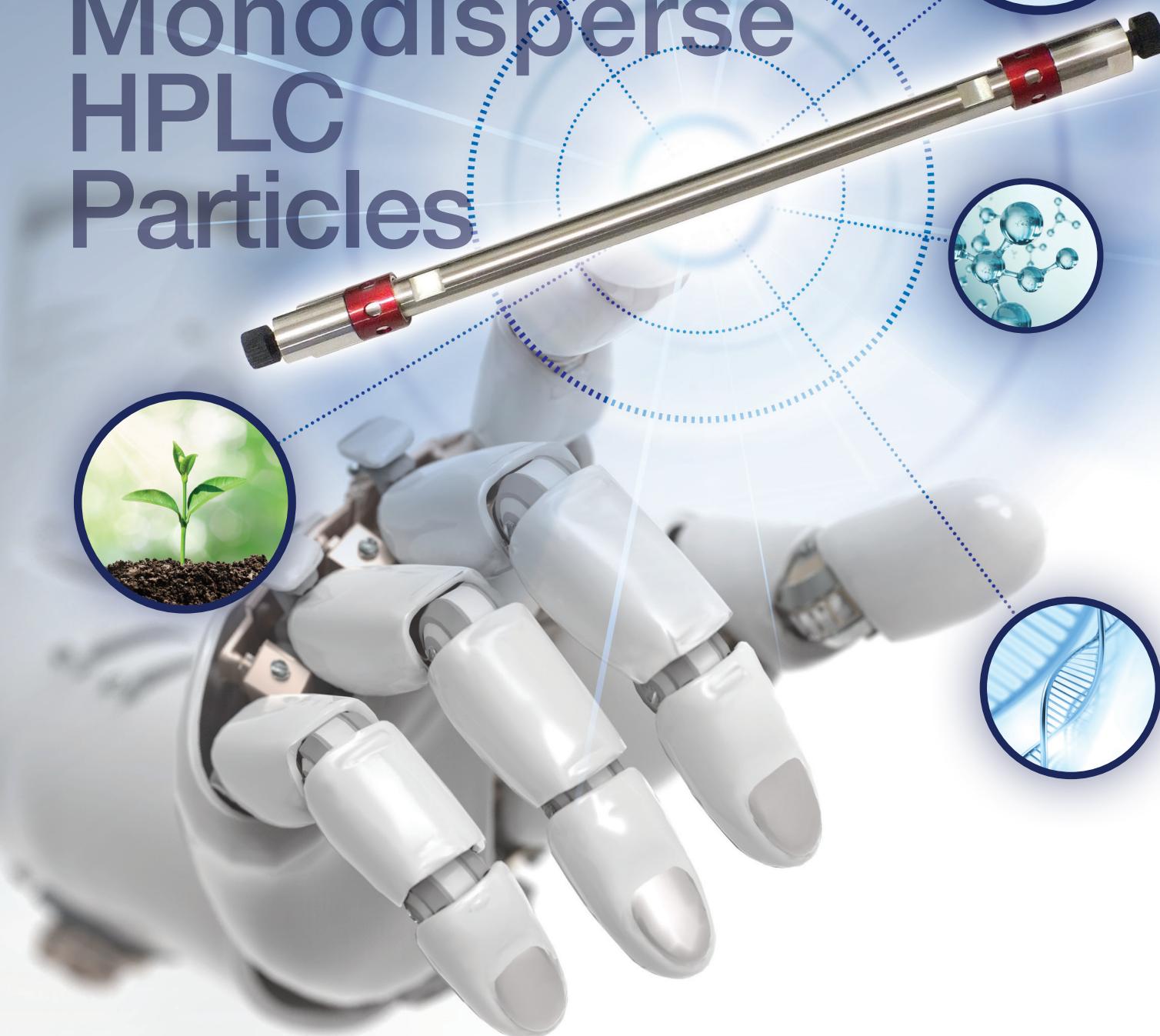


# Monodisperse HPLC Particles

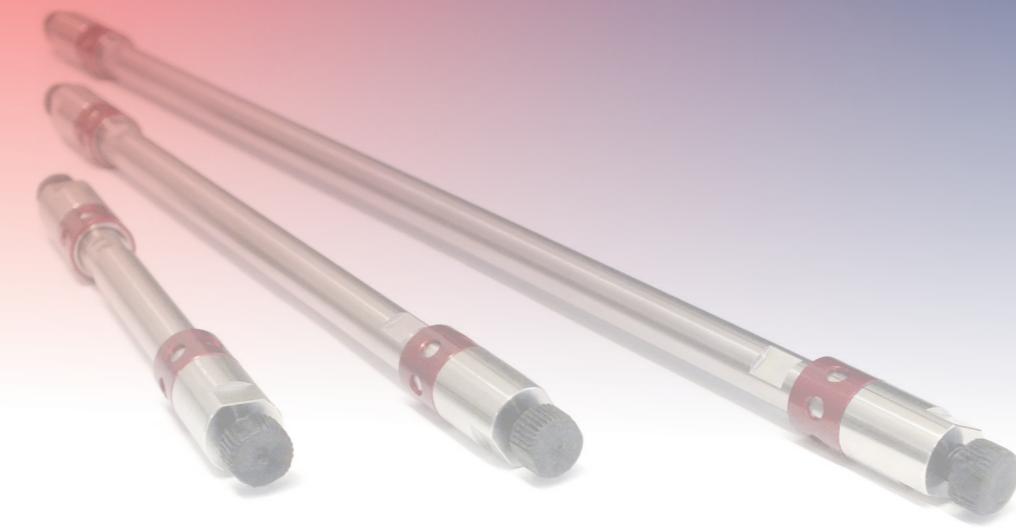


The Evolution of HPLC Columns

**EVOSPHERE**



# EVO SPHERE



## Monodisperse HPLC Columns

Fortis Technologies has designed a new fully porous monodisperse particle for use in HPLC columns. Combining this with a new range of selectivities gives the analyst the ability for high resolution, high efficiency separations.

Based upon a fully porous silica monodisperse particle, Evosphere® is the evolution of particle technology.

Combine a high efficiency particle with low backpressure, high loadability, scaleability and reproducibility and you have the ultimate combination.

Then add in novel selectivity options to provide enhanced resolution and selectivity and you have the capability to separate

more compounds in less time with greater sensitivity.

By building on a pure silica substrate method development and method transfer become more robust and reproducible across platforms as you scale from capillary to preparative.



