---|---|
| Smart Bag PA | Vinyl alcohol polymer film | 120 | 53 | Superior resistance to solvents, heat and permeation | Automobile interior materials, automobile emissions, diffusion gases from materials, inorganic gases |
| Smart Bag 2F | Polyvinylidene fluoride (PVDF) film | 120 | 50 | Superior resistance to solvents and heat | Automobile interior materials, automobile emissions, diffusion gas from materials |
| ANALYTIC-BARRIER™ Bag | Proprietary | 70 | 45 | Good resistance to permeation, low background | Automobile interior materials, inorganic gases |
| Fluororesin Bag | Ethylene-tetrafluoroethylene copolymer film | 110 | 50 | Good resistance to solvents and heat | Organic solvents |
| Aluminium Bag | Laminated film[1] | 65 | 130 | Good permeation resistance to inorganic gas, methane | Inorganic gases |
| Polyester Bag | Polyester film | n/a | 38 | Good permeation resistance to VOCs | Volatile organic compounds (VOCs), odour analysis |

[1] From outer: nylon, polyethylene, aluminium foil, polyethylene

## Selection of Sampling Bag

In order to achieve highly reliable test results, the appropriate sampling bag should be chosen depending on the target compounds required to be collected. The following parameters need to be selected:

**1) Material –** The range of materials used in the Smart Bag product line is summarised in the table above. The choice of material is important, in order to optimise adsorption and to minimise interference from background peaks.

**2) Shape –** Three configurations (A, C and E) are available - see Figure 1.

**3) Connector (PTFE) –** Different diameter sleeves, with or without mini valves or M6 connectors, can be incorporated (see Figure 2).

**4) Volume –** 1, 2, 3, 5 and 10 litre volume bags are available as standard. Other sizes are available on request.

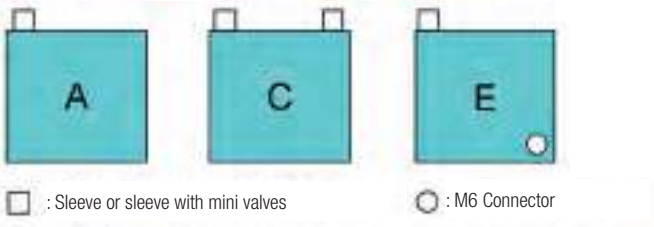



Figure 1. Configurations of Smart Bags


Figure 2. Connectors

## Smart Bag PA

Smart Bag PA, made from vinyl alcohol series polymer film, is one of the most popular sampling bags. It shows excellent resistance to solvents and heat, and very few impurities are generated from the material. Smart Bag PA also avoids the permeation of $O_2$ or $CO_2$. This enables a wide range of sampling from inorganic to organic gases.

Please contact Hichrom for further details and ordering information for these sampling bags.

Smart Bag PA

Automobile interior materials

Environmental air

Automobile emissions

Work environment

# PIPETTE TIPS



- Simple, fast sample preparation with minimal sample loss
- No contamination from supporting matrix
- Sample volumes as small as 0.1µl

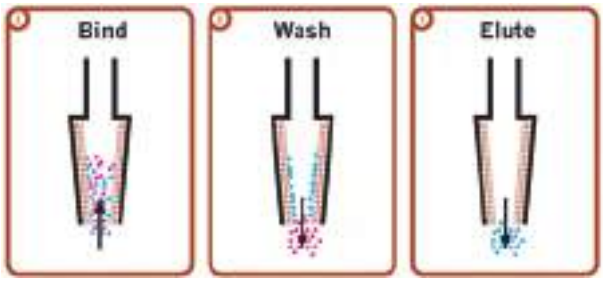
A range of micropipette tips is manufactured by Glygen Corporation for the preparation and separation of biological samples for HPLC, MALDI, CE, desalting and electrophoresis. In addition to non-bonded silica and reversed-phase materials, NuTips and TopTips are available packed with PolyLC ion-exchange materials (see pages 127-130) or with titania, zirconia, graphitic carbon and affinity media.

## NuTip™

NuTip™ enables purification of low concentration or low volume samples by maximising the surface area in contact with the sample. The chromatographic media is directly attached to the inner surface of the pipette tip without using polymers or glue. This helps avoid potential problems with contamination or permeability. Figure 1 shows the basic operating principles of purification using NuTips.

### Specifications

| Tip Volume (µl) | Sample Volume (µl) | Binding Capacity (µg) | Amount Chromatographic Material (mg) |
|---|---|---|---|
| 1 - 10 | 0.5 - 10 | 1 | 30 |
| 10 - 200 | 2 - 25 | 2.5 | 75 |
| 20 - 200 | 5 - 50 | 15 | 400 |



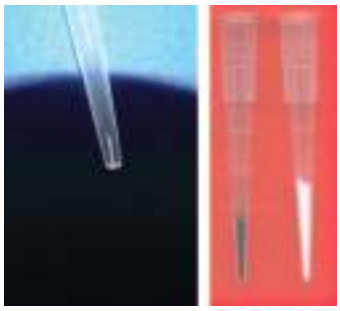

Unpurified sample drawn into NuTip. Target molecule binds.

Impurities expelled. Target molecules remain bound.

Solvent releases bound target molecules. Purified sample collected.

Figure 1. Basic operating principles of NuTip

NuTip



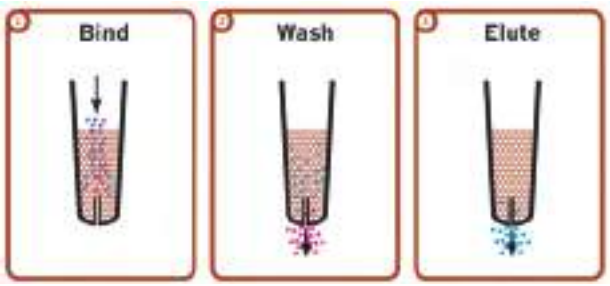
TopTip

## TopTip™

TopTip™ is a pipette tip with a fine slit (1-2µm) at the bottom which enables liquid to pass through but retains chromatographic material (20-30µm) in the tip. This eliminates the need for a filter, thereby reducing dead volume, loss of sample and contamination risk. Pressure can be applied via centrifuge, pipette, syringe or vacuum manifold. Figure 2 shows the basic operating principles of purification using TopTips.

### Specifications

| Tip Volume (µl) | Sample Volume (µl) | Binding Capacity (µg) | Amount Chromatographic Material (mg) |
|---|---|---|---|
| 1 - 10 | 1 - 10 | 400 | 4 |
| 10 - 200 | 2 - 25 | 1000 | 10 |
| 100 - 1000 | 20 - 1000 | 5000 | 50 |




Sample top-loaded. Target molecule binds to media.

Pressure forces impurities out of slit. Target bound.

Target molecules released from media. Purified sample collected.

Figure 2. Basic operating principles of TopTip

Both NuTip and TopTip products are available packed with a range of materials to suit a wide range of applications. Please enquire for further details.

# CHROMAFIL® SYRINGE FILTERS



- Extended solvent compatibility
- Colour coded or colour free labelled
- Low dead volume
- Well suited for automation
- Star-shaped distribution device

**CHROMAFIL® disposable syringe filters** are used for filtration of suspended matter from liquid samples (1 – 100ml) or gases. This is important for preventing column or frit blockage and wear to valve seals. The filters connect to standard Luer hub syringes.

These filters are available in a broad range of pore sizes and membrane types. The polypropylene housings are highly solvent resistant and exhibit low extractables.

**CHROMAFIL Xtra syringe filters** are labelled for method validation and certification. They are imprinted for direct identification of the membrane type, diameter and pore size. They are manufactured in colour-free polypropylene and HPLC test certificates are available for every membrane type. CHROMAFIL Xtra are suitable for use in automated instrumentation. Please enquire for further details and ordering information.

## Recommended filter sizes for different sample volumes

| Sample Volume | Recommended Diameter | Dead Volume |
|---|---|---|
| <1ml | 3mm | 5µl |
| 1 – 5ml | 15mm | 12µl |
| 5 – 100ml | 25mm | <80µl |

## Ordering Information[2]

| CHROMAFIL Type | Membrane Pore Size (µm) | Membrane Diameter (mm) | Catalogue No. | Pack Size[1] | Recommended Applications |
|---|---|---|---|---|---|
| **Cellulose mixed esters (MV)** | | | | | |
| A-20/25 | 0.20 | 25 | 729006 | **100** | Filtration of polar sample solutions |
| A-45/25 | 0.45 | 25 | 729004 | **100** | |
| **Cellulose acetate (CA)** | | | | | |
| CA-20/25 S | 0.20 | 25 | 729024 | **50** | Filtration under sterile conditions |
| CA-45/25 S | 0.45 | 25 | 729025 | **50** | |
| CA-20/25 | 0.20 | 25 | 729026 | **100** | Filtration of water soluble oligomers and polymers, especially biological macromolecules |
| CA-45/25 | 0.45 | 25 | 729027 | **100** | |
| **Regenerated cellulose (RC)** | | | | | |
| RC-20/15 MS | 0.20 | 15 | 729036 | **100** | Filtration of polar and medium polar sample solutions (MS = minispike on filter exit) |
| RC-45/15 MS | 0.45 | 15 | 729037 | **100** | |
| RC-20/25 | 0.20 | 25 | 729030 | **100** | |
| RC-45/25 | 0.45 | 25 | 729031 | **100** | |
| **Polyamide (PA)/Nylon** | | | | | |
| AO-20/3 | 0.20 | 3 | 729010 | **100** | Filtration of medium polarity sample solutions |
| AO-45/3 | 0.45 | 3 | 729011 | **100** | |
| AO-20/25 | 0.20 | 25 | 729012 | **100** | |
| AO-45/25 | 0.45 | 25 | 729013 | **100** | |
| **Polytetrafluoroethylene (PTFE)** | | | | | |
| O-20/3 | 0.20 | 3 | 729014 | **100** | Filtration of nonpolar sample solutions. Flush with alcohol for polar sample solutions. (MS = minispike on filter exit) |
| O-45/3 | 0.45 | 3 | 729015 | **100** | |
| O-20/15 MS | 0.20 | 15 | 729008 | **100** | |
| O-45/15 MS | 0.45 | 15 | 729009 | **100** | |
| O-20/25 | 0.20 | 25 | 729007 | **100** | |
| **Polyvinylidene difluoride (PVDF)** | | | | | |
| PVDF-20/15 MS | 0.20 | 15 | 729043 | **100** | Filtration of polar and nonpolar solutions (MS = minispike on filter exit) |
| PVDF-45/15 MS | 0.45 | 15 | 729044 | **100** | |
| **Polyester (PET)** | | | | | |
| PET-20/15 MS | 0.20 | 15 | 729022 | **100** | Multi-purpose membrane for polar and nonpolar solutions, especially suited for mixtures of water and organic solvents used as eluents in HPLC (MS = minispike on filter exit) |
| PET-45/15 MS | 0.45 | 15 | 729023 | **100** | |
| PET-20/25 | 0.20 | 25 | 729021 | **100** | |
| PET-45/25 | 0.45 | 25 | 729020 | **100** | |
| **Glass fibre (GF)** | | | | | |
| GF-100/15 MS | 1.0 | 15 | 729034 | **100** | For solutions with a high load of particulate matter and highly viscous solutions (MS = minispike on filter exit) |
| GF-100/25 | 1.0 | 25 | 729028 | **100** | |
| **Glass fibre (GF)/Polyester (PET)** | | | | | |
| GF/PET-20/25 | 1.0/0.20 | 25 | 729032 | **100** | |
| GF/PET-45/25 | 1.0/0.45 | 25 | 729033 | **100** | |

[1] BIG BOX (400/pk for 25mm filters and 800/pk for 15mm filters) also available – please enquire

[2] Please enquire for ordering information for CHROMAFIL Xtra syringe filters

# TLC PLATES



- Economical separation method
- High sample throughput
- Pilot procedure for HPLC and flash chromatography
- Versatile range of ready-to-use layers

Thin layer chromatography (TLC) is a simple, fast and highly versatile separation tool for both qualitative and quantitative analyses. The areas of application cover virtually all classes of compounds. About 80% of all TLC separations are performed using unmodified silica as the separation medium. Other commonly used adsorbents include modified silica, aluminium oxide and cellulose. Silica has the advantage of being able to use thicker layers (up to 2mm), suitable for preparative TLC.

## Macherey-Nagel TLC Plates

Macherey-Nagel offers a wide selection of TLC plates. In addition to glass plates, flexible plates are available on aluminium sheets (ALUGRAM®) and polyester sheets (POLYGRAM®). These are more economical than glass plates and have the advantage of being easy to cut. ALUGRAM Xtra aluminium sheets exhibit outstanding wettability even with 100% aqueous eluents. They also show improved cutting properties due to an optimised binder system. TLC plates produced by Macherey-Nagel are coated with the same silica used for Macherey-Nagel flash products.

The properties of a range of unmodified silica adsorbents for TLC and HPTLC are summarised below.

| | |
|---|---|
| **Standard unmodified silica** | |
| SIL G | Silica 60, standard grade, particle size 5-17µm |
| ADAMANT | Silica 60, improved binder system, optimised particle size distribution |
| DURASIL | Silica 60, special binder system, more polar than SIL G |
| SIL N-HR | High purity silica 60, special binder system, higher gypsum content |
| SILGUR | Silica 60 with kieselguhr concentrating zone |
| **Unmodified silica for HPTLC** | |
| Nano-SIL | Nano silica 60, standard grade, particle size 2-10µm |
| Nano-ADAMANT | Nano silica 60, optimised binder system and particle size distribution |
| Nano-DURASIL | Nano silica 60, special binder system |
| Nano-SILGUR | Nano silica 60, with kieselguhr concentrating zone |

### SIL G

Standard grade silica (SIL G) has a pore size of 60Å and a particle size of 5-17µm. The binder is stable in almost all organic solvents and resistant towards aggressive visualisation reagents.

### ADAMANT

ADAMANT glass TLC plates are based on standard grade silica 60, with optimised particle size distribution for increased separation efficiency, and an improved binder system resulting in outstanding hardness and abrasion resistance. They are suitable for trace analyses due to a UV indicator with increased brilliance and a low background noise.

### Nano Silica HPTLC (Nano-SIL) Plates

For high performance TLC separations, the same silica 60 is used, but with a particle size distribution of 2-10µm. This narrower particle size fractionation allows sharper separations, shorter development times, shorter migration distances and increased detection sensitivity compared to SIL G plates.

### TLC and HPTLC Plates with Concentrating Zone (SILGUR and nano-SILGUR)

Plates with concentrating zones are a valuable aid for manual sample application. The kieselguhr concentrating zone is completely inert towards a large number of compounds. Samples always form a narrow band at the interface of the silica and kieselguhr adsorbents, irrespective of shape, size or position of the spots in the concentrating zone (see Figure 1). Separation then takes place in the silica layer.

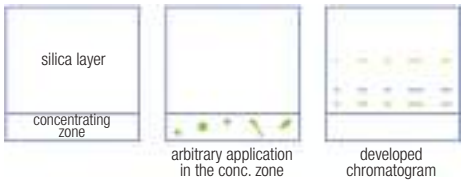



Figure 1. TLC plate with concentrating zone

### Modified Silica Layers

Although the majority of TLC separations are performed on unmodified hydrophilic silica, reversed-phase TLC plates are also available. These are prepared by total (100%) or partial (50%) bonding of C18 groups to the nano silica (2-10µm, 60Å). Nano-SIL CN, $NH_2$ and Diol are also available – please contact Hichrom for details.

### Other Adsorbents

A range of aluminium oxide and cellulose adsorbent TLC plates are available as glass plates, or polyester or aluminium sheets. In addition, a range of alternative adsorbents for specific applications is offered.

Ordering information for unmodified silica plates for TLC and HPTLC is given on the following page.
Please contact Hichrom for details of TLC and HPTLC plates with modified silica or other adsorbents.

# Macherey-Nagel TLC Plates (continued)

## Ordering Information

### Standard Silica (SIL G) TLC plates

| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| **Glass plates** | | | | |
| SIL G-25 | 0.25 | 5 x 10 | 50[2] | 809017 |
| SIL G-25 | 0.25 | 5 x 20 | 100 | 809011 |
| SIL G-25 | 0.25 | 10 x 20 | 50 | 809012 |
| SIL G-25 | 0.25 | 20 x 20 | 25 | 809013 |
| SILGUR-25 | 0.25 | 10 x 20 | 50 | 810012 |
| SILGUR-25 | 0.25 | 20 x 20 | 25 | 810013 |
| **POLYGRAM polyester sheets** | | | | |
| SIL G | 0.20 | 4 x 8 | 50 | 805032 |
| SIL G | 0.20 | 5 x 20 | 50 | 805012 |
| SIL G | 0.20 | 20 x 20 | 25 | 805013 |
| **ALUGRAM aluminium sheets** | | | | |
| SIL G | 0.20 | 5 x 10 | 50 | 818161 |
| SIL G | 0.20 | 10 x 20 | 20 | 818163 |
| SIL G | 0.20 | 20 x 20 | 25 | 818033 |
| **ALUGRAM Xtra aluminium sheets** | | | | |
| SIL G | 0.20 | 5 x 10 | 50 | 818261 |
| SIL G | 0.20 | 5 x 20 | 50 | 818232 |
| SIL G | 0.20 | 20 x 20 | 25 | 818233 |
| SILGUR | 0.20 | 10 x 20 | 20 | 818412 |
| SILGUR | 0.20 | 20 x 20 | 25 | 818413 |

[1] Other dimension plates available [2] Other pack sizes available

### Standard Silica (SIL G) TLC plates with UV indicator

| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| **Glass plates** | | | | |
| SIL G-25 $UV_{254}$ | 0.25 | 5 x 10 | 50[2] | 809027 |
| SIL G-25 $UV_{254}$ | 0.25 | 5 x 20 | 100 | 809021 |
| SIL G-25 $UV_{254}$ | 0.25 | 10 x 20 | 50 | 809022 |
| SIL G-25 $UV_{254}$ | 0.25 | 20 x 20 | 25 | 809023 |
| SILGUR-25 $UV_{254}$ | 0.25 | 10 x 20 | 50 | 810022 |
| SILGUR-25 $UV_{254}$ | 0.25 | 20 x 20 | 25 | 810023 |
| **POLYGRAM polyester sheets** | | | | |
| SIL G/$UV_{254}$ | 0.20 | 4 x 8 | 50 | 805021 |
| SIL G/$UV_{254}$ | 0.20 | 5 x 20 | 50 | 805022 |
| SIL G/$UV_{254}$ | 0.20 | 20 x 20 | 25 | 805023 |
| **ALUGRAM aluminium sheets** | | | | |
| SIL G/$UV_{254}$ | 0.20 | 5 x 10 | 50 | 818160 |
| SIL G/$UV_{254}$ | 0.20 | 10 x 20 | 20 | 818162 |
| SIL G/$UV_{254}$ | 0.20 | 20 x 20 | 25 | 818133 |
| **ALUGRAM Xtra aluminium sheets** | | | | |
| SIL G/$UV_{254}$ | 0.20 | 5 x 10 | 50 | 818360 |
| SIL G/$UV_{254}$ | 0.20 | 5 x 20 | 50 | 818332 |
| SIL G/$UV_{254}$ | 0.20 | 20 x 20 | 25 | 818333 |
| SILGUR $UV_{254}$ | 0.20 | 10 x 20 | 20 | 818422 |
| SILGUR $UV_{254}$ | 0.20 | 20 x 20 | 25 | 818423 |

### Nano-SIL TLC plates

| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| **Glass plates** | | | | |
| Nano-SIL-20 | 0.20 | 5 x 5 | 100 | 811011 |
| Nano-SIL-20 | 0.20 | 10 x 20 | 50 | 811013 |
| Nano-SILGUR-20 | 0.20 | 10 x 10 | 25 | 811032 |
| **ALUGRAM aluminium sheets** | | | | |
| Nano-SIL G | 0.20 | 20 x 20 | 25 | 818141 |
| **ALUGRAM Xtra aluminium sheets** | | | | |
| Nano-SIL G | 0.20 | 5 x 20 | 50 | 818240 |
| Nano-SIL G | 0.20 | 20 x 20 | 25 | 818241 |
| Nano-SILGUR | 0.20 | 10 x 10 | 25 | 818432 |

[1] Other dimension plates available

### Nano-SIL TLC plates with UV indicator

| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| **Glass plates** | | | | |
| Nano-SIL-20 $UV_{254}$ | 0.20 | 5 x 5 | 100 | 811021 |
| Nano-SIL-20 $UV_{254}$ | 0.20 | 10 x 20 | 50 | 811023 |
| Nano-SILGUR-20 $UV_{254}$ | 0.20 | 10 x 10 | 25 | 811042 |
| **ALUGRAM aluminium sheets** | | | | |
| Nano-SIL G/$UV_{254}$ | 0.20 | 20 x 20 | 25 | 818143 |
| **ALUGRAM Xtra aluminium sheets** | | | | |
| Nano-SIL G/$UV_{254}$ | 0.20 | 5 x 20 | 50 | 818342 |
| Nano-SIL G/$UV_{254}$ | 0.20 | 20 x 20 | 25 | 818343 |
| Nano-SILGUR $UV_{254}$ | 0.20 | 10 x 10 | 25 | 818442 |

### ADAMANT Silica TLC plates (glass)

| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| ADAMANT | 0.25 | 5 x 10 | 50[2] | 821040 |
| ADAMANT | 0.25 | 10 x 10 | 25 | 821050 |
| ADAMANT | 0.25 | 20 x 20 | 25 | 821060 |

[1] Other dimension plates available [2] Other pack sizes available

### ADAMANT Silica TLC plates (glass) with UV indicator

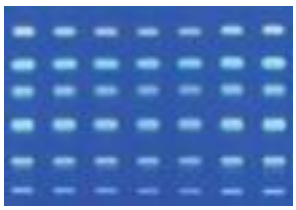
| Description | Layer (mm) | Plate Size[1] (cm) | Pack Size | Cat. No. |
|---|---|---|---|---|
| ADAMANT $UV_{254}$ | 0.25 | 2.5 x 7.5 | 100 | 821005 |
| ADAMANT $UV_{254}$ | 0.25 | 5 x 10 | 50[2] | 821010 |
| ADAMANT $UV_{254}$ | 0.25 | 10 x 10 | 25 | 821020 |
| ADAMANT $UV_{254}$ | 0.25 | 10 x 20 | 50 | 821025 |
| ADAMANT $UV_{254}$ | 0.25 | 20 x 20 | 25 | 821030 |

## Merck Millipore TLC Plates

Merck Millipore offer a wide range of classical TLC plates for manual operation and HPTLC (High Performance TLC) plates for automated operations. Merck Millipore introduced the first pre-coated plates on the market. TLC plates in a wide range of chemistries, sizes and backing materials, to suit a variety of applications, are offered. The features of a selection of these plates are summarised below.