# Monodisperse Particles



## Particle size distribution (D90/10)

When assigning a measurement to characterise a particle size distribution the ratio of D90/10 is often quoted, and as such can be used to gauge the degree of size uniformity of the particles.

The parameter D90 signifies the point in the size distribution, up to and including which, 90% of the total volume of material in the sample is 'contained'. For example; if the D90 is 6 $\mu\text{m}$ , this means that 90% of the sample has size of 6 $\mu\text{m}$  or smaller. The definition for D50, is then the size point below which 50% of the material is contained. Similarly, the D10 is the size below which 10% of the material is contained. This description has long been used in size distribution measurements.

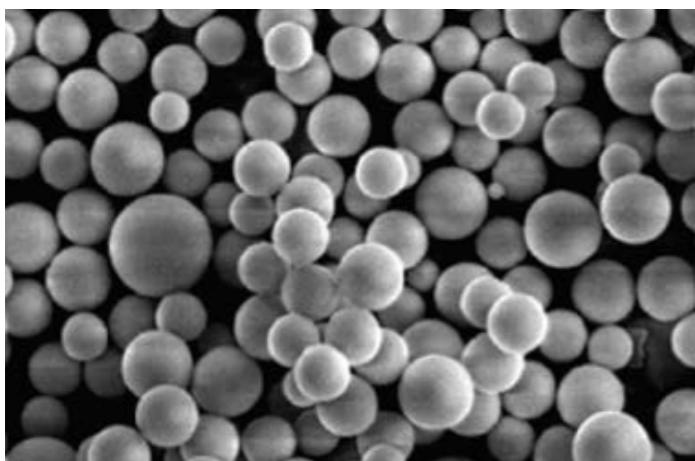
As the particle size distribution for chromatographic silica moves towards monodisperse then the D90 and D10 values become closer together and the D90/10 value tends towards a value of 1.

## Particle Morphology

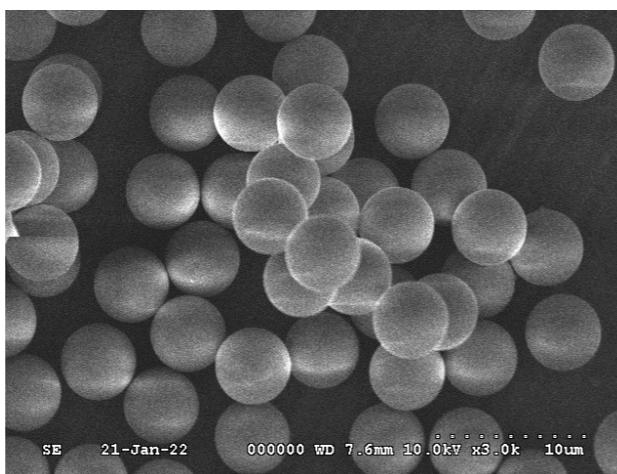
Evosphere silica particles are manufactured to provide a high degree of monodispersity with a uniform smooth surface. Monodispersity generates high efficiency HPLC columns due to the reduced flow path dispersion (Eddy diffusion)

SEM imagery of the Evosphere in comparison with traditional particles highlights the much narrower size distribution.

Monodisperse Evosphere particles are available in 1.7 $\mu\text{m}$ , 3 $\mu\text{m}$  and 5 $\mu\text{m}$  particle sizes.