**Classical Silica TLC Plates.** These are based on Merck silica gel with a pore size of 60Å and surface area of 520$m^2$/g. Classical silica TLC plates have a layer thickness of 250µm (glass plates) or 200µm (aluminium or plastic backed plates) and a mean particle size of 10-12µm.
*Applications: Wide range*

**High Performance Silica Plates (HPTLC).** Merck HPTLC plates offer higher speed and higher sensitivity than classical TLC plates. They utilise optimised silica gel 60 with a particle size of only 5-6µm and thinner layers (<200µm). Glass or aluminium backed plates, with or without fluorescent indicator are available.
*Applications: Identity testing in analysis of herbal medicines, quantitative QC separations of drugs, trace analysis in food*



A. Classical TLC silica gel 60 plate

B. HPTLC silica gel 60 plate

**LiChrospher® HPTLC Plates.** LiChrospher® HPTLC plates are based on spherical silica particles (60Å pore size, 7µm particle size), for the ultimate TLC performance, speed and sensitivity, enabling high throughput analysis of complex samples. They enable lower detection limits and higher resolution to be achieved, compared to TLC.
*Applications: Complex low concentration samples eg. pesticide mixtures, pharmaceuticals*

**RP-Modified Silica Plates (TLC and HPTLC).** RP-modified (RP-2, RP-8 or RP-18) silica layers are suitable for separations insufficiently resolved on unmodified silica. Glass and aluminium backed plates are available.
*Applications: Amides, antibiotics, fatty acids, non-polar substances using aqueous solvents*

**CN-, Diol- and $NH_2$-Modified Plates (TLC and HPTLC).** These are less polar than the classical silica phases and therefore suited for the separation of hydrophilic or charged substances. The amino modified plates offer an alternative to PEI cellulose.
*Applications: CN-silica – benzodiazepines, pesticides, plasticisers, tetracyclines, antibiotics*
*Diol-silica – glycosides, anabolic steroids, aromatic amines, dihydroxybenzoic acids*
*$NH_2$-silica – charged compounds eg. nucleotides, phenols, sulphones*

**Aluminium Oxide TLC Plates.** These TLC plates utilise neutral or basic aluminium oxide of 60Å or 150Å pore size, with or without fluorescence indicator to suit different application needs. Under aqueous conditions basic compounds are best separated on basic aluminium oxide plates, while neutral compounds are best separated on neutral plates.

**Cellulose Plates (TLC and HPTLC).** Cellulose plates are ideal for separating hydrophilic compounds by partition chromatography. Glass, aluminium and plastic backed TLC plates as well as glass and aluminium backed HPTLC plates are available.
*Applications: Amino acids, carbohydrates, phosphates, nucleic acids and nucleic acid derivatives*

**PEI (Polyethyleneimine) Cellulose Plates.** PEI modified cellulose acts as a strong basic anion-exchanger.
*Applications: Amino acids, peptides, nucleotides, nucleosides, sugar phosphates*

**Concentrating Zone Plates (TLC, HPTLC, PLC).** Merck's concentrating zone plates are based on the different adsorption properties of two adsorbents. The first is a large pore concentrating adsorbent where the samples are applied; the second is a selective layer for separation. Regardless of the spots' shape, size, or position, the sample always concentrates as a narrow band where the two adsorbents overlap and where the separation starts.

**Mixed Layer Plates.** Merck's mixed layer plates utilise a combination of classical silica gel 60 and kieselguhr, to provide good separation properties for certain special applications.
*Applications: Inorganic ions, herbicides, some steroids*

**Preparative Layer Plates (PLC).** PLC plates allow the separation of mg to gram samples using up to 2mm thick layers on glass backed plates. Plates are available with layers of silica gel, RP-18 modified silica or aluminium oxide in several layer thicknesses (0.5mm to 2mm), with or without fluorescent indicator.

Please contact Hichrom for further details and ordering information for the above products and alternative TLC plates and accessories not listed.

# RSA™ – Reduced Surface Activity Glass Vials

- Detect low abundance analytes normally adsorbed by glass
- Prevent pH changes in vials before injection
- Prevent sample hydrolysis that can occur in vials
- Excellent choice for LC, LC-MS, LC-MS/MS, GC and GC-MS

RSA Glass™ vials and inserts, supplied by MicroSolv Technology Corporation, are manufactured using a revolutionary patented production process that virtually eliminates all silanols and produces glass which has a greatly reduced surface activity for basic compounds. RSA Glass vials are not coated or silanised. The reduced surface activity of these vials makes them an excellent choice for many applications, including biologicals, natural products and basic compounds, or when accurate quantitation is vital.

## Why use RSA vials?

- **If you are working with low abundance basic samples** with regular glass vials, your components could easily be adsorbed to the glass wall. This can change your quantitation or completely remove the compound from solution preventing injection and ultimately masking its detection and identification. With RSA Glass vials and inserts this will not occur if the adsorption is due to silanol interaction.
- **If you are working with sensitive compounds** commonly found in natural products, biological samples or chemical synthesis, the glass wall could be a problem for basic compounds. With RSA Glass vials this problem is eliminated.
- **If you are working with basic proteins** that adsorb or are affected by silanols, inert RSA Glass vials will be a good solution. In addition, if silanisation is required for very 'sticky' proteins and peptides, the results are superior with RSA Glass over standard borosilicate glass.

## Manufacture of Conventional Glass Vials

When vials are made, they are constructed from long lengths of borosilicate glass tubing, made to exact tolerances and chemical specifications. This tubing is placed into vial converting machines that use heat to tool the tubing into vials and give them the dimensional characteristics such as necks, threads and bottoms. During this process, the intense heat that is used can often cause some of the glass to become a gas, which will quickly cool and sublimate onto the surface of the vial in the form of a silicate material. This material is not covalently bonded to the glass and will vary in amount from vial to vial and lot to lot, depending on the amount of heat used.

This 'silicate' material is pH sensitive, adsorbs basic compounds and will 'delaminate' or come off the vial under some conditions.

## Potential Problems When Using Ordinary Borosilicate Glass Vials

When using ordinary borosilicate glass autosampler vials and inserts, sample diluents such as water can deprotonate the many hydroxyl groups on the wall of the vial, which produces a negative charge on the glass. The acidic functionality of these silanols can cause adsorption of basic compounds, change the pH of the sample solution and/or hydrolyse susceptible compounds making them undetectable.

## Manufacture of RSA Glass Vials

RSA Glass vials are manufactured in the same manner as conventional borosilicate glass vials, except that the proprietary process of precision heat control is used to virtually eliminate the gaseous state formation. Therefore, there is no silicate layer formed and subsequently the problems associated with it are removed.

To maintain the quality and integrity of these vials, they are immediately packaged by robotic arms in a Class 100,000 clean room and are never touched by human hands.

RSA Reduced Surface Activity Glass
Autosampler Vials & Inserts

## Metal Levels

RSA Glass has been tested for metals that may cause sample or diluent contamination. It has ultra low levels of sodium, calcium, aluminium and boron. Sodium and boron levels are 200 to 300 times less concentrated compared to leading manufacturers' vials, with calcium and aluminium almost undetectable. Testing was carried out with ICP using water from the vials which had had a 4 hour 'soak' time.

## Advantages of RSA™ Vials

Table 1 illustrates the advantages of RSA™ vials compared to conventional borosilicate HPLC and GC autosampler vials with regard to sample adsorbance and pH changes. For the sample adsorption test a 5µg/ml aqueous solution of chlorhexidine was used. After 4 hours in the vials, the final concentrations and concentration losses were determined for each brand of vial in the study. MicroSolv have demonstrated that the conventional borosilicate glass vials Brand X and Brand Y showed considerable loss of sample over the 4 hours of the study. The benefits of using RSA vials to minimise sample adsorption to the glass can clearly be seen.

The pH test used deionised water at pH 5.45 (see starting point value in Table 1). After a 4 hour test period, the sample solution in the RSA vials showed no change in pH, whereas Brand X and Brand Y vials showed changes of ≥1.25 pH units.

### Table 1. Adsorption and pH changes[1]

| Brand | Ave. % loss after 4 hrs[2] | pH |
|---|---|---|
| Starting Point | 0 | 5.45 |
| RSA Glass | 0.35 | 5.50 |
| MicroSolv Brand | 17.62 | 6.15 |
| Brand X | 54.14 | 6.65 |
| Brand Y | 45.96 | 6.94 |

[1] Data courtesy of MicroSolv Technologies, USA. The comparative data presented here may not be representative for all applications

[2] Average of 3 vials

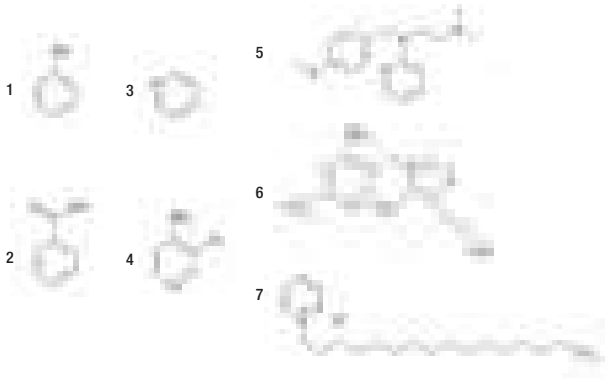
Chlorhexidine

In a further study seven test solutes containing different functional groups were assayed by HPLC in commonly used conventional borosilicate and RSA glass autosampler vials over a 4 hour period. Table 2 indicates that analytes with no basic group showed no loss of peak area during this time for either vial type. The greatest losses due to adsorption to the glass occurred for basic compounds, in particular for the cationic amines thiamine and cetylpyridinium chloride.

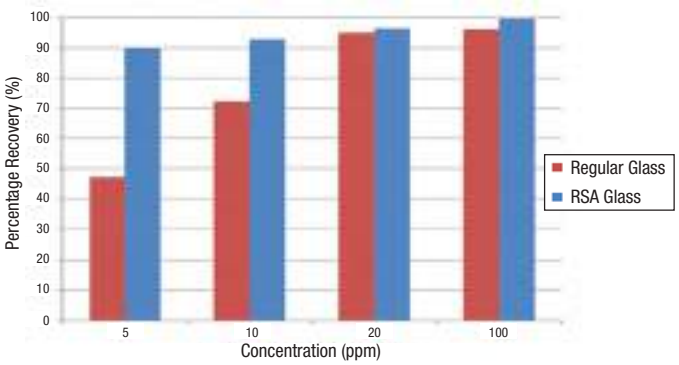
### Table 2. Percent loss after 4 hours[1]

| Analyte | Regular Glass | RSA Glass |
|---|---|---|
| 1. Benzoic acid | 0 | 0 |
| 2. Phenol | 0 | 0 |
| 3. Pyridine | 3.5 | 0.2 |
| 4. 4-Amino-3-chloropyridine | 9.1 | 0.6 |
| 5. Mepyramine | 13.1 | 0.7 |
| 6. Thiamine | 32.6 | 3.1 |
| 7. Cetylpyridinium chloride | 52.7 | 9.8 |

[1] Data courtesy of MicroSolv Technologies, USA. The comparative data presented here may not be representative for all applications

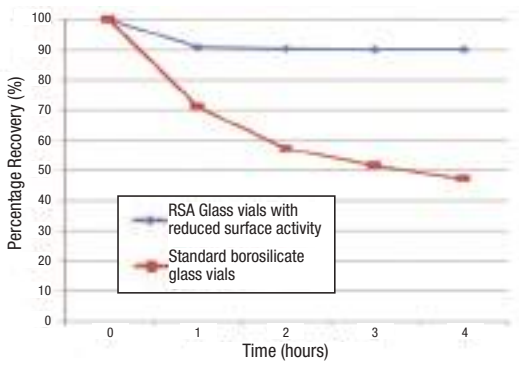



This effect is more significant at lower concentrations. Figure 1 shows the percent recovery for different concentrations of cetylpyridinium chloride solutions after 4 hours using both vial types. Although only a small difference is seen between regular glass and RSA glass vials at the highest concentration studied (100ppm), at the lowest 5ppm level the discrepancy is significant. The losses of a 5ppm solution of cetylpyridinium chloride over a 4 hour period are illustrated further in Figure 2.




Data courtesy of MicroSolv Technologies, USA. The comparative data presented here may not be representative for all applications

Figure 1. Recovery of cetylpyridinium chloride solutions




Data courtesy of MicroSolv Technologies, USA. The comparative data presented here may not be representative for all applications

Figure 2. Losses of 5ppm cetylpyridinium chloride solution

## Conclusions

Compared to conventional glass vials:

- RSA Glass vials show significant reduction in adsorption of basic compounds
- RSA Glass vials give significantly greater recovery for low abundance analytes
- RSA Glass vials result in significantly greater stability of sample solution pHts

## Ordering Information for RSA™ Glass Vials

### RSA Glass Inserts and Vials - AQ™ (Advanced Quality) Brand (100/pack)

| Description | Catalogue No. |
|---|---|
| Inserts, 29 x 6mm, 200µl, with attached plastic springs | 9502S-02N-RS |
| Inserts, 29 x 6mm, 200µl, deactivated, with attached plastic springs | 9502S-02ND-RS |
| 2ml vials, clear 9mm screw top with write-on patch | 9509S-WCV-RS |
| 2ml vials, amber 9mm screw top with write-on patch | 9509S-WAV-RS |
| 2ml vials, clear 9mm screw top | 9509S-0CV-RS |
| 2ml vials, clear, snap/crimp top with write-on patch | 9509C-WCV-RS |
| 2ml vials, amber, snap/crimp top with write-on patch | 9509C-WAV-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, clear, centre draining snap top | 9512C-0CV-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, clear, centre draining 9mm screw top | 9512S-0CV-RS |
| 1.2ml, MRQ™ vials[2], 2µl residual, snap top, clear | 9512C-0CV-T-RS |
| 1.2ml, MRQ™ vials[2], 2µl residual, 9mm screw top, clear | 9512S-0CV-T-RS |
| Vials with 300µl fused insert, snap top, clear with write-on patch | 9532C-0CV-RS |
| Vials with 300µl fused insert, 9mm screw top, clear with write-on patch | 9532S-0CV-RS |
| Vials with 300µl wide tip fused insert, screw top, clear with write-on patch | 9532S-WCV-WT-RS |
| Vials with 300µl wide tip fused insert, screw top, clear with write-on patch, silanised | 9532S-WCDV-WT-RS |


9512C-0CV-RS


9502S-02N-RS


9532S-0CV-RS


9512C-0CV-T-RS

### Screw Caps for RSA Glass Vials - AQ™ Brand

| Description | Catalogue No. |
|---|---|
| Caps, light blue with fitted ultra pure silicone/PTFE septa, 100/pack | 9509S-10C-B |
| Caps, light blue with fitted ultra pure silicone/PTFE septa, 1000/case | 9509S-10C-B-M |
| Caps, light blue with preslit fitted ultra pure silicone/PTFE septa, 100/pack | 9509S-30C-B |
| Caps, light blue with preslit fitted ultra pure silicone/PTFE septa, 1000/case | 9509S-30C-B-M |


9512S-0CV-T-RS


9509S-WCV-RS

### Snap Caps for RSA Glass Vials - MicroSolv Brand

| Description | Catalogue No. |
|---|---|
| Caps, blue with silicone/PTFE septa, 100/pack | 9502C-10CB |
| Caps, blue with silicone/PTFE septa, 1000/case | 9502C-10CB-M |
| Caps, blue with preslit silicone/PTFE septa, 100/pack | 9502C-30CB |
| Caps, blue with preslit silicone/PTFE septa, 1000/case | 9502C-30CB-M |


9512S-0CV-RS


9532C-0CV-RS

### RSA Glass Vial Easy Purchase Packs - AQ™ Brand (100/pack)

| Description | Catalogue No. |
|---|---|
| 2ml amber write-on vials and light blue AQR screw caps with silicone/PTFE septa | 9509S-1WAP-RS |
| 2ml clear write-on vials and light blue AQR screw caps with silicone/PTFE septa | 9509S-1WCP-RS |
| 2ml amber write-on vials and light blue AQR screw caps with preslit silicone/PTFE septa | 9509S-3WAP-RS |
| 2ml clear write-on vials and light blue AQR screw caps with preslit silicone/PTFE septa | 9509S-3WCP-RS |
| 2ml clear vials and light blue AQR screw caps with silicone/PTFE septa | 9509S-1CP-RS |
| 2ml clear vials and light blue AQR screw caps with preslit silicone/PTFE septa | 9509S-3CP-RS |
| 2ml amber write-on vials and snap caps with AQR silicone/PTFE septa | 9509C-1WAP-RS |
| 2ml clear write-on vials and snap caps with AQR silicone/PTFE septa | 9509C-1WCP-RS |
| 2ml amber write-on vials and snap caps with preslit AQR silicone/PTFE septa | 9509C-3WAP-RS |
| 2ml clear write-on vials and snap caps with preslit AQR silicone/PTFE septa | 9509C-3WCP-RS |
| 2ml amber write-on vials and snap caps with preslit red/white silicone/PTFE septa | 9509C-3XWAP-RS |
| 2ml clear write-on vials and snap caps with preslit red/white silicone/PTFE septa | 9509C-3XWCP-RS |
| 1.2ml MRQ™ vials[2], 2µl residual, clear and light blue AQR screw caps with silicone/PTFE septa | 9512S-1MP-RS |
| 1.2ml MRQ™ vials[2], 2µl residual, clear and light blue AQR screw caps with preslit silicone/PTFE septa | 9512S-3MP-RS |
| 1.2ml MRQ™ vials[2], 2µl residual, clear and blue snap caps with silicone/PTFE septa | 9512C-1MP-RS |
| 1.2ml MRQ™ vials[2], 2µl residual, clear and blue snap caps with preslit silicone/PTFE septa | 9512C-3MP-RS |
| 300µl fused insert vials and light blue AQR screw caps with silicone/PTFE septa | 9532S-1CP-RS |
| 300µl fused insert vials and light blue AQR screw caps with preslit silicone/PTFE septa | 9532S-3CP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, amber and light blue AQR screw caps with silicone/PTFE septa | 9512S-1AP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, clear and light blue AQR screw caps with silicone/PTFE septa | 9512S-1CP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, amber and light blue AQR screw caps with preslit silicone/PTFE septa | 9512S-3AP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, clear and light blue AQR screw caps with preslit silicone/PTFE septa | 9512S-3CP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, amber write-on with polyethylene single injection screw cap | 9512S-9AP-RS |
| 1.8ml Max Recovery vials[1], 8µl residual, clear write-on with polyethylene single injection screw cap | 9512S-9CP-RS |


9509S-WAV-RS


Easy Purchase Pack

[1] 1.8ml is the total fill volume. Vial has the external dimensions of a standard 2ml 12 x 32 vial. Recommended max fill volume 1.5ml, 8µl residual

[2] 1.2ml is the total fill volume. Vial has the external dimensions of a standard 2ml 12 x 32 vial. 2µl residual

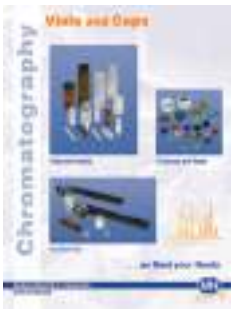
Please contact Hichrom for details of additional MicroSolv vials, caps and inserts not listed above.

# MACHEREY-NAGEL VIALS



Macherey-Nagel offer a wide range of vials and caps compatible with most manufacturers' GC and HPLC autosamplers.

- Crimp top vials with rim diameters of 8, 11, 13 or 20mm (N 8, N 11, N 13 or N 20)
- Screw thread vials in sizes N 8, N 9, N 10, N 13, N 18 and N 24 (EPA)
- Snap top vials in size N 11
- Micro inserts for use with standard sample vials for small sample volumes
- Sample vials with integrated conical micro insert for small sample volumes
- Crimp, screw and snap top caps in different materials and with different septa type
- Headspace vials, micro reaction vials, storage vials, shell vials, crimping tools




Please contact Hichrom for a copy of the Macherey-Nagel Vials and Caps brochure


Crimp vials, caps and inserts


Screw vials, caps and inserts


Snap vials, caps and inserts

A selection of some of the more popular vials is given below. Please enquire for details of vial sizes not included or for information on larger pack sizes.

## Ordering Information

| Description | Qty | Catalogue No. |
|---|---|---|
| **Crimp Top Vials and Caps** | | |
| 1.5ml crimp top vial N 11, clear, wide opening | 100/pk | 70201HP |
| 1.5ml crimp top vial N 11, clear, wide opening, with label and scale | 100/pk | 702885 |
| 1.5ml crimp top vial N 11, amber, wide opening, with label and scale | 100/pk | 702892 |
| Small sample volume (1.1ml) crimp neck vial N 11, clear, 15µl funnel | 100/pk | 702888 |
| Small sample volume (1.1ml) crimp neck vial N 11, clear, conical | 100/pk | 702141 |
| Small sample volume (1.1ml) crimp neck vial N 11, clear, conical with round pedestal glass plate | 100/pk | 702015 |
| Small sample volume crimp neck vial N 11, clear, flat bottom with integrated 0.2ml insert | 100/pk | 702891 |
| Small sample volume crimp neck vial N 11, amber, flat bottom with integrated 0.2ml insert | 100/pk | 702014 |
| N 11 aluminium crimp cap, silver, centre hole, natural rubber/butyl red-orange/TEF colourless septum | 100/pk | 70256 |
| N 11 aluminium crimp cap, silver, centre hole, silicone white/PTFE red septum | 100/pk | 70288 |
| **Screw Cap Vials and Caps** | | |
| 1.5ml screw neck vial N 9, clear, wide opening | 100/pk | 702282 |
| 1.5ml screw neck vial N 9, amber, wide opening | 100/pk | 702293 |
| 1.5ml screw neck vial N 9, clear, with label and scale, wide opening | 100/pk | 702283 |
| 1.5ml screw neck vial N 9, clear, with label and scale, wide opening, silanized | 100/pk | 702078 |
| 1.5ml screw neck vial N 9, amber, with label and scale, wide opening | 100/pk | 702284 |
| 1.5ml screw neck vial N 9, amber, with label and scale, wide opening, silanized | 100/pk | 702079 |
| Small sample volume (1.1ml) screw neck vial N 9, clear, 15µl funnel | 100/pk | 702006 |
| Small sample volume screw neck vial N 9, clear, flat bottom with integrated 0.2ml insert | 100/pk | 702007 |
| Small sample volume screw neck vial N 9, amber, flat bottom with integrated 0.2ml insert | 100/pk | 702008 |
| Small sample volume polypropylene vial N 9, transparent, with 0.3ml inner cone | 100/pk | 702009 |
| N 9 PP screw cap, blue, with red rubber/FEP colourless septum, centre hole | 100/pk | 702732 |
| N 9 PP screw cap, blue, with silicone white/PTFE red septum, centre hole | 100/pk | 702287.1 |
| **Snap Cap Vials and Caps** | | |
| 1.5ml snap top vial N 11, clear | 100/pk | 702714 |
| 1.5ml snap top vial N 11, clear, with label and scale | 100/pk | 702713 |
| 1.5ml snap top vial N 11, amber, with label and scale | 100/pk | 702712 |
| Small sample volume snap top vial N 11, clear, flat bottom with integrated 0.2ml insert | 100/pk | 702709 |
| Small sample volume polypropylene vial N 11, transparent, with 0.3ml conical insert | 100/pk | 702809 |
| N 11 PE snap ring cap, blue with red rubber/TEF colourless septum | 100/pk | 702063 |
| N 11 PE snap ring cap, blue, with silicone white/PTFE red septum | 100/pk | 702710.1 |
| **Inserts** | | |
| 0.2ml Inserts for wide opening vial, clear, conical 15mm tip | 100/pk | 702813 |
| 0.1ml Inserts for wide opening vial, clear, with plastic spring | 100/pk | 702818 |
| 0.3ml Inserts for wide opening vial, clear, flat bottom | 100/pk | 702825 |

Please contact Hichrom for information on vials and caps not listed. Discounts available for bulk orders – please enquire.

# TUBING



Most HPLC systems are plumbed with 1/16″ o.d. tubing, which is produced in a range of materials. PEEK™ tubing has proved to be superior to stainless steel in many HPLC applications, especially where biological samples are analysed or where contact between sample and metal components must be avoided. Tubing length and i.d. should be kept to a minimum to minimise extra-column dead volume and band broadening.

## Properties of different material tubing

| Tubing Material | Tubing o.d. | Max. Recommended Operating Temp. (°C) | Max. Pressure for 1/16″ Tubing (psi) | Chemical Resistance |
|---|---|---|---|---|
| Stainless steel | 1/8″, 1/16″, 1/32″ | 289 | n/a | May corrode with chloride salts or other buffers. May adsorb proteins. pH range 1-14. |
| PEEK™ | 1/16″, 1/32″, 360µm | 100 | 7,000 | Inert to almost all organic solvents. Concentrated nitric and sulphuric acids, DMSO, THF and $CH_2Cl_2$ are not recommended. pH range 0-14. |
| PEEKsil™ | 1/16″, 1/32″, 360µm | 100 | 10,000 | Compatible with most organic solvents. Avoid hydrofluoric acid. pH range 0-10. |
| Fused silica | 360µm | 100 | n/a | pH range 0-10. |
| PTFE | 1/8″, 1/16″ | 100 | 900 | Excellent resistance to all organic solvents, acids and alkalis. pH range 0-14. |

## High Pressure Tubing

Tubing of 0.007″ i.d. is suitable for minimal dead volume injector-column or column-detector connections of microbore (1.0 – 2.1mm i.d.) columns or short columns containing 3µm material. For material of 2µm particle size, tubing of 0.005″ can also be used. Sample and solvent must be filtered to avoid clogging and blockages of very small bore tubing.

The 0.010″ tubing is suitable for connection of analytical and semi-preparative columns containing 5µm and 10µm packing material. It should be used in all parts of the system where dead volume needs to be kept to a minimum.

The 0.020″ and 0.030″ i.d. tubing are suitable for use in preparative systems or in non-critical connections, eg. pump to sample injector, where low flow resistance is more important than low internal volume.

## Stainless Steel Tubing

- Precision bore 316 stainless steel
- Passivated for chemical resistance
- Square, burr-free ends
- Continuous coils or precut lengths

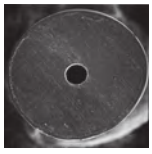
Precision bore 1/16″ o.d. 316 stainless steel tubing is commonly used to connect HPLC components.

Stainless steel tubing can be supplied in continuous coils up to 25 metres in length or as precut lengths. Precut lengths have the advantage of precision finish and cut, which is especially important where low dead volume connections are required.

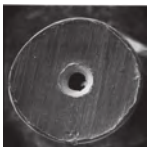
## Ordering Information[1]

### Stainless steel tubing (1/16″ o.d.)

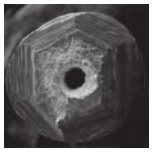
| Tubing i.d. | | Catalogue No. |
|---|---|---|
| Inches | mm | |
| 0.007 | 0.17 | 01-SS-07 |
| 0.010 | 0.25 | 01-SS-10 |
| 0.020 | 0.50 | 01-SS-20 |
| 0.030 | 0.75 | 01-SS-30 |
| 0.040 | 1.00 | 01-SS-40 |



Precut tubing



Tubing cut by a commercially available tubing cutter



File cut tubing

### Precut stainless steel tubing (1/16″ o.d.)

| Tubing i.d. | | Length (cm) | Quantity (/pk) | Catalogue No. |
|---|---|---|---|---|
| Inches | mm | | | |
| 0.005 | 0.125 | 5 | 5 | 05-SS-05 |
| 0.005 | 0.125 | 10 | 5 | 10-SS-05 |
| 0.005 | 0.125 | 20 | 5 | 20-SS-05 |
| 0.007 | 0.17 | 5 | 5 | 05-SS-07 |
| 0.007 | 0.17 | 10 | 5 | 10-SS-07 |
| 0.007 | 0.17 | 20 | 5 | 20-SS-07 |
| 0.010 | 0.25 | 5 | 5 | 05-SS-10 |
| 0.010 | 0.25 | 10 | 5 | 10-SS-10 |
| 0.010 | 0.25 | 20 | 5 | 20-SS-10 |
| 0.020 | 0.50 | 25 | 2 | 25-SS-20 |
| 0.020 | 0.50 | 50 | 1 | 50-SS-20 |

[1] Other dimensions available on request

## Tubing (continued)

### PEEK™ Tubing

- Biocompatible, inert and easily cut
- Good for high pressure applications

PEEK™ (**p**oly**e**ther**e**ther**k**etone) polymer tubing is biocompatible and chemically inert to most solvents. It is completely flexible, can easily be cut to desired lengths and is suitable for a wide range of applications. PEEK tubing can be used with stainless steel or polymer fittings. It has a very smooth internal surface and is the least permeable to gas of all the polymer tubing materials. 1/16″ o.d. tubing (commonly 0.010″ i.d.) is generally suitable for use throughout an analytical HPLC system. Alternative o.d. tubing, eg. 1/8″ for high pressure semi-preparative systems and 360µm for capillary systems, is also available.

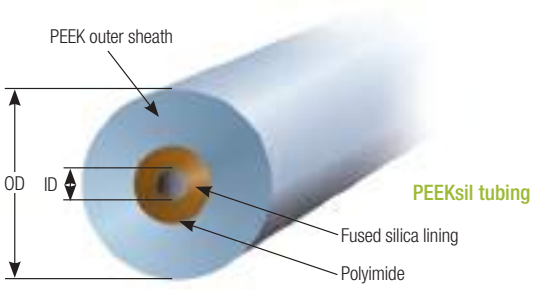
### Ordering Information – PEEK Tubing

| Tubing i.d. (1/16″ o.d.) | | Catalogue No. |
|---|---|---|
| Inches | mm | |
| 0.005 | 0.13 | 16-PEEK-05 |
| 0.007 | 0.17 | 16-PEEK-07 |
| 0.010 | 0.25 | 16-PEEK-10 |
| 0.020 | 0.50 | 16-PEEK-20 |
| 0.030 | 0.75 | 16-PEEK-30 |

### PEEKsil™ Tubing

- PEEK covered fused silica
- Withstands high pressures
- Inert smooth flow path, to minimise band broadening

PEEKsil™ tubing maintains the ease of use of PEEK tubing, but offers tight i.d. tolerances. It has excellent chemical compatibility and is square-cut and polished, to make perfect connections. It is ideal for plumbing capillary and micro LC systems where conventional 1/16″ or 1/32″ fittings (metal or polymer) are used. It is advisable to purchase precut lengths. Please contact Hichrom for further details and ordering information.




### Fused Silica Tubing

Fused silica tubing is manufactured from synthetic fused silica with a polyimide coating. It is used for micro- and nano-scale HPLC and capillary electrophoresis, with the most common o.d. being 360µm. Please contact Hichrom for further details and ordering information.

### Low Pressure PTFE Tubing

- Economical
- Flexible
- Excellent solvent resistance

PTFE (**p**oly**t**etra**f**luoro**e**thylene) is widely used in all low pressure laboratory applications. It is the most common tubing used to connect the solvent reservoir to the pump. It is inert to all chemicals used in HPLC. PTFE is relatively porous and low molecular weight compounds can diffuse through the tubing wall.

### Ordering Information – Low Pressure PTFE Tubing

| Tubing i.d. | | Tubing o.d. (Inches) | Catalogue No. |
|---|---|---|---|
| Inches | mm | | |
| 0.020 | 0.50 | 1/16 | 16-PTFE-20 |
| 0.030 | 0.75 | 1/16 | 16-PTFE-30 |
| 0.062 | 1.58 | 1/8 | 08-PTFE-62 |

### Tubing Cutters

#### Stainless Steel Tubing Cutter

- Ideal for cutting 1/16″ and 1/8″ stainless steel tubing
- Smooth, uniform cuts

HI-192

#### Polymer Tubing Cutter

- Standard cutter for 1/16″ and 1/8″ o.d. tubing
- Compatible with PEEK and PTFE (Teflon) tubing
- Flat burr-free cuts
- Guide holes to ensure precise cutting
- Includes 5 replacement blades

HI-191

### Ordering Information – Tubing Cutters

| Description | Catalogue No. |
|---|---|
| Stainless steel tubing cutter | HI-192 |
| Replacement cutter wheel for HI-192 | HI-193 |
| Standard polymer tubing cutter for 1/16″ and 1/8″ o.d. tubing | HI-191 |
| Replacement blades for HI-191, 5/pk | HI-189 |

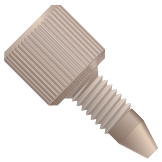
# FITTINGS AND CONNECTORS



## PEEK™ Fingertight Fittings

### One-Piece Fittings

- Universal fitting, suitable for most HPLC connections
- Fingertight to 5,000psi
- Inert and biocompatible
- Excellent chemical resistance
- Void free connection
- Usable with stainless steel and PEEK tubing
- Compatible with all column end fitting types


HI-050


HI-048


HI-031

The following one-piece PEEK™ fingertight fittings are universal fittings for use with all HPLC columns. They are convenient, easy to use and biocompatible. HI-050 is probably the most commonly used fitting in HPLC. They can be used at temperatures up to 150°C. Unlike stainless steel fittings, PEEK fingertight fittings do not permanently lock into place on the tubing, avoiding potential dead volume connection problems. They have 10-32 threads and are designed for use with all 1/16″ o.d. tubing.

| Description | Qty/pack | Cat. No. |
|---|---|---|
| PEEK fingertight fitting, natural, 10-32, for 1/16″ o.d. tubing | 10 | HI-050X |
| PEEK fingertight fitting, natural, 10-32, short, for 1/16″ o.d. tubing | 10 | HI-048 |
| PEEK fingertight fitting, natural, 10-32, long, for 1/16″ o.d. tubing | 10 | HI-031 |

### Two-Piece Fittings

Two-piece fingertight fittings are designed for use with 1/16″ tubing and come complete with ferrule. They are pressure rated to 6,000psi. Instead of the whole fitting, just the ferrule can be replaced, making these fingertights more economical than the one-piece versions.


HI-505

| Description | Qty/pack | Cat. No. |
|---|---|---|
| Double-winged PEEK nuts, natural, with HI-508 ferrules, 10-32 | 10 | HI-505 |
| Replacement PEEK ferrules, for 1/16″ o.d. tubing | 10 | HI-508 |

## PEEK Fingertight Column Coupler

- Universal connector
- Ultra low dead volume
- Inert and biocompatible
- Withstands pressure up to 6,000psi
- Female-female connector

PEEK one-piece fingertight column couplers are ideal for connecting two analytical columns or analytical or semi-preparative columns to guard cartridges.


HI-081

| Description | Cat. No. |
|---|---|
| PEEK fingertight column coupler | HI-081 |

## Column End Plugs

- Extend column lifetime
- Fingertight design
- Fits any column

Column end plugs are used to prevent solvent evaporation from the column bed when the columns are not in use. Before storage, simply attach a column end plug to each end of the column.They are made from a chemically inert polymer and are of fingertight design.


HI-001

| Description | Qty/pack | Cat. No. |
|---|---|---|
| Column end plugs | 10 | HI-001 |

## High Temperature and Pressure Fittings

For high temperature applications, **p**oly**k**etone (PK) fingertights are recommended. Polyketone exhibits excellent biocompatibility and chemical resistance comparable to PEEK. These fingertights are able to withstand pressures up to 12,000psi at room temperature or 6,000psi at 200°C.


HI-035

| Description | Qty/pack | Cat. No. |
|---|---|---|
| PK fingertight fitting 10-32 for 1/16″ o.d. tubing | 10 | HI-035 |

## Fittings and Connectors (continued)

### SLIPFREE™ Connectors

- Void-free and leak-free connections
- Fingertight to 10,000psi
- Compatible with all commercially available end fittings
- For fast, simple multiple connections and reconnections


SLIPFREE™ HPLC connectors offer a simple, rugged way of ensuring good column connections. They are universal for all HPLC columns and consist of a precision-cut length of 1/16″ (0.010″ i.d.) stainless steel tubing, a Vespel ferrule and a stainless steel compression nut/locking cap assembly. Versions with 0.005″ i.d. and 0.020″ i.d. are also available for microbore and preparative analysis. Please enquire for details.

Single connectors are used where one connection is made frequently, such as between injector and guard or analytical column, or column to detector. Double connectors are used where both connections are made frequently, such as between analytical and guard column, or column and filter. Flexible SLIPFREE connectors are designed to bend in the middle, allowing easy connections to be made at any angle.

| Description | Catalogue No. | Description | Catalogue No. |
|---|---|---|---|
| Single 6cm | HI-090 | Flexible single 15cm | HI-039 |
| Single 10cm | HI-091 | Flexible single 28cm | HI-040 |
| Single 20cm | HI-092 | Flexible single 40cm | HI-041 |
| Double 6cm | HI-095 | Flexible double 15cm | HI-042 |
| Double 10cm | HI-096 | Flexible double 28cm | HI-043 |
| Double 20cm | HI-097 | Flexible double 40cm | HI-044 |

### Viper™ Fingertight Fittings

- Zero dead volume fingertight connectors for HPLC and UHPLC
- Fingertight up to 1200 bar (17,400psi)
- Compatible with almost all valves and columns
- Suitable for temperatures up to 120°C

The Viper™ stainless steel fingertight fitting system provides zero dead volume by sealing at the tip of the tubing, without the need for ferrules. The system consists of two stainless steel fingertights combined with a flexible stainless steel capillary, which is available in different lengths from 150 to 950mm and in 0.13mm and 0.18mm i.d. Robust connections are guaranteed even under ultra-high pressures. Viper fittings are easy to use and reconnect easily between different column hardware.

| Length (mm) | 0.13mm i.d. | 0.18mm i.d. | Length (mm) | 0.13mm i.d. | 0.18mm i.d. |
|---|---|---|---|---|---|
| | Cat No. | Cat. No. | | Cat. No. | Cat. No. |
| 65 | 6040.2307 | 6040.2357 | 550 | 6040.2305 | 6040.2355 |
| 150 | 6040.2315 | 6040.2360 | 650 | 6040.2310 | 6040.2395 |
| 250 | 6040.2325 | 6040.2385 | 750 | 6040.2320 | 6040.2370 |
| 350 | 6040.2335 | 6040.2375 | 850 | 6040.2330 | 6040.2380 |
| 450 | 6040.2345 | 6040.2365 | 950 | 6040.2340 | 6040.2390 |

### Hichrom UHPLC Column Connectors

- Compatible with all UHPLC systems and column brands
- Avoids poor connections
- Pressure rated to >1700 bar (>25,000psi)
- Reusable

Hichrom reusable UHPLC column connectors (HI-903) have 10-32 threads suitable for use with 1/16″ o.d. tubing. These fittings are reusable, as they are not permanently swaged onto the inlet tubing. An internal ferrule design prevents tubing slippage, whilst the PEEK-polymer blend front ferrule eliminates the risk of damage to receiving ports.

| Description | Qty/pack | Cat. No. |
|---|---|---|
| UHPLC connector 10-32 thread for 1/16″ o.d. tubing | 1 | HI-903 |
| | 10 | HI-903X |

## Fittings and Connectors (continued)

### PEEK™ Unions

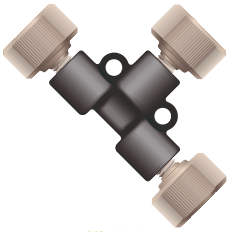
- Use to connect 2 pieces of 1/16″ o.d. tubing
- Chemically inert and biocompatible
- Zero dead volume connection
- Pressure rated to 5,000psi
- Complete with two HI-505 fingertight fittings

HI-059

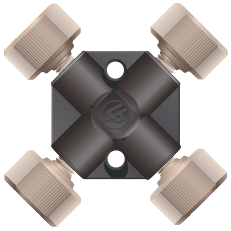
| Description | Thru-hole | Swept Volume | Cat. No. |
|---|---|---|---|
| PEEK ZDV union, 10-32 | 0.010″ | 0.07µl | HI-059 |
| PEEK ZDV union, 10-32 | 0.020″ | 0.28µl | HI-055 |

### PEEK Tees and Crosses

- Use with 1/16″ o.d. PEEK™ or stainless steel tubing
- Maximum operating pressure 3,500psi (241 bar)
- Includes 3 or 4 HI-505 fingertight fittings



HI-032



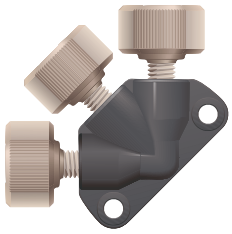
HI-034

| Description | Thru-hole | Swept Volume | Cat. No. |
|---|---|---|---|
| PEEK tee, 10-32 | 0.020″ | 0.57µl | HI-032 |
| PEEK cross, 10-32 | 0.020″ | 0.72µl | HI-034 |

### Static Mixing Tee

- PEEK body with 2-piece fingertight fittings
- Low swept volume
- Pressure rated to 5,000psi (345 bar)

The static mixing tee HI-454 is ideal for microbore or analytical gradient HPLC. It has a low swept volume (2.2µl) and is designed for flow rates of 0.5 to 3ml/min. and a maximum pressure of 5,000psi (345 bar).



HI-454

| Description | Thru-hole | Swept Volume | Cat. No. |
|---|---|---|---|
| PEEK static mixing tee for 1/16″ tubing | 0.020″ | 2.2µl | HI-454 |

### Stainless Steel Nuts and Ferrules

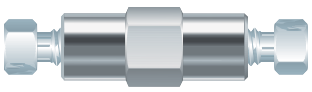
Now largely replaced by PEEK fingertight fittings, except for the most searching applications, these precision machined stainless steel nuts and ferrules (threads 10-32) provide an excellent connection when needed, and are guaranteed to be both burr and contaminant free.

| Description | Cat. No. |
|---|---|
| Male nut 1/16″ | HI-500 |
| Ferrule 1/16″ | HI-502 |

HI-500

HI-502

HI-070



HI-752

### Stainless Steel Connectors

The connector HI-070 consists of a 4cm length of 1/16″ o.d. x 0.010″ i.d. stainless steel tubing with appropriate 1/16″ nuts and ferrules and can be used to connect female endfitting Hichrom guard and analytical columns and make similar connections. The ZDV male-male connectors (unions) have internal threads and provide zero dead volume connections. These ZDV unions are rated to 20,000psi.

| Description | Cat. No. |
|---|---|
| SS 1/16″ female-female connector, 0.010″ | HI-070 |
| SS ZDV union for 1/16″ tubing, 0.010″ thru-hole, male-male | HI-752 |

## Fittings and Connectors (continued)

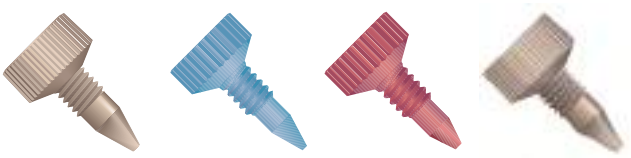
# Microflow Fittings

## MicroTight® PEEK™ Fittings

- For connecting capillary tubing (360µm or 1/32″ o.d. or MicroTight sleeves)
- Pressure rated to 4,000psi (276 bar)
- Temperature stable up to 125°C

MicroTight® 1-piece fingertight PEEK™ fittings, with 6-32 threads, are used to connect capillary tubing to MicroTight unions, adapters or filters.

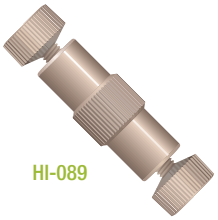
| Description | Qty/pack | Cat. No. |
|---|---|---|
| Standard fitting for MicroTight tubing sleeve | 10 | HI-362 |
| Standard fitting for 360µm o.d. tubing | 10 | HI-365 |
| Standard fitting for 1/32″ o.d. tubing | 10 | HI-799 |
| Fitting plug | 1 | HI-804 |



HI-362 HI-365 HI-799 HI-804

## MicroTight PEEK Connector

- Use to connect 2 pieces of capillary tubing, using tubing sleeves
- True ZDV union
- Includes fittings and gauge plug
- Pressure rated to 4,000psi

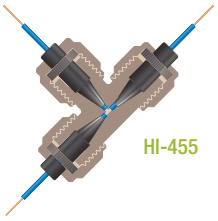


HI-089

| Description | Thru-hole | Swept Volume | Cat. No. |
|---|---|---|---|
| PEEK true ZDV union for MicroTight sleeves | N/A | N/A | HI-089 |
| MicroTee for MicroTight sleeves (fittings included) | 0.006″ | 29nl | HI-455 |
| MicroCross for MicroTight sleeves (fittings included) | 0.006″ | 38nl | HI-456 |

## PEEK MicroTee and MicroCross

- Use to connect 1/16″, 1/32″ and 360µm o.d. tubing
- Low swept volume
- Use tubing sleeves to connect different o.d. tubing



HI-455

## MicroTight PEEK Tubing Sleeves

- Colour-coded for easy i.d. identification
- Pressure rated to 4,000psi (276 bar)
- Temperature stable up to 125°C

PEEK tubing sleeves are used with MicroTight fittings to connect capillary tubing. They have an o.d. of 0.025″ with a range of i.d.s suitable for use with different ports. The i.d. of the sleeve should be 0.001″- 0.002″ larger than the o.d. of the capillary tubing.

| Sleeve i.d.[1] | For Tubing o.d. | Colour | Qty/pk | Cat. No. |
|---|---|---|---|---|
| 125µm | 70-110µm | Red | 10 | HI-132 |
| 180µm | 125-165µm | Yellow | 10 | HI-172 |
| 230µm | 175-215µm | Natural | 10 | HI-181 |
| 395µm | 340-380µm | Green | 10 | HI-171 |

[1] Other i.d. sleeves available – please enquire

## NanoTight Fittings and Tubing Sleeves

- For connecting 1/16″ o.d. or capillary tubing using tubing sleeves to standard 10-32 coned ports
- Pressure rated to 4,000psi (276 bar)

NanoTight fittings and sleeves are designed to connect 70µm – 1mm o.d. capillary tubing to any standard 10-32 coned port using NanoTight tubing sleeves. These fittings are made from PEEK, with the ferrule manufactured from ETFE. NanoTight tubing sleeves are manufactured from FEP fluoropolymer and have a maximum recommended operating temperature of 50°C.

Please contact Hichrom for ordering information for NanoTight fittings and sleeves.