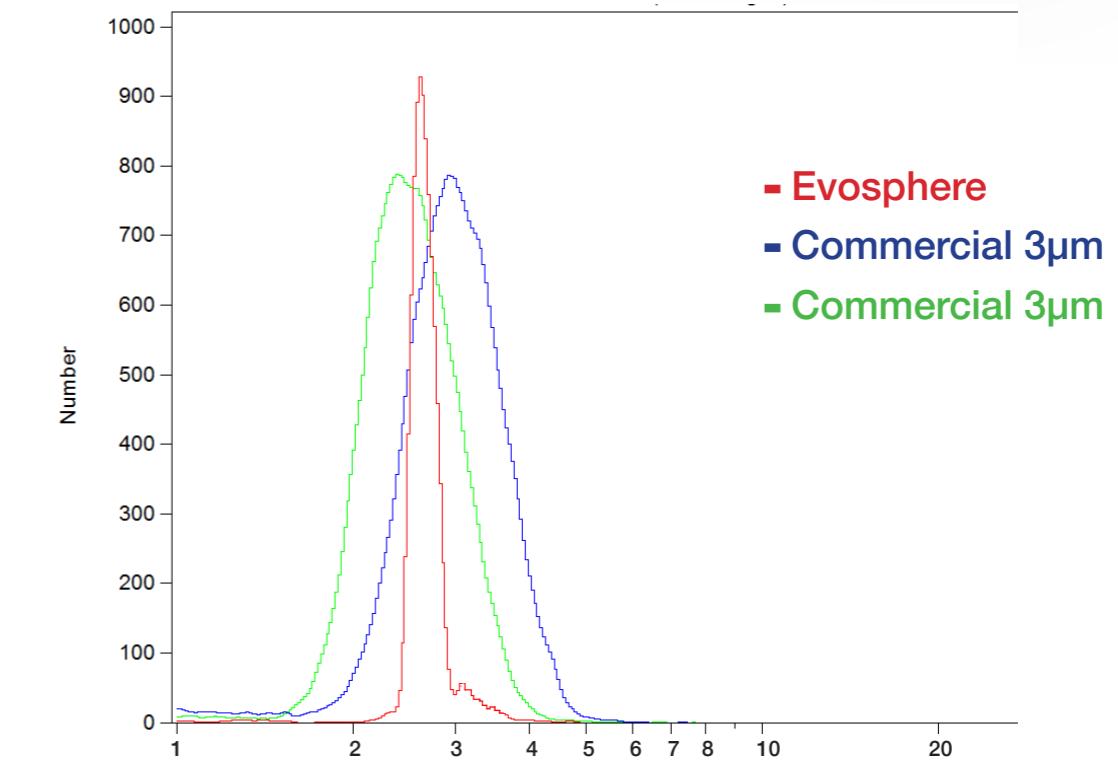


Traditional  
porous particles



Monodisperse  
porous particles

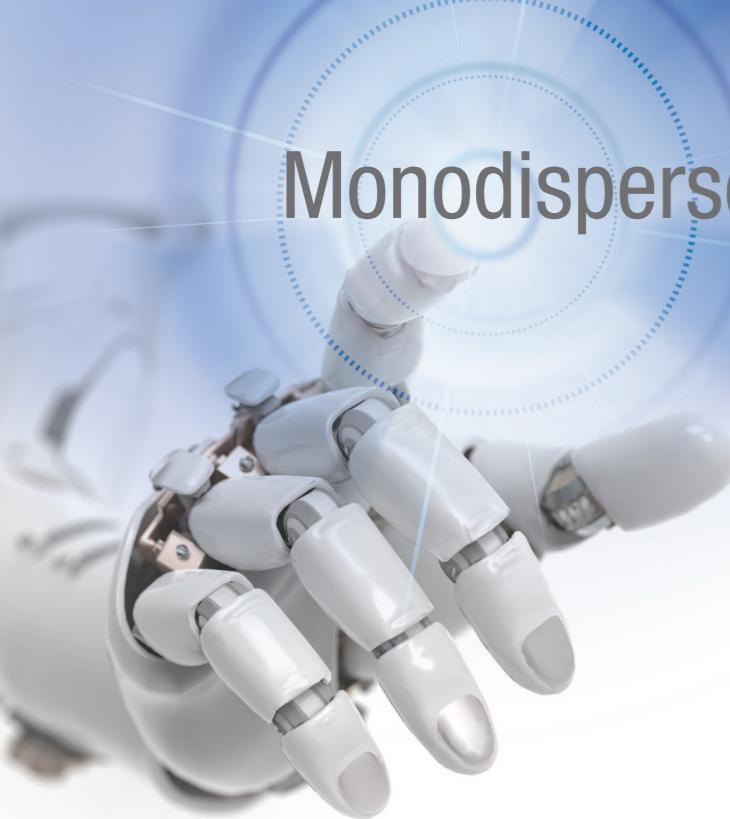
## Particle Size Distribution



	Monodisperse Silica	Commercial 3 $\mu\text{m}$ Silica-A	Commercial 3 $\mu\text{m}$ Silica-B
Median Particle size (d <sub>50</sub> )*	2.66 $\mu\text{m}$ *	2.48 $\mu\text{m}$	2.97 $\mu\text{m}$
SEM Particle Size	3.0 $\mu\text{m}$	2.8 $\mu\text{m}$	3.3 $\mu\text{m}$
D90/10	<b>1.12</b>	<b>1.58</b>	<b>1.61</b>
Pore Volume	0.89	0.88	0.89

\* Measured by Coulter Counter

# Monodisperse Particles



## Loading Capacity

Fortis Evosphere has a high surface area ( $350\text{m}^2/\text{g}$ ) as per many modern Type B porous silica's, this allows loadability of compounds to be high for purification purposes.

Evosphere is available from capillary scale dimensions all the way up to preparative columns.

If you compare this to core-shell particles which typically have a surface area in the region of  $130\text{m}^2/\text{g}$  you will quickly see overload and compromised peak shapes, meaning scale up of methods can be difficult.