# FRITS

## Stainless Steel

All column frits have a carefully controlled porosity and are fabricated from 316 grade stainless steel. A 0.5µm porosity frit is recommended for 3-4µm packing materials. The use of 2µm porosity frits is recommended for retention of 5-20µm packing material. PEEK encapsulated stainless steel frits may be used to prevent sample and eluent from entering dead volume space between the end fitting and the top of the column. This results in improved peak symmetry with 1.0 – 3.2mm i.d. columns.

## Bioanalytical Applications

Titanium or PAT (PEEK alloyed with Teflon) frits are preferred for the analysis of sensitive biomolecules, which may adsorb or decompose on stainless steel frits. Please enquire for further details.


## Ordering Information

| Frit Material | Column i.d. (mm) | Column Diameter (inches) | Frit Thickness (inches) | Porosity (µm) | Catalogue No.[1] |
|---|---|---|---|---|---|
| Stainless steel (PEEK encapsulated) | 1.0 | 1/4 | 0.030 | 0.5 | HI-127 |
| | | | | 2.0 | HI-108 |
| Stainless steel (PEEK encapsulated) | 2.1 | 1/4 | 0.062 | 0.5 | HI-103 |
| | | | | 2.0 | HI-100 |
| Stainless steel (PEEK encapsulated) | 3.2 | 1/4 | 0.062 | 0.5 | HI-104 |
| | | | | 2.0 | HI-145 |
| Stainless steel | 4.0 – 4.6 | 1/4 | 0.031 | 0.5 | HI-105 |
| | | | | 2.0 | HI-120 |
| Stainless steel | 7.75 | 3/8 | 0.040 | 2.0 | HI-126 |
| Stainless steel | 10.0 | 1/2 | 0.040 | 2.0 | HI-133 |
| Stainless steel | 20.0 – 21.2 | 1 | 0.062 | 2.0 | HI-141 |

[1] 10/pk

For details of frits not shown above, please contact Hichrom.

# SPANNERS

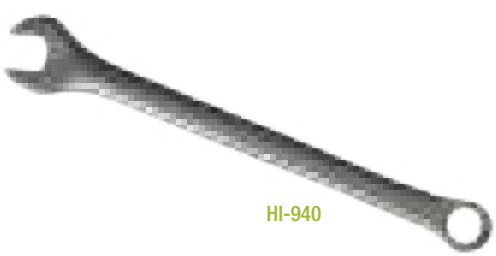
The following spanners can be supplied:

## Ordering Information

| Description | Catalogue No. |
|---|---|
| Spanner ½″ x 9/16″ AF | HI-226 |
| Spanner ¼″ x 5/16″ AF | HI-225 |
| Combination spanner 3/8″ | HI-940 |
| ValvTool | HI-199 |


## ValvTool

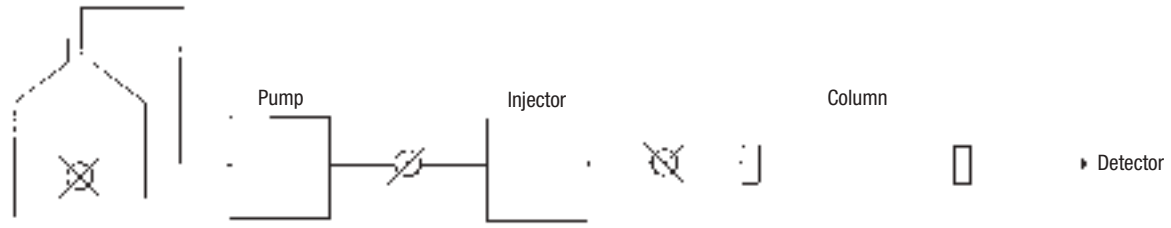
The ValvTool (HI-199) is a uniquely designed slotted wrench which provides easy access to many hard-to-reach areas. This enables fittings, sample loops and most other ¼″ stainless steel and PEEK HPLC fittings to be more easily tightened or loosened.

# FILTERS



The use of filters is strongly recommended for complete protection of different components within an HPLC or UHPLC system. As shown in the figure below, three types of filter are used.




A. Inlet Filter  B. Inline Filter  C. Precolumn Filter

## A. Inlet Filters

- Protect pump check valves
- Help remove dissolved gases

Solvent inlet filters are used at the low pressure inlet side of the pump to help protect check valves, injector and column from particulate contamination from the eluent. It is recommended that inlet filters are replaced at least every six months.

### 1) Stainless Steel Inlet Filter

Made from 10µm porosity stainless steel, these filters are available for connection to either ⅛″ or 1/16″ i.d. tubing. Their large surface area filters out particulate matter from the solvent that may otherwise damage expensive hardware. No tools are required for replacement.

| Description | Catalogue No. |
|---|---|
| 10µm Inlet filter with stem for 1/16″ i.d. tubing | HI-583 |
| 10µm Inlet filter with stem for ⅛″ i.d. tubing | HI-584 |


### 2) Bottom-of-the-Bottle™ Solvent Filters

These biocompatible filters are made from 100% PEEK, including two built-in PEEK frits. The bottom frit (2 or 10µm) will draw solvents from within 2mm of the bottom of the solvent bottle. The 2µm frit on the side may be used for a ⅛″ o.d. helium sparging line.

| Description | Catalogue No. |
|---|---|
| 2µm PEEK filter for ⅛″ o.d. tubing | HI-579 |
| 10µm PEEK filter for ⅛″ o.d. tubing | HI-580 |

### 3) Last Drop™ Inlet Filter

The Last Drop™ eluent filter utilises a flat filter element which sits parallel to the bottom of the reservoir. This design enables the filter to draw all but the last 2% of the eluent from the reservoir without drawing air into the system. The Last Drop filter allows more analyses per batch of eluent and helps reduce waste compared to conventional cylindrical eluent filters. The filter contains a 316 stainless steel or PTFE filter element in an inert Teflon housing. The metal-free version should be used for sensitive biochromatography applications. A tubing connector is supplied with every Last Drop eluent filter.

| Description | Catalogue No. |
|---|---|
| Last Drop Filter (2µm SS filter) | HI-692 |
| Last Drop Filter (10µm SS filter) | HI-690 |
| Last Drop Filter (2.5µm PTFE filter) | HI-695 |
| Last Drop Filter (5µm PTFE filter) | HI-696 |
| Last Drop Filter (10µm PTFE filter) | HI-694 |

## B. Inline Filters

- Protect injectors from particulates
- Compatible with all HPLC systems

Inline filters are placed between the pump and sample injection valve to trap particles released through normal piston seal wear within the pump. Hichrom offers stainless steel and biocompatible inline filters to suit various applications. It is recommended that the frits are checked frequently and replaced as soon as they contribute to a system back pressure.

### 1) Analytical Inline Filters

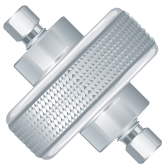
Model HI-702 comprises a stainless steel holder and a 2µm PEEK encapsulated stainless steel frit (supplied). Replacement frits (HI-100) are readily available. The HI-683 is a fingertight bioanalytical alternative, comprising a PEEK holder and a 5µm titanium PEEK-encased frit. Replacement 5µm and 2µm titanium PEEK-encased frits are available.


HI-702


HI-683

### 2) Preparative Inline Filter

The preparative inline filter HI-610 is suitable for use with 10-30mm i.d. HPLC and SFC columns. The filter protects the column and helps maintain performance by removing particulate matter and insoluble material from eluent and sample matrix. This versatile filter can also protect check valves, injectors and detectors.


HI-610

## C. Precolumn Filters

- Protect columns from particulates
- Compatible with all HPLC columns
- Ultra low dead volume

Placed immediately before the column, precolumn filters trap sample particulates. However, for samples that may irreversibly adsorb onto the column, guard cartridges are preferred, or additionally recommended.

### 1) ColumnSaver™ HPLC Precolumn Filter

The ColumnSaver™ fingertight precolumn filter is universally compatible with all manufacturers' endfittings. Both 2µm and 0.5µm versions are available, for the protection of columns containing packings of 5µm and 3µm respectively. The ColumnSaver precolumn filter is leakproof to over 6,000psi.


### 2) Hichrom UHPLC Precolumn Filter

Hichrom UHPLC Precolumn Filters are engineered specifically for use with fast, high efficiency UHPLC columns. The low dispersion of these filters ensures that the efficiency of the UHPLC column is maintained, assuring no loss of critical resolution. These filters can be installed simply on any analytical UHPLC or UPLC® column in seconds, providing leak-free filter protection to 1000 bar (15,000psi).


HI-602

### 3) Stand-alone Precolumn Filter

Model HI-704 comprises a 'stand-alone' holder with a 2µm PEEK encapsulated stainless steel frit. Replacement 2µm and 0.5µm frits are available, providing protection for 5µm and 3µm columns respectively. The use of a fingertight coupler (HI-081, see p. 188) is recommended to connect the holder to the column inlet.


HI-704

### Ordering Information – Inline Filters

| Description | Cat. No. |
|---|---|
| 2µm Stainless steel inline filter assembly | HI-702 |
| 2µm Replacement frits for HI-702 (10/pk) | HI-100 |
| Fingertight Ti PEEK encased inline filter (complete with 5µm frit) | HI-683 |
| Replacement Ti PEEK encased frits (5µm) (5/pk) | HI-684 |
| Replacement Ti PEEK encased frits (2µm) (5/pk) | HI-674 |
| Preparative inline filter | HI-610 |
| 2µm frits for preparative inline filter (10/pk) | HI-611 |

### Ordering Information – Precolumn Filters

| Description | Cat No. |
|---|---|
| 2µm ColumnSaver (10/pk) for 5µm columns | HI-685 |
| 0.5µm ColumnSaver (10/pk) for 3µm columns | HI-686 |
| 0.5µm Precolumn filter for UHPLC columns (1/pk) | HI-602 |
| 0.5µm Precolumn filter for UHPLC columns (10/pk) | HI-602X |
| Precolumn filter holder with 2µm frit | HI-704 |
| Replacement 2µm frits (10/pk) | HI-101 |
| Replacement 0.5µm frits (10/pk) | HI-102 |

# RHEODYNE®



- Automated and manual injectors and switching valves
- Fittings and accessories

Rheodyne® valves fit virtually any flow control application. There are valves for preparative, analytical, nano and microscale analyses in a variety of flow configurations and flow rating ranges.

## MX Series II™ Automated Switching Valves

- Increase laboratory productivity
- Increase reproducibility
- Flexible automation
- Increase equipment reliability

Rheodyne MX Series II™ automated fluidic valves provide productivity enhancing solutions for today's demanding analytical methods. These modular valves can be combined with current instrumentation to support complex fluid switching and sample injection needs.


### Flexible Automation

The MX Series II modular valves are flexible, with several options available for connecting the valves to an analytical instrument or PC, including contact closure, BCD, serial and USB. Commands can be sent to the MX Series II valves using your chromatography software or TitanMX™ software (included) for timed-events programmability. MX Series II valves can be controlled remotely or operated manually using the push-button front panel with LED position indicator.

### Reduce Downtime

The high pressure and fast chromatography MX Series II modular valves feature the Rapid Replacement Pod™ design for easy maintenance. The Rapid Replacement Pod is a complete factory assembled and tested liquid-end, providing virtually zero downtime maintenance. Traditional RheBuild® kits (see page 344) to service your MX Series II valve are also available.

The low pressure MX Series II modular valves make changing fluidic connections quick and easy with their patented TitanEX™ fittingless tubing connection system. These long-life polymer valves meet the reliability needs of demanding applications.

### Typical Applications of MX Series II Valves

1. Sample injection
2. Two column selection
3. Alternating column regeneration
4. High speed sample enrichment and clean-up
5. Sample clean-up/sample enrichment
6. Column backflushing
7. Multi-dimensional proteomic peptide separation
8. Solvent selection
9. Fraction collection
10. Six column selection

### Example of MX Series II Valve Application

Figure 1 shows a schematic diagram of multi-dimensional proteomic peptide separation using a 2-position 10-port switching valve. Salt fractions eluted from the SCX column are trapped on a RP trap column. Switching the valve from Position A to Position B elutes the peptides from the trap column for further resolution on the nano RP column and detection by MS. In Position A, eluent from the SCX column traps on the RP trap column 1. While nano RP column 1 analyses the sample, RP trap column 2 traps the next salt fraction. In Position B, the SCX column is in-line with RP trap column 2, while RP trap column 1 traps the next salt fraction. Please contact Hichrom for full details of other switching applications of MX Series II valves.

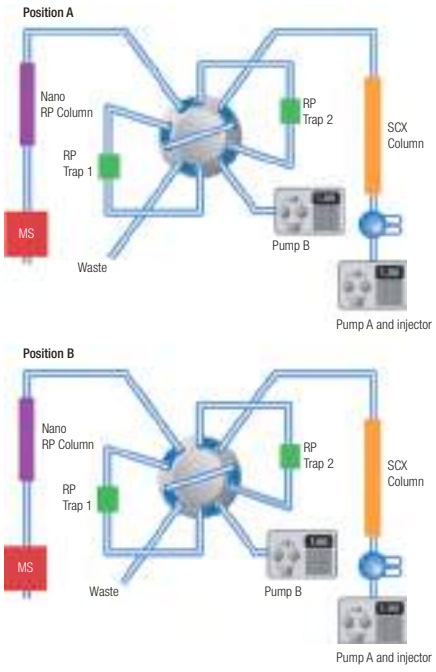



Figure 1. Multi-dimensional proteomic peptide separation using a 2-position 10-port switching valve

### Increase Laboratory Capability

The MX Series II modules are available in a variety of flow paths including options for nano, analytical and semi-preparative, as well as low pressure and fast chromatography applications and UHPLC conditions up to 15,000psi (1034 bar) - see page 196. These modules feature the reliable automation of Titan valves, saving valuable resource time and increasing overall productivity.

## Ordering Information – MX Series II™ Automated Switching Valves

### MXT for Fast Ultra High Pressure Chromatography Applications, up to 15,000 psi (1034 bar)

| Description | Stator Passages | Typical Applications | Part Number |
|---|---|---|---|
| 2-Position 6-port switching valve Stainless steel UltraLife[1] | 0.011″ (0.28mm) | Sample injection<br>2 column selection<br>Sample clean-up/sample enrichment<br>Column backflushing | MXT715-000 |
| 2-Position 10-port switching valve Stainless steel UltraLife[1] | 0.011″ (0.28mm) | 2 column selection<br>High speed sample enrichment and clean-up | MXT715-102 |
| 6-Position 7-port selector valve Stainless steel UltraLife[1] | 0.011″ (0.28mm) | Solvent selection<br>Fraction collection<br>6 column selection | MXT715-105 |

### MXP for High Pressure Applications, up to 6,000 psi (410 bar)

| Description | Stator Passages | Typical Applications | Part Number |
|---|---|---|---|
| 2-Position 6-port switching valve Stainless steel DuraLife[1] | 0.012″ (0.30mm) | Sample injection<br>2 column selection<br>Sample clean-up/sample enrichment<br>Column backflushing | MXP7900-000 |
| 2-Position 6-port vertical port valve Stainless steel DuraLife[1] | 0.012″ (0.30mm) | Sample injection | MXP7920-000 |
| 2-Position 6-port switching valve PEEK | 0.012″ (0.30mm) | Sample injection<br>2 column selection<br>Sample clean-up/sample enrichment<br>Column backflushing | MXP9900-000 |
| 2-Position 10-port switching valve Stainless steel DuraLife[1] | 0.010″ (0.25mm) | 2 column selection<br>Alternating column regeneration<br>High speed sample enrichment and clean-up<br>Multi-dimensional proteomic peptide separation | MXP7960-000 |
| 6-Position 7-port selection valve Titanium DuraLife II[1] | 0.012″ (0.30mm) | Solvent selection<br>Fraction collection<br>6 column selection | MXP7970-000 |
| 2-Position 10-port switching valve PEEK | 0.010″ (0.25mm) | 2 column selection<br>Alternating column regeneration<br>High speed sample enrichment and clean-up<br>Multi-dimensional proteomic peptide separation | MXP9960-000 |
| 2-Position 6-port nano switching valve Titanium DuraLife II[1] | 0.004″ (0.10mm) | 2 column selection<br>Sample clean-up/sample enrichment<br>Column backflushing | MXP7980-000 |
| 2-Position 10-port nano switching valve Titanium DuraLife II[1] | 0.004″ (0.10mm) | Alternating column regeneration<br>High speed sample enrichment and clean-up<br>Multi-dimensional proteomic peptide separation | MXP7986-000 |

### MXX for Low Pressure Applications, up to 125 psi (9 bar)

| Description | Stator Passages | Typical Applications | Part Number |
|---|---|---|---|
| 2-Position 6-port switching valve RPC-7[2] | 0.016″ (0.41mm) | Sample injection<br>Column selection | MXX777-601 |
| 2-Position double three-way valve RPC-7[2] | 0.016″ (0.41mm) | Detector selection<br>Waste diversion | MXX777-603 |
| 6-Position 7-port selection valve RPC-7[2] | 0.040″ (1.0mm) | Solvent selection<br>Fraction collection | MXX777-605 |
| 2-Position 6-port switching valve RPC-7[2] | 0.060″ (1.5mm) | Sample injection<br>Column selection | MXX777-612 |
| 6-Position 7-port selection valve RPC-7[2] | 0.060″ (1.5mm) | Solvent selection<br>Fraction collection | MXX777-616 |
| 10-Position 11-port selection valve RPC-7[2] | 0.060″ (1.5mm) | Solvent selection<br>Fraction collection | MXX778-605 |

[1] Proprietary coating materials [2] Proprietary polymer combination

Please enquire for details of Rapid Replacement Pods™.

## Manual Sample Injectors

The Rheodyne® Series-25 models are dual-mode injectors, which can be used in either a partial or complete loop filling method. In the former, a syringe determines the sample volume without wasting sample. In the complete loop filling method, the loop determines sample volume. Single-mode or fixed loop injectors use only the complete filling method.

Analytical models are for use with conventional HPLC columns with samples from 1µl to 5ml. Micro models are for 1mm and 2mm i.d. columns. Preparative models are for columns with diameters from 1cm to 10cm, operated at high flow rates, with samples from 100µl to 20ml. Models with an 'i' suffix designate the inclusion of a built-in position sensing switch to mark the injection start. Table 1 compares the characteristics of Rheodyne sample injectors, to aid selection of the most suitable model.

### Table 1. Characteristics of Rheodyne® Manual Sample Injectors

| Analysis Scale | Partial Filling Volumes (Range) | Sample Loop Sizes (Range) | Liquid-contact Materials | MBB[1] | Model |
|---|---|---|---|---|---|
| **Dual Mode** | | | | | |
| Analytical | 1µl - 2.5ml | 2µl - 5.0ml | 316 SS, Vespel | Yes | 7725, 7725i |
| | 1µl - 5.0ml | 2µl - 10.0ml | PEEK, Tefzel, ceramic | Yes | 9725, 9725i |
| Micro | 0.1µl - 500µl | 5µl - 1.0ml | 316 SS, Vespel, ceramic, PEEK | No | 8125 |
| Preparative | 100µl - 10.0ml | 2.0ml - 20.0ml | 316 SS, PEEK | Yes | 3725-038, 3725i-038 |
| | | | PEEK | Yes | 3725i |
| **Single Mode** | | | | | |
| Analytical | N/A | 5µl - 5.0ml | 316 SS, Vespel | No | 7010[2] |
| | | 5µl - 10.0ml | PEEK, Tefzel, ceramic | No | 9010 |

[1] MBB stands for Make-Before-Break

[2] MX Series II valves are recommended for new instrument installations - see pages 195-196

## Dual Mode Injectors

### Models 7725 and 7725i

- Make-Before-Break design
- Wide port angles for improved access to fittings
- 5µl to 5ml removable sample loops
- 2µl internal sample loop accessory

The stainless steel models 7725 and 7725i (along with the PEEK versions 9725 and 9725i) are Rheodyne's most advanced manual sample injectors for HPLC. They are based on the model 7125 which they have largely superseded. These versatile injectors can use either the partial filling or complete filling method. They can inject from 1µl to 5ml with high accuracy and precision.

The Rheodyne patented Make-Before-Break (MBB) design virtually eliminates pressure transients. Flow switching at a flat interface between a polymeric rotor seal and a ceramic stator face allows over 30,000 injections before rotor seal replacement is necessary in a clean system. Channels in the rotor seal make new connections before the old ones break. As a result flow is not interrupted when the injector is switched between LOAD and INJECT positions (see Figure 2), a benefit when using flow-sensitive detectors, fragile columns or pumps that are disturbed by flow or pressure transients.

A patented needle port design connects the tip of the syringe needle directly to the end of the sample loop, resulting in zero sample loss and no cross contamination.

7725i

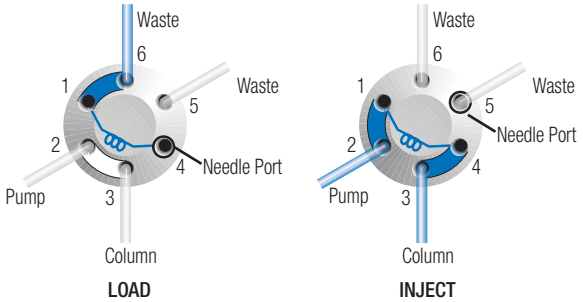



Figure 2. Flow path for typical dual mode injector

## Dual Mode Injectors (continued)

### Models 9725 and 9725i

- Metal-free inert flow passages
- Unaffected by buffers, acids, bases or halide salts
- For HPLC and low pressure soft gel chromatography
- Biocompatible

The Model 9725 is highly inert and well suited to the chromatography of biological molecules, including those with aggressive mobile phases. No metal components come into contact with the mobile phase. The injector is identical to Model 7725 stainless steel injector, but the loop, nuts, ferrules and stator are made of PEEK. The rotor seal polymer is Tefzel, which has an operating pH range of 0 to 14. The stator face is alumina ceramic, which is inert and resistant to wear. To avoid exposing sample to the metal syringe needle, a suction loading technique can be used.

### Model 8125

- Suitable for microbore and analytical HPLC
- Accurately injects as little as 0.1µl of sample
- Improves peak resolution
- Built-in position switch to signal injection

The Model 8125 is designed for use with 1mm and 2mm microbore columns, as well as analytical columns. It can inject sample volumes from 0.1µl to 500µl with zero sample waste. Small diameter flow channels produce low dispersion, maintaining the high mass sensitivity inherent in micro columns. The 8125 has a 5µl sample loop attached, allowing partial loading of up to 2.5µl. To facilitate low-dispersion performance, the 5µl, 10µl, 20µl and 50µl sample loops use 0.020″ o.d. tubing instead of conventional 1/16″ o.d. tubing.

3725-038

### Models 3725i and 3725-038

- For preparative HPLC columns
- Suitable for large sample volumes and high flow rates
- Stainless steel or PEEK design
- Flow rates from 10 to 800ml/min

These injectors are as easy to use as Rheodyne's analytical scale injectors but have the capacity for larger sample volumes and flow rates. The Model 3725i has only non-metal (PEEK) parts in contact with the eluent, and is suitable for applications involving purification of biological molecules. The 3725-038 and 3725i-038 contain both PEEK and stainless steel parts. Models 3725i and 3725i-038 have a built-in position sensing switch.

## Single Mode Injectors

### Models 7010 and 9010

- Simplest type of injection valve
- Complete filling method of loading sample loop

Models 7010 (stainless steel) and 9010 (PEEK) are conventional single-mode 6-port injectors for the complete filling method of loading the sample loop. These are the simplest injectors, ideal for routine analysis. Loops from 5µl to 5ml are available. The loop can be filled by pressure or suction loading. The Models 7012 and 9012 loop filler ports are recommended for use with the 7010 and 9010 respectively. They allow the valves to be directly filled by syringe.

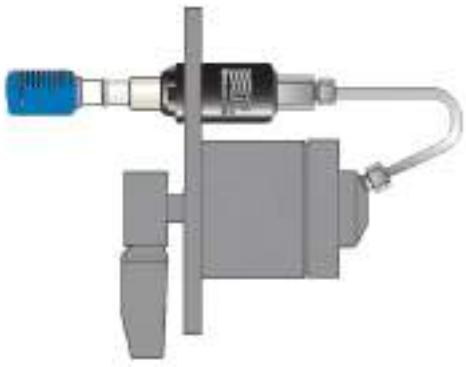


Figure 3. Suggested mounting arrangement of Model 7010 with filler port 7012

## Manual Switching Valves

High pressure switching valves are used to simplify procedures and improve the speed, resolution and sensitivity of HPLC analyses. There are two major areas of application: column selection and column switching.

Column selection valves substitute one column for another without the need to manually disconnect the plumbing. This makes it easy to dedicate a separate column for each analysis. Dedicated columns eliminate equilibration delays, reduce interferences and prolong column life. The columns switched off-line are automatically sealed at both ends.

Column switching valves re-route the eluent flow during the chromatographic run. This can be done with no change in the mode of separation, such as in backflushing and fraction cutting. Alternatively, it can be multi-dimensional when the sample is sequentially separated on two or more columns, usually using different packings or eluents.

## Two Position Switching Valves – Model 7000

The Model 7000 is a stainless steel six-port two-position switching valve, compatible with 1/16″ fittings. Rotating the handle by 60° allows the rotor flow passages to connect different ports. The valve has many column switching uses.

### Applications include:

#### 1. Sample enrichment
**Position A.** Pre-column concentrates components of interest
**Position B.** Pre-column concentrate is flushed by second pump on to analytical column

#### 2. Pre-column backflushing
**Position A.** Flow continues through pre-column and analytical column in normal direction
**Position B.** Only pre-column is backflushed

#### 3. Sample clean-up
**Position A.** Flow through pre-column and analytical column
**Position B.** Pre-column flushed by second pump to remove highly retained unwanted sample components

#### 4. Two column selection
**Position A.** Flow through column 1
**Position B.** Flow through column 2

Models accepting 1/8″ fittings and large bore 'L' versions of these switching valves are available. These are used to avoid excessive pressure drops when using high flow rates. Please contact Hichrom for details.

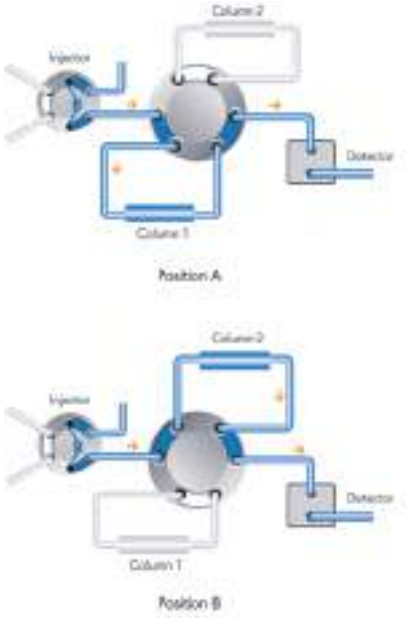



Figure 4. Two column selection using Model 7000 switching valves

## Six-Position Switching Valves - Model 7060

The Model 7060 is a manually operated stainless steel selection valve, accepting 1/16″ fittings. The centre port of this valve connects to the injector. Turning the valve handle directs flow into one of up to six columns connected to the six peripheral ports. A second valve is placed at the column outlets to select the operating column effluent and direct it to the detector, as shown in Figure 5. It is useful for the sixth port to be used for a bypass/flush-out tube.

| Model (Catalogue No.) | Description | Stator Material |
|---|---|---|
| 7000 | 2-position 6-port switching valve | Stainless steel |
| 7030 | 2-position 6-port switching valve, double 3-way | Stainless steel |
| 7040 | 2-position 6-port switching valve, 4-way | Stainless steel |
| 7060 | 6-position 7-port switching valve, 6-way | Stainless steel |

For automated switching valves, please see pages 195-196.

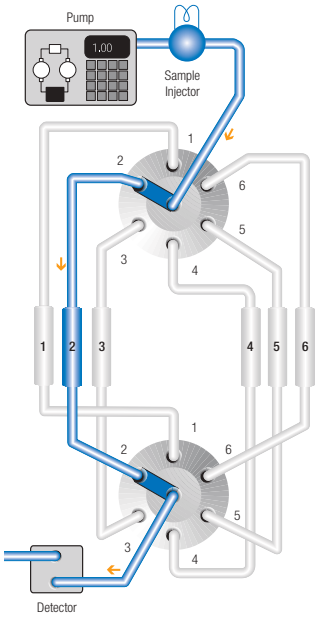



Figure 5. Six column selection using two Model 7060 switching valves

## Replacement Items and Accessories

The exploded diagram (Figure 6) illustrates the components of a Rheodyne® Model 7725 sample injector. Replacement parts and accessories for all major Rheodyne injectors can be supplied.

### Rotor Seals

The rotor seal is the most commonly replaced part. Vespel blend rotor seals have an operating pH range from 0 to 10. Tefzel blend and PEEK blend rotor seals have a pH range from 0 to 14.

### Stators

Stators need replacement only if the ports or sealing surfaces become damaged. They are available in stainless steel and PEEK.

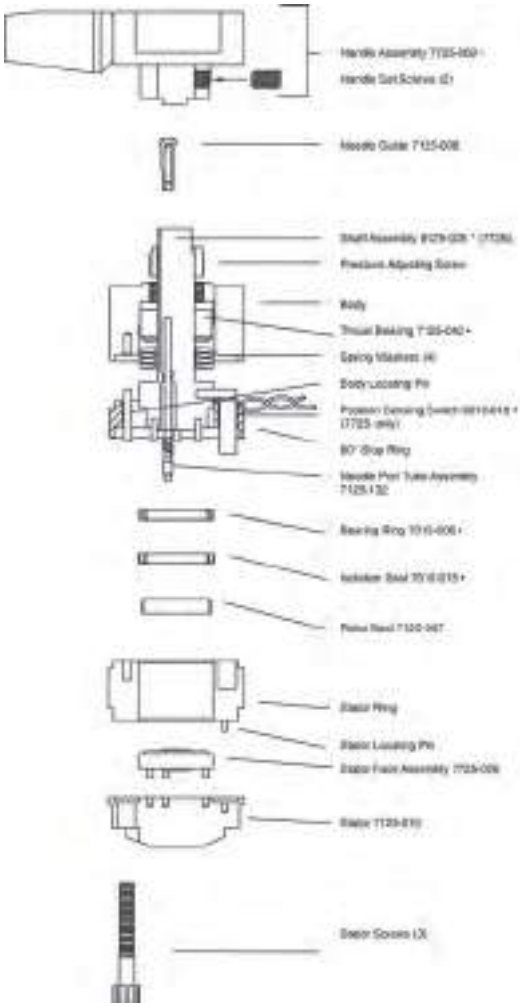


Figure 6. Replacement parts for Model 7725 valve

### Sample Loops

Stainless steel and PEEK sample loops are factory-cut and finished to the highest quality. The stainless steel loop ends have a square cut. They are burr-free for a flush connection to the valve and are supplied with unswaged fittings. The flexible PEEK loop ends are provided with a clean and straight cut for low dead volume connection. A wide range of sample loops from 2µl to 20ml are available.

### RheFlex® PEEK Fittings

RheFlex® tube fittings provide inert, non-metal connections, and can be used with all Rheodyne injectors, valves and sample loops. The nut and ferrule are PEEK and sold as a set. The fitting holds with finger tightening on a variety of plastic and metal tubing.

Further replacement items and accessories are listed on page 201.

# Manual Valves and Accessories

## Ordering Information

| Valve Series<br>Model Description | 7725 | 7125 | 8125 | 9725<br>PEEK | 3725<br>PEEK | 3725-038 | 7010 | 9010<br>PEEK |
|---|---|---|---|---|---|---|---|---|
| Manual | 7725 | - | - | 9725 | - | 3725-038 | 7010 | 9010 |
| Injection Mark Model[1] | 7725i | - | 8125 | 9725i | 3725i | 3725i-038 | - | - |
| **Replacement Parts[4]** | | | | | | | | |
| Vespel Rotor Seal | 7125-047 | 7125-047 | 8125-038 | - | - | - | 7010-039 | - |
| Tefzel Rotor Seal | 7125-079 | 7125-079 | 8125-097 | 9125-082 | - | - | 7010-071 | 9010-051 |
| PEEK Rotor Seal | - | - | - | - | 3725-018 | 3725-018 | - | - |
| Stator | 7725-010 | 7010-040 | 8125-098 | 9125-043 | 3725-006 | 3725-085 | 7010-040 | 9125-043 |
| Stator Face Assembly | 7725-026 | 7125-067 | 8125-074 | 7725-026 | 3725-039 | 3725-039 | - | - |
| Nut (Bushing) + Ferrule (10/pk) | 6000-209 | 6000-209 | 6000-209 | 6000-055[2] | - | - | 6000-209 | 6000-055[2] |
| Long Nut + Ferrule (10/pk) | 6000-211 | 6000-211 | 6000-211 | 6000-054[2] | - | - | 6000-211 | 6000-054[2] |
| Extra Long Nut + Ferrule (10/pk) | 6000-262 | 6000-262 | 6000-262 | 6000-066[3] | - | - | 6000-262 | 6000-066[3] |
| Ferrule (10/pk) | 6000-210 | 6000-210 | 8125-084[3] | 6000-051[2] | - | - | 6000-210 | 6000-051[2] |
| Isolation Seal | 7010-015 | 7010-015 | 7010-015 | 7010-015 | - | - | 7010-015 | 7010-015 |
| Stator Screws (10/pk) | - | - | - | - | - | - | 7010-144 | - |
| RheBuild Kit | 7725-999 | 7125-999 | 8125-999 | 9725-999 | 3725-999 | 3725-999 | 7010-999 | 9010-999 |
| **Sample Loops** | | | | **PEEK** | **PEEK** | | | **PEEK** |
| 2µl Sample Loop | 7755-015 | - | - | 7755-015 | - | - | - | - |
| 5µl Sample Loop | 7755-020 | 7020 | 8020 | 9055-020 | - | - | 7020 | 9055-020 |
| 10µl Sample Loop | 7755-021 | 7021 | 8021 | 9055-021 | - | - | 7021 | 9055-021 |
| 20µl Sample Loop | 7755-022 | 7022 | 8022 | 9055-022 | - | - | 7022 | 9055-022 |
| 50µl Sample Loop | 7755-023 | 7023 | 8023 | 9055-023 | - | - | 7023 | 9055-023 |
| 100µl Sample Loop | 7755-024 | 7024 | 7755-024 | 9055-024 | - | - | 7024 | 9055-024 |
| 200µl Sample Loop | 7755-025 | 7025 | 7755-025 | 9055-025 | - | - | 7025 | 9055-025 |
| 500µl Sample Loop | 7755-026 | 7026 | 7755-026 | 9055-026 | - | - | 7026 | 9055-026 |
| 1ml Sample Loop | 7755-027 | 7027 | 7755-027 | 9055-027 | - | - | 7027 | 9055-027 |
| 2ml Sample Loop | 7755-028 | 7028 | 7755-028 | 9055-028 | 3055-018 | 3065-018 | 7028 | 9055-028 |
| 5ml Sample Loop | 7755-029 | 7029 | 7755-029 | 9055-029 | 3055-019 | 3065-019 | 7029 | 9055-029 |
| 10ml Sample Loop | - | - | - | 9055-033 | 3055-023 | 3065-023 | - | 9055-033 |
| 20ml Sample Loop | - | - | - | - | 3055-025 | 3065-025 | - | - |

[1] Recommended models [2] 5/pk [3] 1/pk [4] For both manual and injection mark models

## Manual Valves and Accessories (continued)

### Ordering Information (continued)

| Description | Valve Series | |
|---|---|---|
| | 7410 | 7520 |
| Vespel Rotor Seal | 7410-038 | - |
| Tefzel Rotor Seal | 7410-075 | - |
| Stator | 7410-041 | - |
| 0.2µl Rotor | - | 7520-011 |
| 0.5µl Rotor | - | 7520-012 |
| 1µl Rotor | - | 7520-013 |
| 0.5µl Loop Disc | 7410-070 | - |
| 1µl Loop Disc | 7410-071 | - |
| 2µl Loop Disc | 7410-072 | - |
| RheBuild Kit | 7410-999 | 7520-999 |

| Accessory | Catalogue No. |
|---|---|
| Suction Needle Adapter | 9125-076 |
| Loop Filler Port | 7012 |
| PEEK Loop Filler Port | 9012 |
| PEEK Needle Port | 9013 |
| Needle Guide | 7125-008 |
| Needle Port Cleaner | 7125-054 |
| Mounting Panel | 7160 |
| Valve Angle Bracket | 7160-010 |
| RheFlex Standard Fittings set (5/pk) | 6000-054 |
| RheFlex Short Fittings set (5/pk) | 6000-055 |
| 1/16″ RheFlex PEEK ferrules (5/pk) | 6000-051 |
| ValvTool | HI-199 |

### ValvTool

The ValvTool (HI-199) is a uniquely designed slotted wrench which provides easy access to many hard-to-reach areas. This enables fittings, sample loops and most other 1/4″ stainless steel and PEEK HPLC fittings to be more easily tightened or loosened.

### RheBuild® Kits

RheBuild® kits contain a complete selection of all the parts necessary for general valve maintenance. For front-loading injection valves, the kit includes rotor seal, stator face assembly, isolation seal, needle guide, needle port cleaner, 2 hex keys and repair instructions.

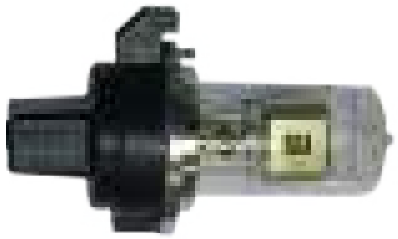

### Needle Port Accessories

The Rheodyne adaptable loop filler ports (7012 and 9012) are used to load sample from syringe needles or luer tips. The needle port (9013) conserves sample by minimising the volume between the needle and the valve.

# HPLC DETECTOR LAMPS

- All lamps from Quality Assured manufacturers
- All lamps tested to guarantee performance

In addition to supplying a range of Agilent UV detector and spectrophotometer lamps, Hichrom offers a range of replacement lamps for Agilent and many other commonly used HPLC UV detectors. Please enquire for instrument lamps not listed.



## Agilent UV Detector and Spectrophotometer Lamps

| Detector Type | Configuration | Lamp Type | Catalogue No. |
|---|---|---|---|
| VWD | G1314D/E/F | Long life deuterium with RFID tag | G1314-60101 |
| | G1314A/B/C, 1120, 1220 Infinity LC | Long life deuterium | G1314-60100 |
| DAD/MWD | G1315C/D, G1365C/D | Long life deuterium with RFID tag | 2140-0820 |
| | G1315A/B, G1365A/B | Long life deuterium | 2140-0813 |
| | G1315A/B, G1365A/B | Long life deuterium | 5182-1530 |
| | G1315A/B/C/D, G1365A/B/C/D | Tungsten | G1103-60001 |

## Replacement Lamps for Other Detectors and Spectrophotometers

| Instrument Manufacturer[1] | Model[2] | Catalogue No. |
|---|---|---|
| Agilent | G1315A/B, G1365A/B, G1314A/B/C | LAG-1001 |
| | | LAG-1002 |
| Applied Biosystems | 757, 759, 783A, 785A, 980 + others | LAP-1001 |
| Cecil Instruments | 1000, 1070, 2000, 5000 and 6000 Series + others | LCE-1001 |
| Gilson | 115, 116, 117, 118, 119, 151, 152, 153, 155 | LGI-1001 |
| Knauer | 8700, 9700 | LKA-1001 |
| | Wellchrom Series | LKA-1002 |
| Perkin Elmer | Int. 2000, Int. 4000, LC55, LC55B, LC65T, LC75, LC85, LC135 | LPE-1001 |
| | Lambda Series | LPE-1007 |
| Shimadzu | LC3, LC4A, LC6A | LSH-1001 |
| | UV- Series | LSH-1004 |
| Varian | Spectra A10, Spectra A20, Spectra A30, Spectra A40, 75 Series | LVA-1002 |
| Waters | 480, 481, 480LC, 481LC, Lambda Max | LWA-1001 |
| | 484 | LWA-1002 |

[1] Lamps for other manufacturers' models also supplied [2] Lamps for other models also supplied

# SYRINGES

HPLC valve injection syringes utilise needles (point style 3) characterised by a 90° square tip whose edges are chamfered and polished to eliminate damage to the rotor seal and stator face of the injector valve. This style of needle tip is also suitable for pipetting liquids. Please contact Hichrom for details of other syringes available (including syringes for GC).

## Hamilton Syringes

Hamilton MICROLITER® 700 series syringes are the standard syringe used in many laboratories throughout the world. For optimum reproducibility, it is recommended that the barrel should always be filled to its maximum capacity, but the volume injected should not exceed 80% of the syringe volume.

Hamilton GASTIGHT® 1700 series syringes use teflon as the inert plunger seal. With these syringes, liquids only come into contact with glass and teflon. They are recommended for bioanalytical applications.

Glass barrels and syringe plungers of the same capacity are interchangeable for the 1700 series syringes, but not for the 700 series syringes.

## Luer Tip Syringes

To remove contamination from valve sample loops, flushing with a larger volume Luer tip syringe is reccommended.

Please contact us for ordering information details for syringes.

## SGE Syringes

SGE manufacture an extensive range of HPLC syringes for use with Rheodyne or Valco syringe-loading injection valves. Syringes with either a fixed needle or a removable needle and repeating adaptor are available.


### Ordering Information

| Luer tip syringe | Syringe Code | Cat. No. |
|---|---|---|
| 2 ml | - | HI-252 |
| 5 ml | - | HI-255 |

# SMART CAPS

## Smart Healthy Caps (for inlet solvent bottles)

- Protect health
- Minimise solvent evaporation
- Maintain accurate solvent concentrations
- No external contamination of eluent

Smart Healthy Caps were designed to comply with the increasing safety regulations of the modern laboratory. They are made from inert, chemically resistant materials, such as PTFE and polypropylene. Figure 1 shows a Smart Healthy Cap screwed onto a solvent inlet reservoir.

The use of Smart Healthy Caps can reduce harmful solvent evaporation from solvent inlet reservoirs by up to 80%, protecting health and keeping the HPLC eluent concentration accurate for several days. This is achieved by the use of a one-way check valve (Figure 2), which allows air to enter the bottle for pressure equalization, but eliminates solvent vapour emissions.

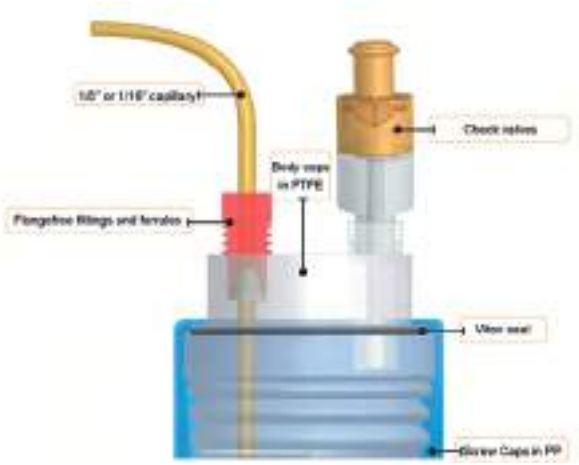


Figure 1. Smart Healthy Cap on solvent inlet reservoir


Figure 2. One-way check valve

Smart Healthy Caps also protect solvents against external contamination and are available to fit a wide range of bottle types and sizes. They can be supplied with 1 to 4 outlet ports to accommodate multiple HPLCs. Tubing (⅛″ or 1/16″ o.d.) is securely fixed by use of a ferrule and flange-free fitting. Smart Healthy Caps are designed to fit the standard GL45 bottle thread. For alternative bottle threads such as GL40 and GL32, adaptors are available (see Figure 3).

## Ordering Information

### Smart Healthy Caps[1] (to fit standard GL45 bottle thread)

| No. Outlet Ports | Catalogue No. |
|---|---|
| 1 | SHC-1 |
| 2 | SHC-2 |
| 3 | SHC-3 |
| 4 | SHC-4 |

[1] Supplied with check valve, fittings and ferrules for ⅛″ o.d. tubing (fittings and ferrules for 1/16″ o.d. tubing supplied on request)


Figure 3. Smart Healthy Caps adaptors

### Smart Healthy Caps Adaptors (to convert a GL40 or GL32 thread for use with GL45 Smart Healthy Caps)

| Adapter Thread | Material | Cat. No. |
|---|---|---|
| GL40 | Teflon | AD-40-T |
| GL40 | Polypropylene | AD-40-PP |
| GL32 | Teflon | AD-32-T |
| GL32 | Polypropylene | AD-32-PP |

Please contact Hichrom for Smart Healthy Cap items not listed above.

### Starter Kit – contains:

1 x Smart Healthy Cap GL45 thread with 2 x ⅛″ outlet ports
2 x Fittings and ferrules for ⅛″ tubing
1 x Port plug for unused ports
1 x check valve

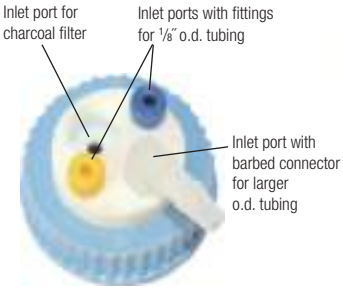
Cat. No. SHC-2-SK
**(Contains everything required)**

## Smart Waste Caps (for waste solvent bottles)

- Protect health
- 95% reduction in harmful emissions
- Activated charcoal filter cartridge

Smart Waste Caps reduce harmful substance emissions from waste bottles by up to 95% and are available to fit a wide range of waste bottle sizes, including drums. They allow waste solvents from an HPLC to enter the waste bottle but prevent solvent vapours from escaping into the laboratory, by use of an activated charcoal filter cartridge. Smart Waste Caps (see Figure 4) are available to fit waste solvent containers with GL45 to S90 threads (and can be custom manufactured for all applications).