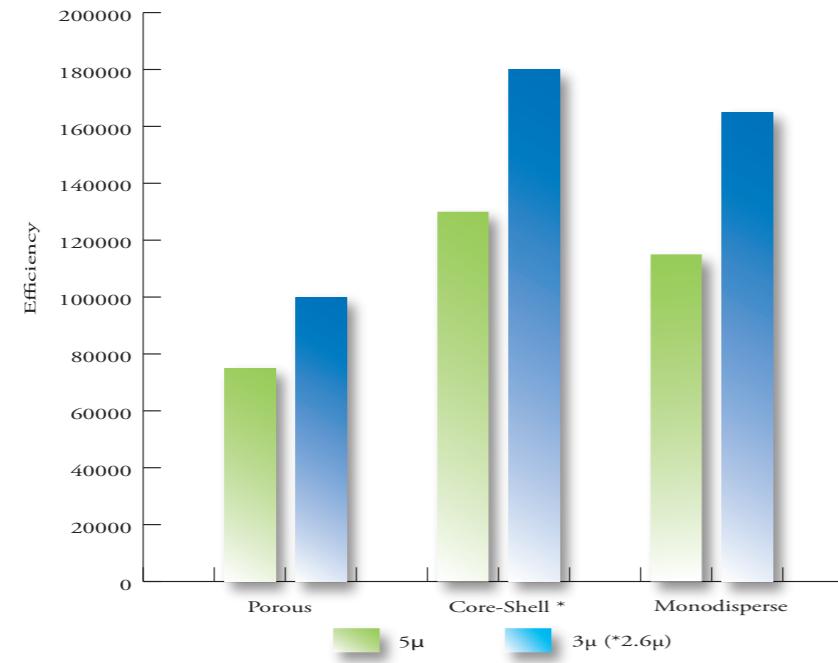
## Efficiency of Monodisperse particles

Analyst have had two ways of improving efficiency in the past. Move to a smaller particle with associated high backpressure and the need to buy a UHPLC instrument, or move to core-shell particles but with a compromise in loading and scalability.

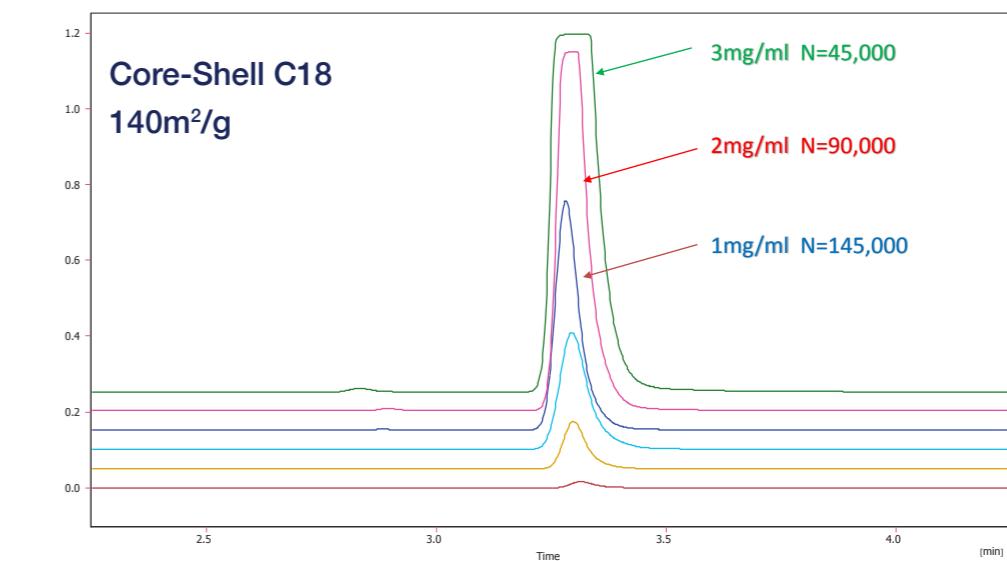
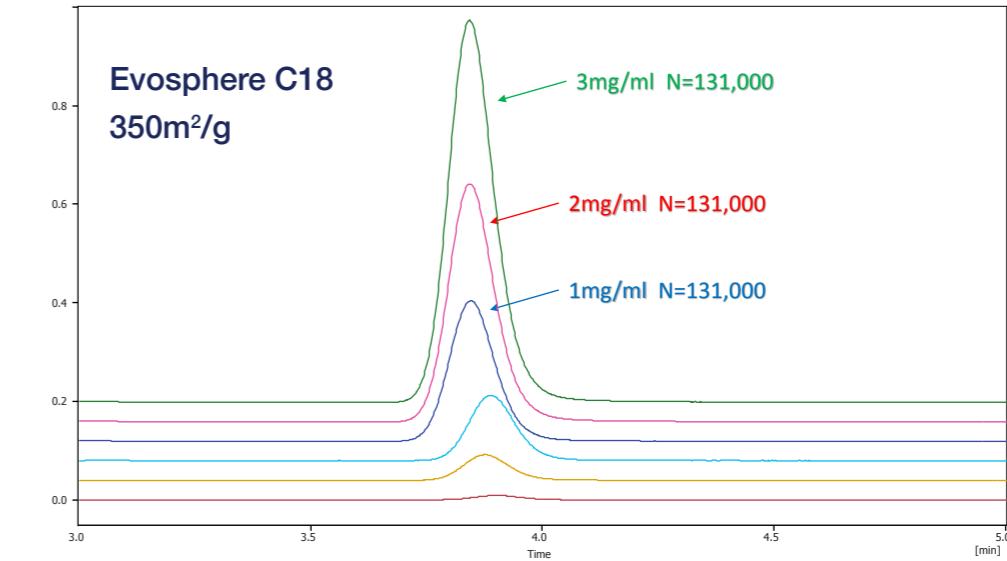
Evosphere fully porous monodisperse particles have vastly increased efficiency over equivalent porous particle sizes. Due to maintaining high surface area, loading and retention time are not compromised as seen with core-shell particles.