Smart Waste Caps can be supplied with multiple inlet ports to accommodate multiple HPLC lines. Tubing (⅛″ or 1/16″ o.d.) is securely fixed by use of a ferrule and flange-free fitting. Larger o.d. tubing (up to 9mm) can be connected by use of a barbed connector.




SW45-2-C-1L
Smart Waste Cap

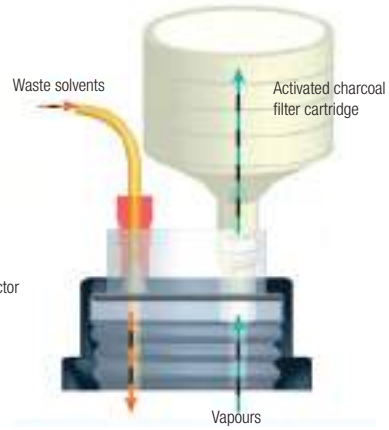



Figure 4. Smart Waste Cap on solvent waste bottle

### Ordering Information

### Smart Waste Caps (charcoal filter cartridge purchased separately – see below)

| No. and Type of Inlet Ports | Thread Size | | | |
|---|---|---|---|---|
| | GL45 | S51/S55 | S60/S61 | S90 |
| 3 x ⅛″ (or 1/16″) inlet ports[1] | SW45-3-C | SW55-3-C | SW60-3-C | SW90-3-C |
| 4 x ⅛″ (or 1/16″) inlet ports[1] | SW45-4-C | - | - | - |
| 1 x ⅛″ (or 1/16″) + 3 barbed inlet ports[1] | - | SW55-1-C-3L | - | - |
| 2 x ⅛″ (or 1/16″) + 1 barbed inlet port[1] | SW45-2-C-1L | SW55-2-C-1L | SW60-2-C-1L | - |
| 3 x ⅛″ (or 1/16″) + 1 barbed inlet port[1] | SW45-3-C-1L | - | - | SW90-3-C-1L |
| 3 x ⅛″ (or 1/16″) + 2 barbed inlet ports[1] | - | - | - | SW90-3-C-2L |
| 3 x ⅛″ (or 1/16″) + 3 barbed inlet ports[1] | - | - | SW60-3-C-3L | - |
| 4 x ⅛″ (or 1/16″) + 1 barbed inlet port[1] | - | - | SW60-4-C-1L | SW90-4-C-1L |

[1] Comes with fittings and ferrules for ⅛″ o.d. tubing and 90° angled barbed connectors for 6-9mm o.d. tubing as standard. Fittings and ferrules for 1/16″ o.d. tubing and 90° angled and straight barbed connectors for 3-4mm, 5-6mm and 9-11mm o.d. tubing, plus straight barbed connectors for 6-9mm o.d. tubing are available on request.

### Charcoal Filter Cartridges

| Wt. Charcoal (g) | Lifetime (months) | Cat. No. |
|---|---|---|
| 25 | 3 | FC25 |
| 50 | 6 | FC50 |
| 100 | 12 | FC100 |

Ref: FC25 Ref: FC50 Ref: FC100

Figure 5. Charcoal filter cartridges


Please contact Hichrom to request your Smart Caps catalogue

### Barbed Connectors

| Tubing o.d. (mm) | 90° Angled | Straight |
|---|---|---|
| 3 - 4 | RC-34 | RD-34 |
| 5 - 6 | RC-56 | RD-56 |
| 7 - 9 | RC-69 | RD-69 |
| 9 - 11 | RC-911 | RD-911 |

### Starter Kit – contains:

1 x Smart Waste Cap GL45 thread with 3 x ⅛″ inlet ports
3 x Fittings and ferrules for ⅛″ tubing
2 x Port plugs for unused ports
1 x 25g Charcoal filter cartridge

Cat. No. SW45-3-C-SK
**(Contains everything you need)**

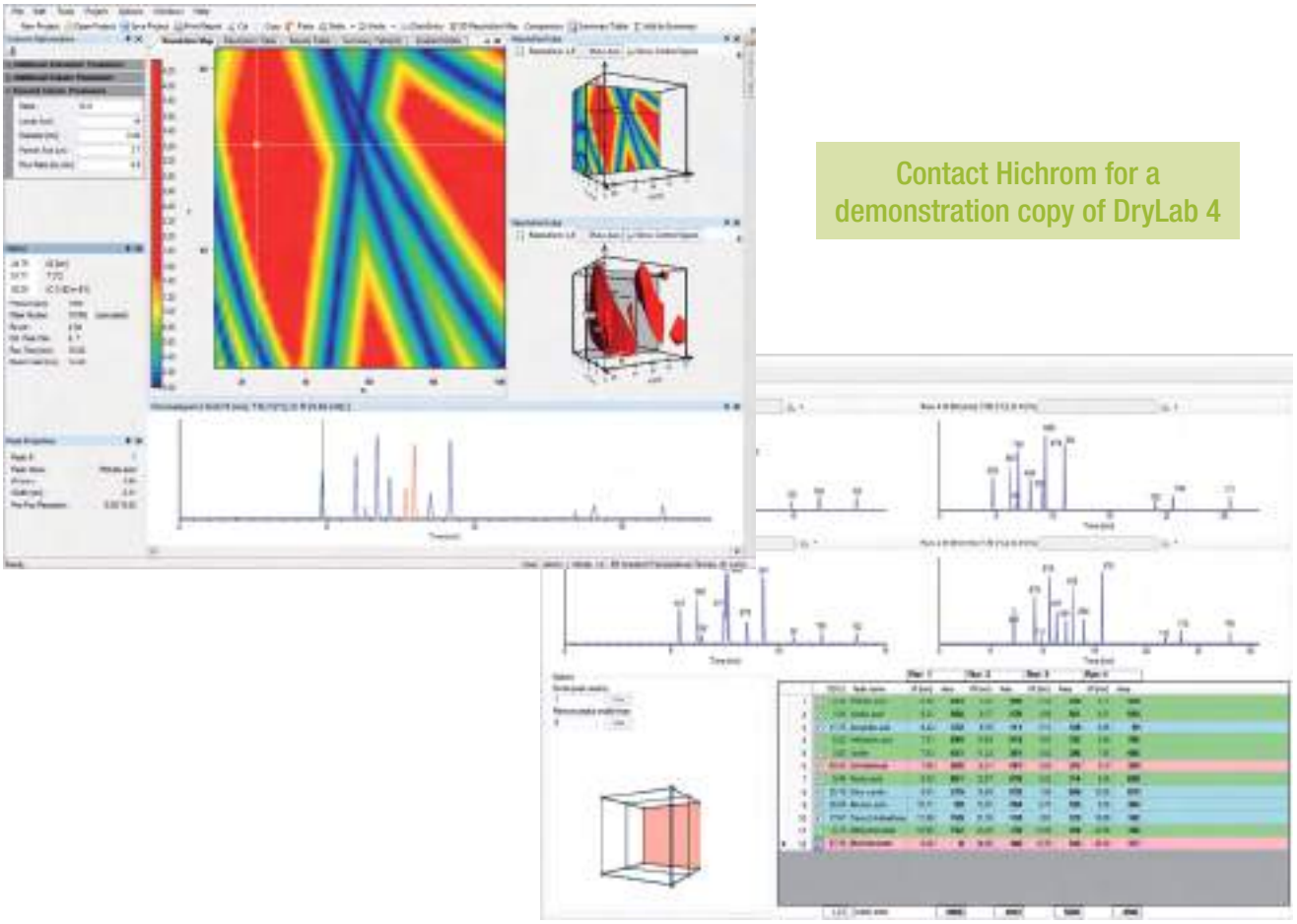
# CHROMATOGRAPHY MODELLING AND SIMULATION SOFTWARE

Many chemical, pharmaceutical, environmental and other products are quality controlled and tested by HPLC methods. A reliable and robust HPLC method is therefore a vital and integral component of the production and quality process. DryLab®4 is a tool which helps the chromatographer to produce better more reliable HPLC and UHPLC methods in a shorter time. It helps you to structure and organise your work, so enabling you to save time and consumables used during method development and make the final results more predictable, reliable, transferable and successful.

## DryLab®4 – Key Points

- Latest version of the acclaimed DryLab chromatography modelling software, which has evolved over 25 years.
- Allows you to develop better, more robust HPLC/UHPLC methods faster and more efficiently.
- Also allows you to optimise or modify existing HPLC methods for successful transfer to alternative UHPLC/HPLC equipment.
- Features improved "peak tracking" to speed up data entry and improve correct peak identification on imported chromatograms.
- Improved functionality allows for 1, 2 and 3 chromatographic parameters (e.g. % organic, temp, pH etc.) to be modelled simultaneously.
- Allows modelling results to be easily visualised as a "2D colour resolution map" or "3D colour resolution cube".
- From just 12 runs you can model 1,000,000 possible solutions.
- New robustness module allows you to simply and quickly explore the boundaries of robustness, cumulatively or independently, for all specified parameters.
- Fully compatible with the principles of Quality by Design (QbD).

### A Truly Powerful Tool for HPLC Method Development



**Contact Hichrom for a demonstration copy of DryLab 4**

## Product Description

DryLab software consists of a core module and two other optional modules. Depending on the modules purchased, DryLab 4 will provide the functions described below. It is available in single or multi-licence format.

**1) The Core Module** allows 1D & 2D modelling and is required for all applications in DryLab 4.

**2) The 3D Design Space Module** is required to enable 3D modelling and for the results to be visualised as a 3D cube.

**3) The Robustness Module** gives you a statistical evaluation of just how robust your method is and highlights which parameters are more important than others. This includes an evaluation of those parameters that are not being directly modelled such as 'flow rate' for example.

To discuss individual requirements and to obtain pricing information please contact Hichrom Limited.

## Chromatography Modelling and Simulation Software (continued)

# DryLab®4

## Why should you invest in DryLab® 4?

DryLab® helps you find the best solutions for your sample separation challenges. For twenty-five years, scientists from major international pharmaceutical and chemical companies, as well as universities and other organisations, have utilised DryLab to create high quality HPLC methods to meet the needs of their demanding applications. Anybody working in the realm of HPLC who wishes to economise on the resources spent developing and running methods will benefit from the advantages offered by DryLab. The most important reasons for adopting DryLab technology into your HPLC laboratory are considered to be as follows:

### • To save time and cost

Method development and optimisation with DryLab is a streamlined and efficient process. Methods developed in DryLab often have run times that are 50% shorter compared to methods developed by traditional 'trial and error' approaches. This can mean significant increases in productivity for both equipment and staff. A DryLab model uses real data to accurately simulate literally thousands of experiments, meaning experimental work normally requiring valuable time in the laboratory can be done instantly. How many times has a whole day or week of HPLC method development effort left you no further ahead than you were at the beginning? DryLab can prevent this from happening by providing you with information which allows you to decide which experiments are worth pursuing and which are dead-ends not worthy of your time.

The pharmaceutical industry spends up to 16% of the R&D cost of a new drug on the development and execution of HPLC methods. A non-robust method will be overly sensitive to the fluctuations in parameters typical when a method is deployed across a multitude of instruments, locations, and users. The time spent compensating and adjusting for the problems arising from a non-robust method will cost time and money and may result in production delays or even whole batches of drug substance being lost.

DryLab's method development motto is to make it right the first time. High quality robust HPLC methods can be developed faster than ever before, and the Design Space built into the DryLab model provides a comprehensive knowledge that gives you long-term security and confidence in any future method amendments and validations. In many cases, optimisation of a methanol/acetonitrile/buffer ternary solvent blend in the mobile phase achieves a degree of selectivity not possible with just acetonitrile alone.

Shorter run times mean that more samples can be analysed per column and the use of expensive solvents and mobile phase additives minimised. The use of cheaper alternative solvents such as methanol can be considered from the beginning, often with no sacrifice in method benchmarks like resolution and robustness. Waste disposal costs will therefore fall.

### • To help update and transfer methods

DryLab's Gradient Editor predicts the optimum linear gradient for your separation and lets you model multi-segmented gradients or adjust methods for different instrument configurations. It is straightforward to transfer a method to an instrument with a different dwell volume. In addition, you can use DryLab to facilitate updating methods from traditional long columns (250 x 4.6mm, 5µm) onto shorter UHPLC columns with smaller particle sizes.

### • To help conform to FDA 'Quality by Design' standards

The new Quality by Design (QbD) concept of the FDA requests the scientific elaboration of the Design Space for the proposed HPLC method. With its comprehensive resolution and robust mapping abilities, Drylab is perfectly geared to this task. Peak movements can be modelled making it easy to determine the tolerance thresholds that define method robustness. You will be able to comfortably assure the FDA regulators about the high quality of your methods using a DryLab model to illustrate the science based reasoning for your choice of working conditions.

### • To help teach and train new chromatographers

Training new staff requires a big investment in time and resources, and mistakes made at the instrument can be costly. DryLab lets novices do their initial learning on a computer. As they alter experimental conditions, DryLab updates the chromatogram instantaneously, helping old and new chromatographers alike to quickly gain an insight into a method's properties. Such immediate feedback makes it a powerful tool to teach the fundamental principles of chromatographic retention mechanisms. DryLab comes complete with a detailed Tutorial Guide and many useful examples for practical instruction to help all users get a successful start to DryLab HPLC method development.

*The more you use DryLab, the more time and money you save – and the more confidence you have in the methods you develop!*

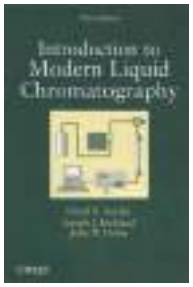
# BOOKS

## Introduction to Modern Liquid Chromatography

3rd Edition
L.R. Snyder, J.J. Kirkland and J.W. Dolan

The third edition of this popular book features new HPLC applications and technologies, including chiral compound separations and bioseparations. An update of HPLC computer software and technology is also included, making this book a complete compendium of HPLC methods and applications.

**978-0470167540**




## Practical HPLC Method Development

2nd Edition
L.R. Snyder. J.J. Kirkland and J.L. Glajch (Wiley)

This essential reference describes the practical application of chromatographic theory to method development. Organised examples show the analyst how to control the many separation variables in a simplified and easy-to-follow format. Sample preparation, separation optimisation, detection and quantitation are discussed in detail, along with more specialised applications such as chiral and preparative separations.

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## UHPLC in Life Sciences

Ed. D. Guillarme and J. Veuthey

This new book discusses the basics of UHPLC and its application in the field of drug analysis. A general overview of instrumentation and columns is given. Method transfer between HPLC and UHPLC, coupling of UHPLC and MS and a comparison of the performance of totally porous and superficially porous particles are discussed.

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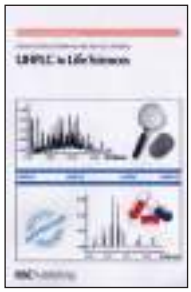

## A Practical Handbook of Preparative HPLC

Donald Wellings (Elsevier)

This book concentrates on practical, hands-on advice for preparative HPLC from laboratory to process scale. It will help ensure optimal design, equipment and separation results for those in the pharmaceutical, agrochemical and biotechnology industries.

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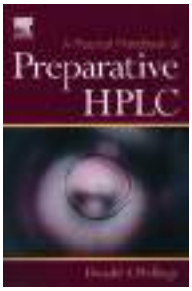


## Handbook of Chemistry and Physics

W. M. Haynes (CRC Press)

This 93rd edition of the CRC Handbook of Chemistry and Physics is an indispensable sourcebook for chemists. It features many new or completely revised tables, providing the most comprehensive and up-to-date data, as well as advances in the chemical and physical sciences.

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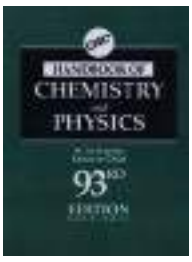


## The HPLC Solvent Guide

2nd Edition
P. Sadek (Wiley)

This revised and expanded edition is a valuable working reference offering experimentalists an overview of the solvent's role in HPLC. It provides detailed coverage of all commonly used HPLC solvents.

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# BOOKS (continued)



## High Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model

Lloyd R. Snyder and John W. Dolan (Wiley)

Written by two well-known authorities in HPLC, this book takes the mystery out of the practice of gradient elution, by giving a comprehensive description of the theory, methodology and applications across many fields of HPLC. The focus is on the use of linear-solvent-strength theory in predicting gradient LC behaviour.


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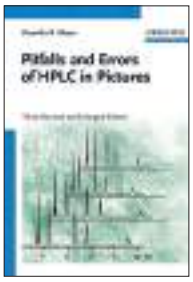


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## Modern HPLC for Practicing Scientists

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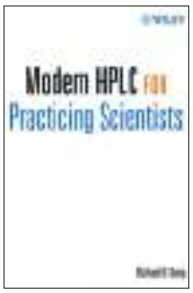
This is a concise text, which presents the most important fundamentals, applications and developments of HPLC. It describes basic theory and terminology for the novice, and reviews relevant concepts, best practices and modern trends for the experienced practitioner.

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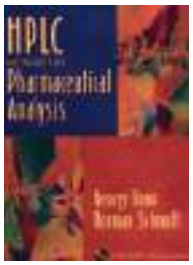
Ed. G. Lunn and N. Schmuff (Wiley)

This useful reference tool provides a readily accessible source of abstracts of the most up-to-date literature on HPLC methods developed for the most commonly prescribed pharmaceutical compounds. Each drug covered includes a number of detailed procedures, enabling analysts to replicate the technique without reference to the original. Extensively annotated bibliographies are included.

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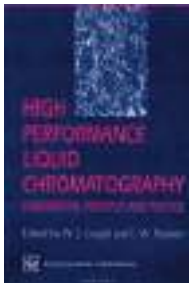

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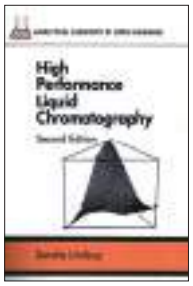
This ACOL (Analytical Chemistry by Open Learning) text provides the beginner with a working knowledge of the basic principles and theory behind HPLC techniques. The book covers the foundations of liquid chromatography, instrumentation, column packings and many important applications.

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## Liquid Chromatography-Mass Spectrometry

R.E. Ardrey (Wiley)

This indispensable reference source is the first book to concentrate on principles of LC-MS. It also describes instrumental aspects of LC-MS and discusses the current applications and practical benefits of the technique.

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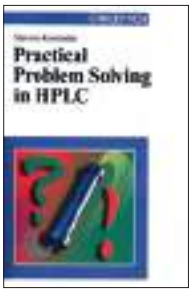
## Practical Problem Solving in HPLC

S. Kromidas (Wiley)

This guide addresses 45 common questions of HPLC users. It explains simple equipment tests and selection criteria for columns and buffers. As well as dealing with specific problems and how to solve them, it features addresses of manufacturers, references, data tables and check lists.

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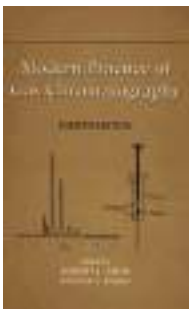

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# GLOSSARY



**Activity.** In adsorption chromatography, the relative strength of the surface of the packing. For silica gel, the more exposed the silanol groups, the more active the surface. Activity can be controlled by adding water or another polar modifier, which is hydrogen-bonded to the active sites, thereby reducing the surface activity.

**Additive.** A substance added to the mobile phase to improve the separation or detection characteristics.

**Adsorption.** The process of retention in which the interactions between the solute and the surface of an adsorbent dominate.

**Affinity Chromatography.** A technique in which, for the macromolecule of interest, a biospecific adsorbent is prepared by coupling a specific ligand (such as an enzyme, antigen or hormone) to a solid support (or carrier). This immobilised ligand will interact only with molecules that can selectively bind to it. Molecules that will not bind elute unretained. The retained compound can later be released in a purified state. Affinity chromatography is seen as an on-off technique.

**Asymmetry (As).** Asymmetry (skew) is a factor describing the shape of a chromatographic peak. Theoretically it is assumed peaks are symmetrical and of Gaussian shape. Practically, the peak asymmetry factor is measured as shown on p.10. A value of >1 indicates a tailing peak and <1 a fronting peak.

**Back Pressure.** The difference in pressure between the inlet and outlet of a column.

**Bar.** A unit of pressure equal to one atmosphere. It is equivalent to 14.5 pounds per square inch (psi) or 0.1 Megapascal.

**BET Method.** A method developed by Bruner, Emmett and Teller for measuring surface area. The level of liquid nitrogen adsorption within the pores of the phase is measured at very low temperatures. Pore volume and pore size distribution methods can also be obtained by this method.

**Biocompatible.** A term used to indicate that a tubing or fitting material will not change the biological activity of material coming into contact with it during the HPLC analysis time. It frequently means metal-free components.

**Bonded.** Term which implies that the stationary phase is chemically bonded to the surface of the supporting material.

**Bonded Phase Coverage.** Refers to the amount of bonded phase on a silica support. Coverage is usually described in mmol/$m^2$ or in terms of percentage carbon.

**Capacity Factor (k').** An old term for a chromatographic parameter that measures the degree of retention ($t_R$). Now defined as the retention factor (k) by the IUPAC.

**Channelling.** Occurs when voids created in the packing material of a column may cause eluent and accompanying solutes to move more rapidly than the average flow velocity, resulting in band broadening. The voids are created by poor packing or by erosion of the packed bed.

**Check Valve.** A device built into an HPLC pump which allows the flow of eluent in one direction only.

**Chiral Stationary Phase (CSP).** Stationary phase designed to separate enantiomeric mixtures.

**Column Dead-time ($t_0$).** The time taken for solvent molecules or other non-retained peaks to move through the column.

**Column Efficiency (N).** A term used to express the width of a peak produced by a column. Efficiency is measured in terms of the number of plates, a parameter which is inversely related to the square of the peak width. See p.10 for the full calculation.

**Counterion.** In an ion-exchange process, the ion in solution used to displace the ion of interest from the ionic site. In ion-pairing, it is the ion of opposite charge added to the eluent to form a neutral ion pair in solution.

**Dead Volume.** A measure of solvent accessible volume between the injector and detector after the space occupied by the column packing material has been subtracted. Both interstitial column volumes and system (injector, detector, connecting tubing and end fittings) volumes contribute. The dead volume can be determined by injecting an inert compound (i.e. a compound that does not interact with the column packing) and measuring its retention volume.

**Degassing.** The practice of removing dissolved gases from the eluent. It can be achieved by helium sparging, applying vacuum to the eluent, ultrasonification or heating.

**Denaturing.** The process of destroying the tertiary and quaternary structure of a protein.

**Desalting.** A technique in which low molecular weight salts and other compounds are removed from non-ionic and high molecular weight compounds. An example is the use of size exclusion columns to exclude large molecules and retain lower molecular weight salts.

**Dwell Volume.** The volume between the point of mixing of solvents (usually in the mixing chamber or at the proportioning valves of the HPLC instrument) and the head of an LC column. Particularly important in gradient elution.

**Eluotropic Series.** A series of solvents with an increasing degree of polarity, generally used to explain solvent strength.

**Elution Volume.** Refers to the volume of eluent necessary to elute a solute from a column. For a symmetrical peak, it is the volume from the point of injection to the volume at maximum concentration.