- High Efficiency
- High Loading
- Scalable - capillary to Prep
- Robust
- Reproducible

## Typical Efficiencies of HPLC particles



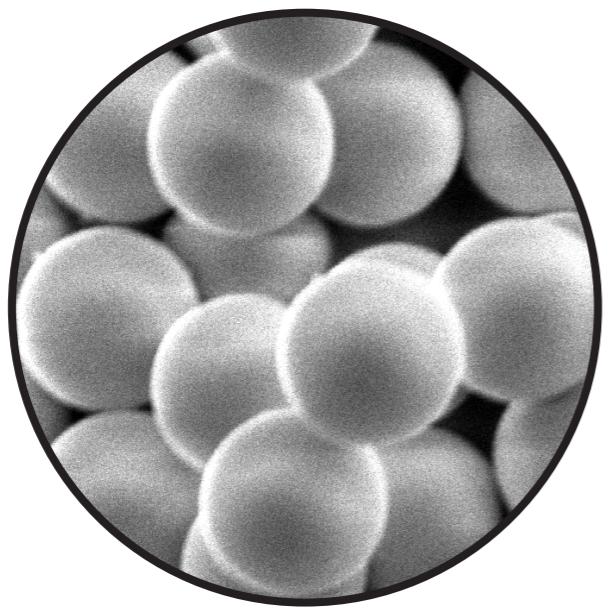
## Comparison of loading capacity



# Selectivity Choices



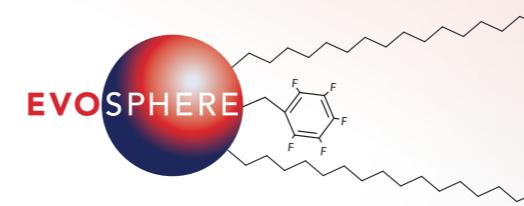
Fortis Evosphere® columns are the very latest in HPLC particle technology. Incorporating our optimised bonding and packing practices with a fully porous monodisperse particle provides the analyst with the ability to speed up analysis, increase efficiency and improve resolution over 'traditional' 3µ & 5µ particles even on normal 400bar HPLC systems.