# GLOSSARY (continued)



**Endcapping.** The reaction of a silylating reagent with unreacted accessible silanols remaining on the silica surface after the initial bonding reaction. The process may reduce undesirable adsorption of basic or polar molecules which otherwise may cause peak tailing.

**Exclusion Limit.** In SEC, the upper limit of molecular weight (or size) beyond which molecules will elute at the same retention volume (exclusion volume). Many SEC packings are referred to by their exclusion limit. For example, a $10^5$ column of porous silica gel will exclude any compounds with a molecular weight higher than 100,000 based on a polystyrene calibration standard.

**Flash Chromatography.** A very fast form of classic LC used by synthetic organic chemists for rapid purification. Performed primarily in the normal-phase mode, sometimes with reversed-phase chromatography.

**Fractionation Range.** In SEC, refers to the range in which the packing can separate molecules based on their size. Molecules that are too large to diffuse into the pores are excluded. Molecules that can diffuse into all of the pores totally permeate the packing, eluting unseparated at the permeation volume.

**Fronting.** A term describing a peak shape whose front has a leading edge.

**Gel Filtration Chromatography (GFC).** SEC carried out with aqueous eluents. It is sometimes referred to as aqueous GPC. Most gel filtration separations involve biopolymers.

**Gel Permeation Chromatography (GPC).** SEC carried out with organic eluents. Used for the separation and characterisation of polymers.

**Gradient Elution.** The process by which the strength and composition of the eluent is increased during the chromatographic run, thereby reducing analysis time. Binary, ternary and quaternary solvent gradients are routinely used.

**Guard Column.** A short column placed between sample injector and the inlet of the main column. It protects the analytical column against contamination from sample particulates and strongly retained solutes. The guard column is usually of cartridge format requiring a holder and packed with the same material as in the main column.

**Helium Sparging.** The process of bubbling helium through the eluent to remove dissolved gas.

**High Pressure Mixing.** A procedure in which two or more solvents are mixed on the high pressure side of the pumping system to form a final eluent. One pump is required per solvent.

**Hybrid Silica.** Silica gel comprising both organic and inorganic moieties with hybrid properties of polymeric packings and silica packings. Offers different selectivity but better high pH stability than bare or uncoated silica gel.

**Hydrophilic.** A description of compounds, solvents or bonded phases that either readily dissolve in water or prefer water to non-polar organic solvents, ie. 'water-loving'.

**Hydrophilic Interaction Chromatography (HILIC).** The use of polar stationary phases and partially aqueous eluents to separate compounds in order of increasing hydrophilicity (polarity).

**Hydrophobic.** A term describing compounds, solvents or bonded phases that dissolve easily in non-polar organic solvents such as hexane or prefer such solvents to water, ie. 'water-hating'.

**Hydrophobic Interaction Chromatography (HIC).** A protein separation technique in which reversed-phase materials are used with eluents containing high salt concentrations. Gradients are run by decreasing salt concentrations with time.

**Hyphenated Techniques.** Refers to the family of techniques best known by their acronyms, including LC-mass spectrometry (LC-MS), LC-Fourier transform IR spectroscopy (FTIR) and LC-MS/MS.

**Injection Solvent.** The solvent the sample is dissolved in prior to chromatographic analysis.

**Interstitial Volume.** The volume between the particles. It does not include the volume in the pores of the particles.

**Ion Chromatography.** An ion-exchange technique in which low concentrations of organic and inorganic anions or cations are determined using ion-exchangers of low ion-exchange capacity with dilute buffers.

**Ion-Exchange Capacity.** A measure of the number of ionic sites that can take part in the exchange process. Exchange capacity is expressed in mequiv/g.

**Ion Exclusion.** The process in which ionised solutes can be separated from non-ionised or partially ionised solutes using ion-exchange resins. Ionised solutes will move faster down the column.

**Ion-Pair Chromatography.** A form of reversed-phase chromatography in which ions in solution can be paired or neutralised prior to separation as an ion-pair. Ion-pairing reagents are usually ionic compounds that contain a hydrocarbon chain. The latter imparts a certain hydrophobicity to the resultant ion-pair allowing it to be retained on a reversed-phase column.

**Ion Suppression.** Buffering in an aqueous eluent at a particular pH to suppress solute ionisation. Useful for improving peak shape of weak acids and bases in reversed-phase chromatography.

# GLOSSARY (continued)



**Irreversible Adsorption.** When a compound with a very strong affinity for an adsorbent is injected onto a column, it can be adsorbed so strongly that it cannot be eluted from the column.

**Isocratic.** Chromatographic conditions in which a constant composition eluent is used.

**Isoelectric Point.** The pH point at which a molecule no longer has a net charge.

**Ligand-exchange Chromatography.** A technique in which chelating ligands are added to the eluent. On adsorption onto the stationary phase they act as chelating agents. An example is the use of copper amine chelates for the separation of amino acids.

**Loadability.** The maximum amount of analyte that can be injected onto a column that no longer permits the isolation of product at the desired level of purity or recovery level. Important in preparative chromatography.

**Low Pressure Mixing.** A pumping procedure in which two or more solvents are mixed on the low pressure side of the pump. Only one pump is required.

**Mass Transfer.** The process of solute movement between the moving and stationary zones. The faster the mass transfer process, the better the column efficiency. Mass transfer is represented by the C-term in the van Deemter equation.

**Mean Pore Diameter.** A term that refers to the average diameter of the pores within a phase.

**Megapascal (MPa).** A unit of pressure. One MPa equals about 10 bar (atmospheres) or 145 pounds per square inch (psi).

**Modifier.** A chemical added to reversed-phase solvent systems designed to optimise the chromatographic separation.

**Monomeric Phase.** A bonded phase in which individual molecules are bonded to a support. For silica, monomeric phases are typically prepared by the reaction of an alkyl- or aryl-monochlorosilane or alkoxysilane.

**Nano LC.** LC carried out with columns less than 100µm in internal diameter. Usually requires specialised instrumentation.

**Overload.** A saturation of the stationary phase by the solute which is evidenced by band broadening, tailing and flat edged chromatographic peaks.

**Particle Size (dp).** This term refers to the average particle size of the material packed into a column.

**Particle Size Distribution.** A measure of the distribution of the particles used to manufacture a column. In HPLC a narrow particle size distribution is desirable. For a 10µm size particle, a particle size distribution of dp±10% means that 90% of the particles have a 9-11µm size.

**Peak Broadening.** The tendency of a chromatographic peak to broaden as it passes through the column. It is also known as peak spreading or peak dispersion. The peak width or the number of theoretical plates in the column (N) is a measure of peak broadening.

**Peak Capacity.** The number of equally well-resolved peaks that can fit in a chromatogram between the hold-up volume and some upper limit in retention.

**Polarity.** A measure of the separation of charges within a molecule. Polar molecules interact more strongly with and are best separated on polar stationary phases.

**Polymeric Packing.** Packings based on polymeric materials, usually in the form of spherical beads. Common polymers include polystyrene-divinylbenzene, polymethylmethacrylate and polyvinylalcohol.

**Polymeric Phase.** A bonded phase in which typically a di- or trichlorosilane is reacted with more than one reactive silanol group on the surface of silica.

**Pore Size (Mean Pore Diameter).** The average diameter of the pore in a porous packing. The pore diameter is important in that it must allow free diffusion of solute molecules into and out of the pore so that the solute can interact with the stationary phase. In SEC, the packings have different pore diameters, and therefore molecules of different sizes can be separated. For a typical adsorbent such as silica gel, 60Å and 100Å pore diameters are most popular. For packings used for the separation of biomolecules, pore diameters >300Å are used.

**Pore Volume.** The total volume of the pores in a porous packing, usually expressed in ml/g. It is measured by the BET method of nitrogen adsorption or by mercury intrusion porosimetry, where Hg is pumped into the pores under high pressure.

**Precolumn.** A column packed with silica placed between the pump and the injector. It presaturates the eluent with stationary phase minimising loss of the latter from the main column. It will also remove particulate material.

**Pressure Drop.** The difference in pressure between the inlet and outlet of a column during flow caused by the hydrodynamic resistance of the packed bed.

**Process Scale Chromatography.** Refers to the use of LC at the industrial scale level. Generally requires specially designed columns (eg. internal diameters > 5cm), recoverable solvents, low cost packings and overloaded operating conditions compared with laboratory scale HPLC.

**Residual Silanols.** These are the silanol (-SiOH) groups that remain on the surface of a silica after a bulky phase is chemically bonded to its surface. Their numbers can be reduced by further reacting (endcapping) the silica surface with a small organosilane.

# GLOSSARY (continued)



**Resolution ($R_S$).** A measure of the separation of two adjacent peaks. The higher the resolution value the greater the separation (see p.216).

**Retention Factor (k).** The period of time that the sample component resides in the stationary phase relative to the time it resides in the mobile phase (see p.216).

**Retention Time.** The elapsed time between sample injection and the appearance of the chromatographic peak apex.

**Sample Capacity.** The term refers to the amount of sample that can be injected onto a column without overloading it. In preparative applications it is typically expressed as grams of solute per gram of stationary phase.

**Scalability.** In going from analytical to preparative chromatography, refers to the reproducibility of results on columns of different internal diameters and/or particle sizes when using the same bonded phase. A linear scale-up process minimises time required to optimise preparative separations.

**Separation Factor ($\alpha$).** A thermodynamic factor that is a measure of relative retention of two substances. Formerly called 'selectivity' or 'selectivity factor'.

**Siloxane Bond.** The main –Si-O-Si- bond found in silica.

**Size Exclusion Chromatography (SEC).** A mode of HPLC used mainly to separate high molecular weight samples and to determine their molecular weight distribution by virtue of their size in solution. Also known as gel permeation, gel filtration or steric exclusion chromatography.

**Superficially Porous Particle.** A particle of typical diameter 2 to 5µm, with a solid core and a porous outer shell.

**Surface Area.** The total area of the phase's solid surface, as determined by an accepted measurement technique such as the BET method, which uses nitrogen adsorption. For silica it is typically 100-600 $m^2/g$.

**Surface Coverage.** Usually refers to the mass of stationary phase per unit area bonded to a chromatographic support. Often expressed in micromoles per square metre of surface. Sometimes the percentage of carbon is given as an indicator of surface coverage.

**Tailing.** The phenomenon in which a peak has an asymmetry factor >1. The downside of the peak will be skew.

**Theoretical Plate.** Measure of column efficiency. Length of column relating to this concept is called height equivalent to a theoretical plate (HETP).

**Trace Enrichment.** Technique in which trace amounts of compounds are retained on an HPLC or precolumn packing out of a weak eluent or solution and then are eluted by adding a stronger eluent, in a concentrated form.

**Van Deemter Equation.** Equation used to explain band broadening in chromatography. The equation represents the height of a theoretical plate (HETP) and has three terms. The A-term describes eddy dispersion or diffusion. The B-term represents the contribution of molecular or longitudinal diffusion of the solute passing through the column. The C-term is the contribution from interphase mass transfer of solute between stationary phase and eluent.

**Void.** The formation of a space, usually at the head of the column, caused by a settling or dissolution of the packing. A void in the column leads to decreased efficiency and loss of resolution.

**Void Volume ($V_0$).** The total volume of eluent in the column, the remainder being taken up by packing material. Can be determined by injecting an unretained substance.

**Zero Dead Volume (ZDV).** It refers to a fitting or component which adds no extra volume to the system. In practice ZDV fittings have a finite but insignificant volume.

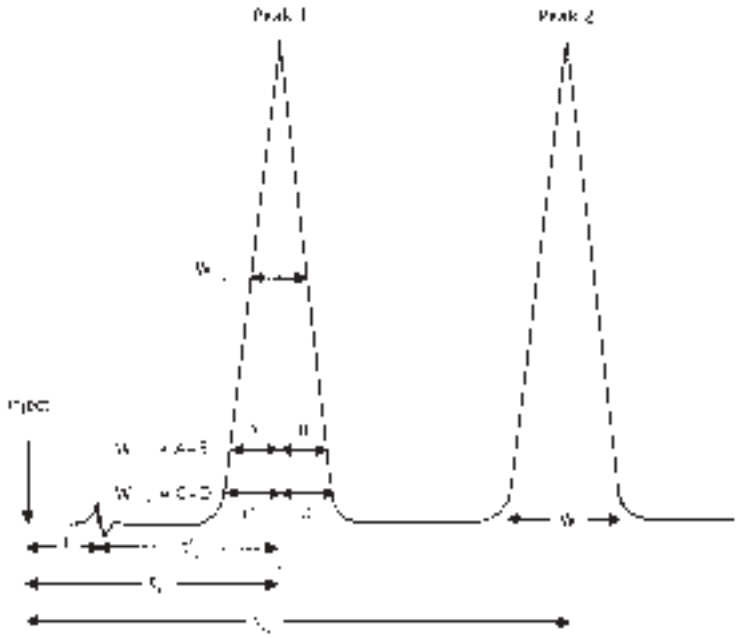
**Zwitterions.** Compounds that carry both positive and negative charges in solution.

# CHROMATOGRAPHIC ABBREVIATIONS



| | |
|---|---|
| A | Absorbance |
| AA | Amino acid |
| ACN | Acetonitrile |
| ADP | Adenosine 5'-diphosphate |
| AGP | $\alpha_1$-acid glycoprotein |
| amu | Atomic mass unit |
| APS | Aminopropylsilyl |
| ATP | Adenosine 5'-triphosphate |
| AU | Absorbance units |
| AUFS | Absorbance units full scale |
| BDS | Base deactivated silica |
| BSA | Bovine serum albumin |
| CBH | Cellobiohydrolase |
| CCC | Countercurrent chromatography |
| CE | Capillary electrophoresis |
| CEC | Capillary electrochromatography |
| CFC | Chlorofluorocarbon |
| CI | Chemical ionisation |
| CPS | Cyanopropylsilyl |
| CSP | Chiral stationary phase |
| CT | Charge transfer |
| CZE | Capillary zone electrophoresis |
| DAC | Dynamic axial compression |
| DACC | Donor-acceptor complex column |
| DAD | Diode array detection |
| Dabsyl | 4-Dimethylaminoazobenzene-4-sulphonyl chloride |
| Dansyl | 5-Dimethylaminonaphthalene-1-sulphonyl chloride |
| DCM | Dichloromethane |
| DEAE | Diethylaminoethyl |
| DEG | Diethylene glycol |
| DHPLC | Denaturing HPLC |
| DMSO | Dimethyl sulphoxide |
| DNA | Deoxyribonucleic acid |
| DNPH | 2,4-Dinitrophenylhydrazine |
| DRI | Differential refractive index |
| EC | Electrochemical |
| ECD | Electrochemical detection |
| EDTA | Ethylenediaminetetraacetic acid |
| EI | Electron impact (or electron ionisation) |
| ELISA | Enzyme-linked immunosorbent assay |
| ELSD | Evaporative light-scattering detector |
| EOF | Electroosmotic flow |
| ESI | Electrospray ionisation |
| FAB | Fast atom bombardment |
| FAME | Fatty acid methyl ester |
| FFAP | Free fatty acid phase |
| FIA | Flow-injection analysis |
| FID | Flame ionisation detection |
| FLEC | 1-(9-Fluorenyl)ethyl chloroformate |
| FMOC | 9-Fluorenylmethoxycarbonyl chloride |
| FPD | Flame photometric detection |
| FPLC | Fast protein liquid chromatography |
| FTIR | Fourier transform infrared |
| GC-MS | Gas chromatography-mass spectrometry |
| GFC | Gel filtration chromatography |
| GLC | Gas-liquid chromatography |
| GPC | Gel permeation chromatography |
| HAC | Hydroxyapatite chromatography |
| Hb | Haemoglobin |
| HEMA | Hydroxyethylmethacrylate |
| HETP | Height equivalent to a theoretical plate |
| HFBA | Heptafluorobutyric acid |
| HFIP | Hexafluoroisopropanol |
| HIC | Hydrophobic interaction chromatography |
| HILIC | Hydrophilic interaction chromatography |
| HPIC | High performance (pressure) ion chromatography |
| HPLC | High performance liquid chromatography |
| HPTLC | High performance thin layer chromatography |
| HRGC | High resolution gas chromatography |
| HSA | Human serum albumin |
| IAM | Immobilised artificial membrane |
| IC | Ion chromatography |
| ICP | Inductively coupled plasma |
| ICR | Ion cyclotron resonance |
| IEC | Ion-exchange chromatography |
| IMAC | Immobilised metal-ion affinity chromatography |
| IPA | Isopropanol (propan-2-ol) |
| IPC | Ion pair chromatography |
| ISRP | Internal surface reversed-phase |
| IUPAC | International Union of Pure and Applied Chemistry |
| LC-MS | Liquid chromatography-mass spectrometry |
| LDR | Linear dynamic range |
| LDV | Low dead volume |
| LEC | Ligand exchange chromatography |
| LIF | Laser-induced fluorescence |
| LLE | Liquid/liquid extraction |
| MALDI | Matrix assisted laser desorption ionisation |
| MECC | Micellar electrokinetic capillary chromatography |
| MEKC | Micellar electrokinetic chromatography |
| MEPS | Microextraction in packed syringe |
| MMSE | Monolithic material sorptive extraction |
| MS | Mass spectrometer |
| MSD | Mass-selective detection |
| MOS | Monooctylsilane |
| NIR | Near infrared |
| NP | Normal-phase |
| NPR | Non-porous resin |
| ODS | Octadecylsilane |
| OPA | o-Phthalaldehyde |
| PAD | Pulsed amperometric detection |
| PAGE | Polyacrylamide gel electrophoresis |
| PAH | Polycyclic aromatic hydrocarbon |
| PAT | PEEK alloyed with Teflon |
| PCR | Polymerase chain reaction |
| PCR | Post column reaction |
| PEEK | Polyetheretherketone |
| PEG | Polyethylene glycol |
| PEI | Polyethyleneimine |
| PEO | Polyethylene oxide |
| PID | Photoionisation detection |
| PK | Polyketone |
| PLOT | Porous-layer open-tubular |
| PMMA | Polymethylmethacrylate |
| PRP | Polymeric reversed-phase |
| PS-DVB | Polystyrene-divinylbenzene |
| PTC | Phenylthiocarbamyl |
| PTFE | Polytetrafluoroethylene |
| PTH | Phenylthiohydantoin |
| PVA | Polyvinylalcohol |
| PVDF | Polyvinylidene difluoride |
| RAM | Restricted access media |
| RI | Refractive index |
| RP | Reversed-phase |
| SAX | Strong anion-exchanger |
| SCOT | Support-coated open-tubular |
| SCX | Strong cation-exchanger |
| SDS | Sodium decyl (or dodecyl) sulphate |
| SEC | Size (or steric) exclusion chromatography |
| SFC | Supercritical fluid chromatography |
| SFE | Supercritical fluid extraction |
| SIM | Selected (single) ion monitoring |
| SLE | Solid-supported liquid-liquid extraction |
| SMB | Simulated moving bed |
| SPE | Solid phase extraction |
| SPME | Solid phase microextraction |
| SPP | Superficially porous particle |
| SPS | Semi-permeable surface |
| SRM | Standard reference material |
| TEAA | Tetraethylammonium acetate |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMS | Trimethylsilyl |
| UHPLC | Ultra high performance liquid chromatography |
| USP | United States Pharmacopoeia |
| WAX | Weak anion-exchanger |
| WCX | Weak cation-exchanger |
| WCOT | Wall-coated open-tubular |
| ZDV | Zero dead volume |

# HPLC CALCULATIONS



where:

$t_0$ = Retention time of unretained peak
$t_{R1}$ = Retention time of component 1
$t_{R2}$ = Retention time of component 2
$w_{0.5}$ = Peak width at 50% peak height
$w_{0.1}$ = Peak width at 10% peak height
$w_{0.05}$ = Peak width at 5% peak height
w = Peak width at base
A = Peak front distance at 10% of peak height
B = Peak tail distance at 10% of peak height
C = Peak front distance at 5% of peak height
D = Peak tail distance at 5% of peak height

## The following equations are based on the terms defined above.

Adjusted (net) Retention Time ($t'_R$)

$$t'_{R1} = t_{R1} - t_0$$

## Selectivity

Retention Factor (k)

$$k = \frac{t_R - t_0}{t_0} = \frac{t'_R}{t_0}$$

Separation Factor ($\alpha$)

$$\alpha = \frac{k_2}{k_1}$$

## Performance

Column Efficiency (N)
(plate number or number of theoretical plates)

$$N_{0.5} = 5.54\left(\frac{t_R}{w_{0.5}}\right)^2 \text{ or } N_{0.1} = 18.55\left(\frac{t_R}{w_{0.1}}\right)^2$$