**Evosphere Monodisperse Particles**

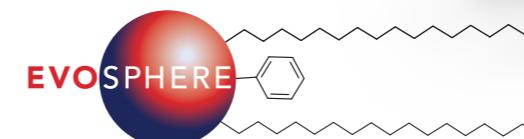
	Particle Size	Surface Area	Pore Size	% C	pH range	USP
Evosphere C12	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	17	1-9	L87
Evosphere C18/AR	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	17	2-9	L1
Evosphere C18/PFP	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	17	2-9	L1
Evosphere RP18-Amide	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	20	2-9	L60
Evosphere Phenyl-Hexyl	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	14	2-9	L11
Evosphere Diphenyl	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	15	2-9	L11
Evosphere PFP	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	13	2-9	L43
Evosphere AQUA	1.7µm 3µm 5µm	350m <sup>2</sup> /g	100Å	18	2-9	L96

## Stationary Phase Choice



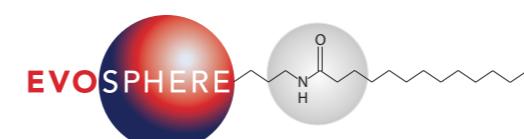
- Evosphere C18/PFP
- Orthogonal Selectivity
- Method development starting point

Evosphere C18/PFP is designed to provide characteristics which will enhance method development. It provides the ability to obtain sharp peak shapes whilst retaining and separating a wide variety of compounds both hydrophobic and hydrophilic.



- Evosphere C18/AR
- Orthogonal Selectivity
- Method development starting point

Evosphere C18/AR is designed to provide increased resolution between compounds, having a combination of hydrophobicity and aromatic selectivity will lead to enhanced resolution. USP L1 column.



- Evosphere RP18-Amide
- Orthogonal Selectivity
- Excellent method development option

Evosphere RP18-Amide is designed to provide polar characteristics which will enhance resolution in method development. It provides orthogonal selectivity to alkyl chain phases due to its polar-embedded group. Sharp peak shapes, extra selectivity and increased retention can all be obtained.



- Evosphere Diphenyl
- Separate positional isomers
- Stable ligand, No "MS" bleed

Evosphere Diphenyl is designed to provide pi-pi, steric and hydrophobic characteristics which will enhance selectivity and the ability to develop methods. Particularly suited to positional isomers and other closely related species such as metabolites.



- Evosphere Phenyl-Hexyl
- Separate metabolites
- Excellent resolution

Evosphere Phenyl-Hexyl is designed to provide characteristics which will enhance selectivity. It provides alternate selectivity to a pure hydrophobic stationary phase whilst still maintaining the key attributes of robustness and reproducibility.



- Evosphere AQUA
- Separate polar species
- Excellent stability

Evosphere AQUA is designed to provide characteristics which will enhance retention of highly polar analytes. Reproducible surface characteristics provide robust separations. Combination of hydrophobic and hydrophilic nature.

# Selectivity Choices



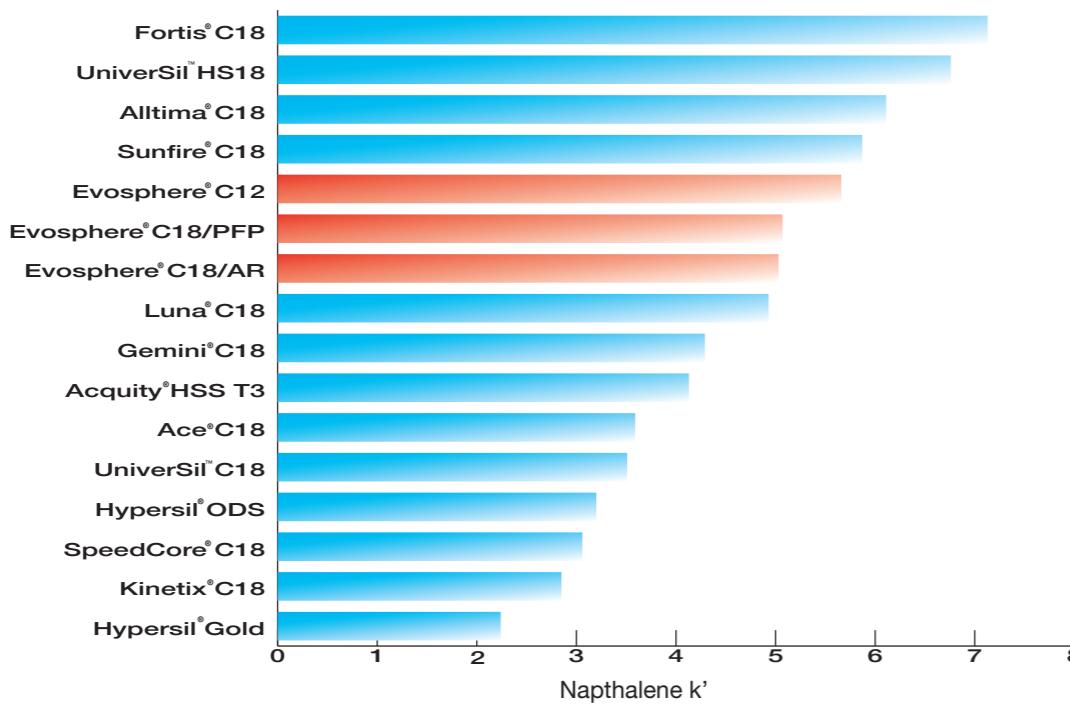
- **Evosphere C12**
- **Ultra High Efficiency**
- **Method development starting point**