Height Equivalent to a Theoretical Plate (HETP)

$$H = \frac{L}{N}$$

where L = length of column

Reduced Plate Height (h)

$$h = \frac{H}{dp}$$

where dp = average particle diameter

## Peak Shape

Asymmetry Factors (see p.10)

$$As_1 = \frac{N_{0.1}}{N_{0.5}} \qquad As_2 = \frac{B}{A}$$

USP Tailing Factor

$$T = \frac{w_{0.05}}{2C}$$

Resolution

$$R_s = 2\left(\frac{t_{R2} - t_{R1}}{w_1 + w_2}\right)$$

or

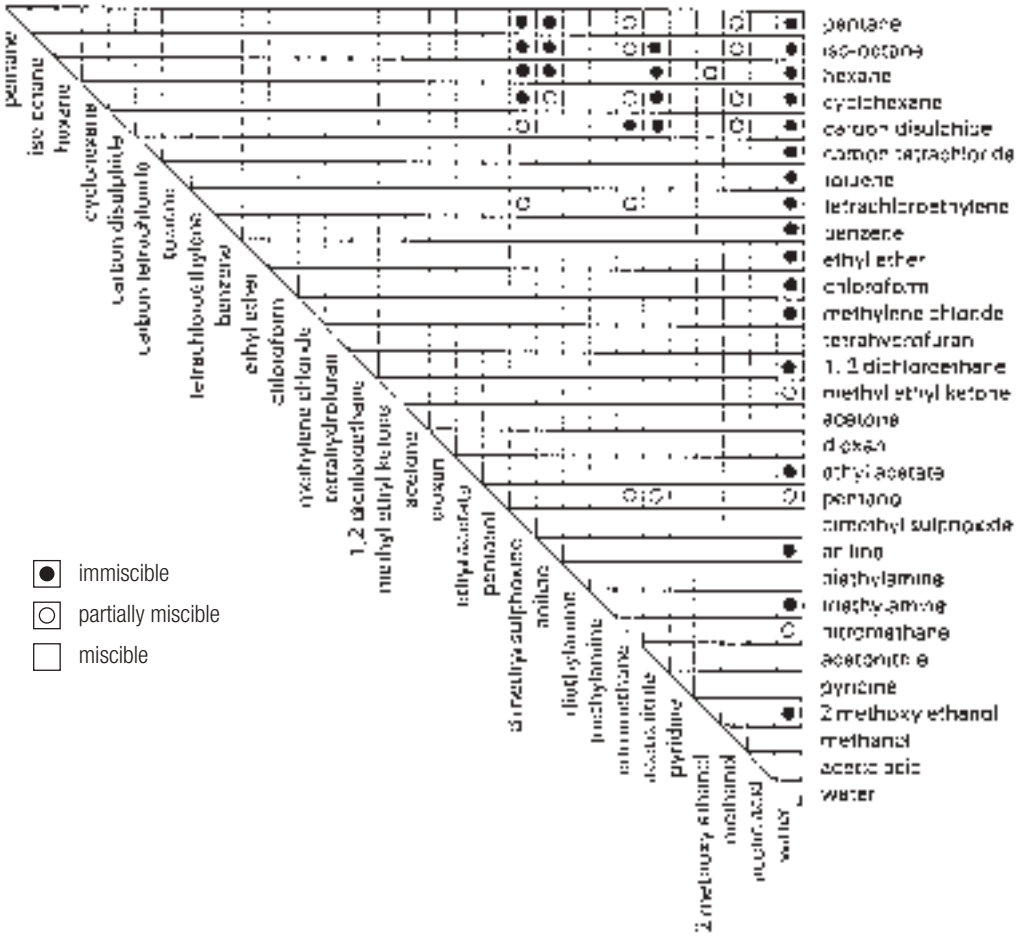
$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

where k is the average retention factor for peaks 1 and 2

# HPLC SOLVENT PROPERTIES



## Miscibility




## Viscosity, Refractive Index and UV cut-off

| Solvent | Viscosity (cPoise) | Refractive Index | UV cut-off (nm) |
|---|---|---|---|
| pentane | 0.23 | 1.358 | 210 |
| iso-octane | 0.50 | 1.404 | 210 |
| hexane | 0.33 | 1.375 | 200 |
| cyclohexane | 1.0 | 1.427 | 210 |
| carbon disulphide | 0.37 | 1.625 | 380 |
| carbon tetrachloride | 0.97 | 1.466 | 265 |
| toluene | 0.59 | 1.496 | 285 |
| tetrachloroethylene | 0.90 | 1.505 | 240 |
| benzene | 0.65 | 1.501 | 280 |
| diethyl ether | 0.23 | 1.353 | 220 |
| chloroform | 0.57 | 1.443 | 235 |
| dichloromethane | 0.44 | 1.424 | 235 |
| tetrahydrofuran | 0.55 | 1.445 | 215 |
| 1, 2 dichloroethane | 0.79 | 1.445 | 225 |
| methyl ethyl ketone | 0.40 | 1.381 | 330 |
| acetone | 0.32 | 1.359 | 330 |
| dioxan | 1.54 | 1.422 | 220 |
| ethyl acetate | 0.45 | 1.370 | 250 |
| 1 pentanol | 4.1 | 1.410 | 210 |
| dimethyl sulphoxide | 2.24 | 1.478 | 290 |
| aniline | 4.4 | 1.586 | 350 |
| diethylamine | 0.38 | 1.387 | 275 |
| triethylamine | 0.38 | 1.401 | 280 |
| nitromethane | 0.67 | 1.394 | 380 |
| acetonitrile | 0.37 | 1.344 | 190 |
| pyridine | 0.94 | 1.510 | 305 |
| 2 methoxy ethanol | 1.72 | 1.401 | 220 |
| methanol | 0.60 | 1.329 | 205 |
| acetic acid | 1.26 | 1.372 | |
| water | 1.0 | 1.330 | – |

# COLUMN CLEANING, REGENERATION AND STORAGE OF SILICA BASED COLUMNS

The routine use of in-line filters and/or guard cartridges (see pages 193-194 and 20-21) is strongly recommended by Hichrom for protection of HPLC columns from both frit blockage and irreversible sample adsorption. However, even with the use of guard cartridges, over a period of time columns may become contaminated by strongly adsorbed sample components. This may be indicated by a steadily increasing back pressure generated by the column, a sudden increase in the back pressure, loss of column efficiency, increased tailing and shouldering or even peak splitting.

The protection of UHPLC columns is also recommended and guard cartridges should be used where available. Guard cartridges capable of withstanding the high pressures required are not available for all manufacturers' UHPLC columns. In these cases, it is advisable to use a precolumn filter which can operate at high pressures (see page 194).

In order to maximise column lifetime, particularly with UHPLC columns, the following tips should be considered:

- Use only ultra-pure UHPLC/HPLC grade solvents
- Use freshly prepared aqueous mobile phases to discourage bacterial growth
- Filter all samples, standards and mobile phases (eg. 0.2µm filter)
- Use an in-line filter system
- Perform sample clean-up on dirty samples

Please note that in cases of irreversible compound adsorption or column voiding, it may not be possible to regenerate the column.

## Column Flushing

If a deterioration in column performance or increase in back pressure is observed, then a column cleaning or regeneration procedure can be undertaken, using a series of stronger solvent combinations. It is strongly advised to read the 'Care and Use' instructions provided by the manufacturer before commencing this procedure. While the majority of spherical 3, 5 and 10µm particle size columns can be reverse flushed or even used in the reverse direction, some 3µm columns can only be reverse flushed for a short time and should not be used in the reverse direction afterwards. For irregular particle columns, it is advisable to flush in the normal direction of flow.

For UHPLC (≤2µm) columns, the manufacturer's 'Care and Use' instructions must be consulted before considering reverse flushing the column. In some cases, it may be preferable to flush the UHPLC column in the normal direction of flow.

It is recommended that the column efficiency is measured before and after any clean-up procedure or long term storage, using either the column test conditions given on the manufacturer's test chromatogram or conditions from the method being followed. This enables the effectiveness of any cleaning procedure to be monitored.

## Column Cleaning Procedures

The following general procedures are recommended for regeneration of column performance.

1. Disconnect and if applicable reverse the column.
2. Connect the column to the pump, but not the detector.
3. Follow the appropriate flushing procedure for the type of column (see page 219), using 10-20 column volumes of each solvent (see table below). Always make sure that the last solvent used will be compatible with the mobile phase.
4. The flow rate should not exceed that specified on the QC chromatogram for the particular column, but preferably should be maintained at 25-50% of the normal working flow rate.

### Column Volumes (ml)

| I.D. \ Length | 50mm | 150mm | 250mm |
|---|---|---|---|
| 2.1mm | 0.17 | 0.52 | 0.87 |
| 3.2mm | 0.4 | 1.2 | 2.0 |
| 4.6mm | 0.83 | 2.5 | 4.2 |
| 10.0mm | 3.9 | 11.8 | 19.6 |
| 21.2mm | 19.3 | 57.8 | 96.3 |

## Column Cleaning, Regeneration and Storage of Silica Based Columns (continued)

### Flushing Procedures for Various Types of Column

#### Reversed-phase columns
(eg. C18, C8, C4, Phenyl, CN, 'AQ' type)
- a) Mobile phase without buffer
- b) Methanol
- c) Acetonitrile
- d) Acetonitrile/IPA (75:25)
- e) IPA
- f) Dichloromethane
- g) Hexane

In many cases, the sequence a) to e) may be sufficient. If step f) or g) is necessary, flush with IPA before returning to mobile phase.

If metal ions are thought to be causing contamination, flush with aqueous 0.05M EDTA followed by water.

Columns which have been used with ion-pairing reagents are best dedicated to that method and kept for this purpose.

#### Reversed-phase columns used for protein/peptide analysis
- a) Mobile phase without buffer
- b) Gradient of 10 – 90% B where
  A = 0.1% TFA in water
  B = 0.1% TFA in acetonitrile

#### Unbonded silica columns (SIL)
- a) IPA
- b) Methanol
- c) Ethyl acetate

#### Bonded normal-phase columns
(CN, $NH_2$, Diol)
- a) Chloroform
- b) IPA
- c) Methylene chloride
- d) Hexane

#### Anion-exchange columns
(SAX, WAX)
- a) Water
- b) Methanol
- c) Chloroform
- d) Methanol
- e) Water

#### Cation-exchange columns
(SCX, WCX)
- a) Water (inject 4x 200µl DMSO during flush)
- b) Tetrahydrofuran

#### Size exclusion columns for proteins
For weakly retained proteins
- a) 0.1M phosphate buffer, pH 3

For strongly retained proteins
- a) Gradient of 100% water to 100% acetonitrile over 60 minutes

If you require further information on flushing procedures for specific columns, please contact the Technical Support group at Hichrom.

### Storage conditions for silica based HPLC columns

The conditions under which a column is stored will affect its lifetime. All buffers, salts and ion-pairing reagents should be flushed from the column before storage. Ideally, the storage solvent should be as shown on the initial column test chromatogram provided by the manufacturer. Column end plugs (see page 188) should be fitted to prevent solvent evaporation and the subsequent drying out of the packing bed.

# TRADEMARKS



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| | |
|---|---|
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| Accucore | Thermo Fisher Scientific Inc. |
| ACE | Advanced Chromatography Technologies Ltd |
| AKTA | GE Healthcare BioSciences AB |
| All-Guard | Alltech Associates, Inc. |
| ALUGRAM | Macherey-Nagel GmbH & Co. KG |
| Aluspher | Merck KGaA, Darmstadt, Germany |
| AmyCoat | Akzo Nobel Pulp & Performance Chemicals |
| ANALYTIC-BARRIER | Mitsubishi Chemical Corp. |
| AQ (Advanced Quality) | MicroSolv Technology Corporation |
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| Core Enhanced Technology | Thermo Fisher Scientific Inc. |
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| COSMOSIL | Nacalai Tesque |
| CROWNPAK | Daicel Corporation |
| Cyclobond | Sigma-Aldrich Co. |
| Defender | Thermo Fisher Scientific Inc. |
| Denali | Alltech Associates, Inc. |
| Develosil | Nomura Chemical Co. |
| DiamondBond | ZirChrom Separations, Inc. |
| DNAPac | Thermo Fisher Scientific Inc. |
| DNASwift | Thermo Fisher Scientific Inc. |
| DryLab | Molnar-Institut, Germany |
| EBT | Advanced Chromatography Technologies Ltd |
| Endeavorsil | Dikma Technologies Inc. |
| Epic | ES Industries |
| Eternity | Akzo Nobel Pulp & Performance Chemicals |
| Everest | Alltech Associates, Inc. |
| Excel | Advanced Chromatography Technologies Ltd |
| Exsil | W.R. Grace & Co.-Conn. |
| FlashMaster | Biotage AB |
| Fluophase | Thermo Fisher Scientific Inc. |
| FunCap | GL Sciences, Inc. |
| Fused-Core | Advanced Materials Technology, Inc. |
| GammaBond | ES Industries |
| GASTIGHT | Hamilton Co. |
| Genesis | W.R. Grace & Co.-Conn. |
| GL-Tip | GL Sciences, Inc. |
| GlycanPac | Thermo Fisher Scientific Inc. |
| Grace | W.R. Grace & Co.-Conn. |
| GraceSmart | Alltech Associates, Inc. |
| GreenSep | ES Industries |
| Guard-Pak | Waters Corporation |
| HALO | Advanced Materials Technology, Inc. |
| Hibar | Merck KGaA, Darmstadt, Germany |
| HSC | Advanced Chromatography Technologies Ltd |
| HYDRODEX | Macherey-Nagel GmbH & Co. KG |
| Hypercarb | Thermo Fisher Scientific Inc. |
| HyperSep | Thermo Fisher Scientific Inc. |
| Hypersil | Thermo Fisher Scientific Inc. |
| Hypersil GOLD | Thermo Fisher Scientific Inc. |
| Impaq | Silicycle Inc. |
| InertCap | GL Sciences, Inc. |
| InertSep | GL Sciences, Inc. |
| Inertsil | GL Sciences, Inc. |
| InertSustain | GL Sciences, Inc. |
| Inspire | Dikma Technologies Inc. |
| Isco | Teledyne Isco Inc. |
| Kromasil | Akzo Nobel Pulp & Performance Chemicals |
| Last Drop | VICI Jour, VICI AG International |
| Leapsil | Dikma Technologies Inc. |
| LiChroCART | Merck KGaA, Darmstadt, Germany |
| LiChroprep | Merck KGaA, Darmstadt, Germany |
| LiChrosorb | Merck KGaA, Darmstadt, Germany |
| LiChrospher | Merck KGaA, Darmstadt, Germany |
| LIPODEX | Macherey-Nagel GmbH & Co. KG |
| manu-CART | Merck KGaA, Darmstadt, Germany |
| MCI GEL | Mitsubishi Chemicals |
| MICRA | Eprogen, Inc. |
| MICROLITER | Hamilton Co. |
| MicroTight | IDEX Health & Science LLC |
| MonoSpin | GL Sciences, Inc. |
| MonoTip | GL Sciences, Inc. |
| MonoTrap | GL Sciences, Inc. |
| MRQ | MicroSolv Technology Corporation |
| nanoViper | Thermo Fisher Scientific Inc. |
| Nova-Pak | Waters Corporation |
| NUCLEOCEL | Macherey-Nagel GmbH & Co. KG |
| NUCLEODEX | Macherey-Nagel GmbH & Co. KG |
| NUCLEODUR | Macherey-Nagel GmbH & Co. KG |
| NUCLEOGEL | Macherey-Nagel GmbH & Co. KG |
| NUCLEOGEN | Macherey-Nagel GmbH & Co. KG |
| NUCLEOSHELL | Macherey-Nagel GmbH & Co. KG |
| NUCLEOSIL | Macherey-Nagel GmbH & Co. KG |
| NuTip | Glygen Corp. |
| Obelisc | SIELC Technologies, Inc. |
| OPTIMA | Macherey-Nagel GmbH & Co. KG |
| OPTIMAPAK | RStech Corp. |
| Partisil | Hichrom Ltd |
| Partisphere | Hichrom Ltd |
| PEEK | Victrex Plc |
| PEEKsil | SGE Analytical Science Pty Ltd |
| PepMap | Thermo Fisher Scientific Inc. |
| PepSwift | Thermo Fisher Scientific Inc. |
| PLgel | Agilent Technologies Inc. |
| PLRP-S | Agilent Technologies Inc. |
| POLYGRAM | Macherey-Nagel GmbH & Co. KG |
| Primesep | SIELC Technologies, Inc. |
| PrincetonSFC | Princeton Chromatography, Inc. |
| PrincetonSPHER | Princeton Chromatography, Inc. |
| ProPac | Thermo Fisher Scientific Inc. |
| ProSwift | Thermo Fisher Scientific Inc. |
| ProTain | ZirChrom Separations, Inc. |
| Purospher | Merck KGaA, Darmstadt, Germany |
| Reacti-Therm | Thermo Fisher Scientific Inc. |
| Regis | Regis Technologies, Inc. |
| Regisil | Regis Technologies, Inc. |
| RegisCell | Regis Technologies, Inc. |
| RegisPack | Regis Technologies, Inc. |
| Resolve | Waters Corporation |
| RheBuild | IDEX Health & Science LLC |
| Rheodyne | IDEX Health & Science LLC |
| RSA | MicroSolv Technology Corporation |
| Sachtopore | Sachtleben Chemie GmbH |
| SeQuant | Merck KGaA, Darmstadt, Germany |
| Sentry | Waters Corporation |
| Servo | Thermo Fisher Scientific Inc. |
| SHARC | SIELC Technologies, Inc. |
| Shodex | Showa Denko K.K. |
| SiliaSphere | Silicycle Inc. |
| SilTite | SGE Analytical Science Pty Ltd |
| Slipfree | Thermo Fisher Scientific Inc. |
| SOLA | Thermo Fisher Scientific Inc. |
| SolventTrak | Axxiom Chromatography Inc. |
| Spherisorb | Waters Corporation |
| Spursil | Dikma Technologies Inc. |
| SuperC18 | Advanced Chromatography Technologies Ltd |
| SuperPhenylHexyl | Advanced Chromatography Technologies Ltd |
| Superspher | Merck KGaA, Darmstadt, Germany |
| Symmetry | Waters Corporation |
| SymmetryShield | Waters Corporation |
| SynChropak | Eprogen, Inc. |
| Syncronis | Thermo Fisher Scientific Inc. |
| Systec AF | IDEX Health & Science LLC |
| Titansphere | GL Sciences, Inc. |
| TopTip | Glygen Corp. |
| TOYOPEARL | Tosoh Corporation |
| ToyoScreen | Tosoh Corporation |
| TRACE | Thermo Fisher Scientific Inc. |
| TraceGOLD | Thermo Fisher Scientific Inc. |
| TracePLOT | Thermo Fisher Scientific Inc. |
| Trinity | Thermo Fisher Scientific Inc. |
| TSKgel | Tosoh Corporation |
| TYPE-C | MicroSolv Technology Corporation |
| UltraCore | Advanced Chromatography Technologies Ltd |
| Ultrasphere | Hichrom Ltd |
| Ultron | Shinwa Chemical Industries |
| Uniguard | Thermo Fisher Scientific Inc. |
| UPLC | Waters Corporation |
| Verify | Thermo Fisher Scientific Inc. |
| Viper | Thermo Fisher Scientific Inc. |
| VisionHT | Alltech Associates, Inc. |
| Vydac | Alltech Associates, Inc. |
| Wakopak | Wako Chemicals GmbH |
| Whelk-0 | Regis Technologies, Inc. |
| ZIC | Merck KGaA, Darmstadt, Germany |
| ZirChrom | ZirChrom Separations, Inc. |
| ZORBAX | Agilent Technologies Inc. |
| ZWIX | Daicel Corporation |

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# NOTES

# TERMS AND CONDITIONS OF SALE



## Governing Terms

These terms and conditions of sale (Terms) shall apply to all contracts for the sale and supply of products and services by Hichrom Ltd (Hichrom). Except as expressly agreed by authorized representatives of Hichrom in writing, no other terms and conditions, including any terms and conditions attached to the buyer's request for quotation, acknowledgement, purchase order or other contract documentation, shall apply to the sale. Each order for goods shall be considered to be an offer by the buyer to purchase the goods from Hichrom subject to these Terms and no order will be deemed to be accepted by Hichrom until a written acknowledgement of order is issued or the goods are delivered to the buyer (if earlier).

## Delivery and Risk

Hichrom makes every effort to deliver all goods in a timely and cost efficient manner, but any dates specified for delivery of the goods are intended to be an estimate and time for delivery shall not be made of the essence by notice. Within the UK orders less than £500 in value will be delivered by first class post. Goods worth in excess of £500 will be dispatched via overnight courier service. If requested, urgent deliveries can be made by overnight or same day courier for an additional charge. Risk of loss for all UK orders will pass to the customer on arrival at the specified delivery address. In the case of orders for supply outside of the UK, risk of loss shall pass to the customer upon collection by the carrier.

## Lost or Damaged Goods

In the case of orders for supply within the UK, Hichrom will make good or replace (at its option) all goods which are lost or damaged during transit, provided that notification of damage or non-delivery is received within 3 days after delivery in the case of damage, and within 7 days after receipt of invoice in the case of non-delivery.

## Returns and Cancellation

Hichrom may, at its sole discretion, accept returns. Returns will only be accepted following prior authorization from Hichrom, after which they must be returned within 30 days. Hichrom is not liable for goods returned without authorization. Returns are subject to payment of a handling charge if required by Hichrom. Hichrom may at its sole discretion, accept cancellation of orders subject to a payment of a cancellation charge if required by Hichrom.

## Title

Ownership of the goods will not pass to the buyer until Hichrom has received in full (in cash or cleared funds) all sums due to it in respect of the goods and all other sums which are or become due to Hichrom from the buyer on any account.

## Prices

Unless otherwise agreed by Hichrom, the price for the goods shall be the price set out in Hichrom's published price list current as at the date of order. Hichrom reserves the right to change the published price list without notice. However, Hichrom will attempt to notify the customer for approval prior to shipment in the event of significant price changes. All prices are quoted exclusive of any value added tax and all costs of packaging, carriage and insurance all of which amounts the buyer will pay in addition at the appropriate rate at the time that payment for the goods or services is due. In addition, in the case of orders for supply outside the UK, the buyer is responsible for the payment of all taxes, charges, levies or duties of any kind payable on the supply of the goods. A minimum value of £50 (net of tax, packaging, carriage, insurance and all other charges which may apply) applies to all orders.

## Payment

All invoices are payable in full in UK pounds sterling 30 days from the invoice date. Time for payment shall be of the essence and no payment shall be deemed to have been received until Hichrom has received cleared funds. Accounts outstanding for more than 60 days from invoice date may be placed on hold until payment is made. Hichrom may change credit or payment terms at any time when, in Hichrom's opinion, the customer's financial condition, previous payment record, or nature of customer's relationship with Hichrom so warrants. Hichrom reserves the right to place any account on a prepayment basis. Payment may be made by bank transfer, credit card or cheque.

## Warranty

Hichrom warrants that, as at the date of delivery to the buyer, all products manufactured and distributed by Hichrom will meet Hichrom's publicly disclosed performance and quality standards, be free from defects in materials and workmanship and be of satisfactory quality within the meaning of the Sale of Goods Act 1994. For items distributed but not manufactured by Hichrom, the warranty is limited to the terms of the original manufacturer's warranty. For Hichrom manufactured products notification of any defect must be made to Hichrom within 90 days after delivery of the goods. At its discretion, Hichrom will replace products proven to be defective. If this is not achievable in a reasonable time, the buyer shall be entitled to a refund of the purchase price. This warranty shall not apply to any defect, failure or damage caused by the buyer's failure to follow Hichrom's oral or written instructions as to storage, use or maintenance of the goods or by any other improper use or improper or inadequate maintenance or care. Except as set out above, all warranties, conditions and other terms which may be implied by statute or common law are, to the fullest extent permitted by law, excluded from these Terms.

## Limitation of Liability

Nothing in these Terms excludes or limits the liability of Hichrom for death or personal injury caused by Hichrom's negligence or for fraudulent misrepresentation. Subject to the first sentence of this paragraph, Hichrom shall not be liable to the buyer for any indirect or consequential liability, loss or damage (including without limitation, loss of profit, loss of business or depletion of goodwill), costs, expenses or claims for consequential compensation arising from the use of, or in conjunction with, the goods supplied. In addition, but subject to the first sentence of this paragraph, Hichrom's total liability in contract, tort (including negligence or breach of statutory duty), misrepresentation or otherwise arising in connection with the performance or contemplated performance of this contract will be limited to the invoice price of the product.

## Severance

If any provision of these Terms is found by any court or other authority of competent jurisdiction to be invalid or unenforceable in whole or in part, it will be deemed severable and the remainder of such provision will continue in full force and effect.

## Governing Law

All contracts incorporating these Terms shall be subject to English law and the exclusive jurisdiction of the English courts.

# HICHROM

## Chromatography Technical Advice Desk

**Contact our experts on any chromatography issue for friendly, no obligation, free of charge, professional support and advice:**

- Troubleshooting
- Method development
- Reproducibility advice
- Column identification
- Batch reservation
- Column selection
- Method optimisation
- Application support
- Cost reduction
- Custom packing
- Method transfer
- Custom bonding

HiCHROM

Contact us on +44 (0)118 930 3660
or technical@hichrom.co.uk

# HICHROM

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- Trinidad
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- Turkey
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- Uganda
- Ukraine
- Uruguay
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- US Virgin Islands
- Venezuela
- Vietnam
- Zimbabwe

Hichrom products are available worldwide through our International Network of Distributors...

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