Evosphere C12 is designed to provide characteristics which will enhance method development. The dense C12 ligand provides the ability to obtain sharp peak shapes whilst retaining and separating a wide variety of acid, base and neutral compounds with excellent robustness.

- **Evosphere PFP**
- **Orthogonal Selectivity**
- **Combined with Ultra High Efficiency particles**

Evosphere PFP (PentaFluoroPhenyl) is designed to provide characteristics which will enhance selectivity. It provides alternate selectivity to a hydrophobic stationary phase whilst still maintaining the key attributes of robustness and reproducibility.

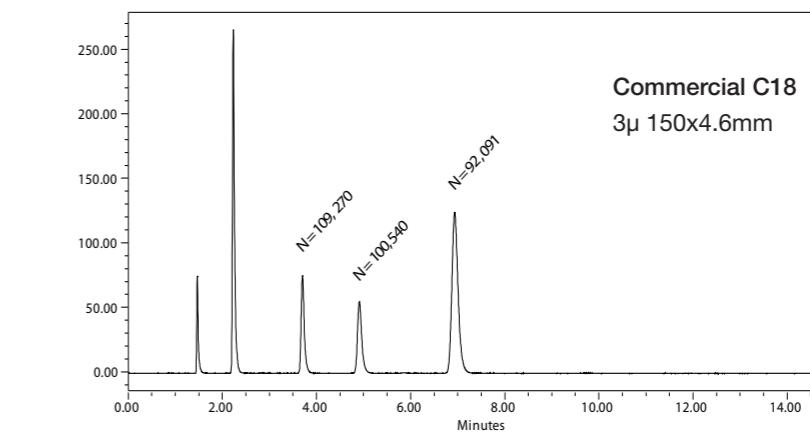
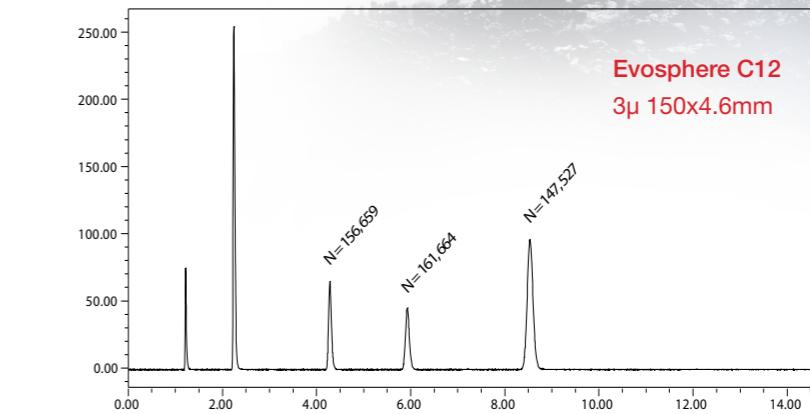
## Hydrophobicity



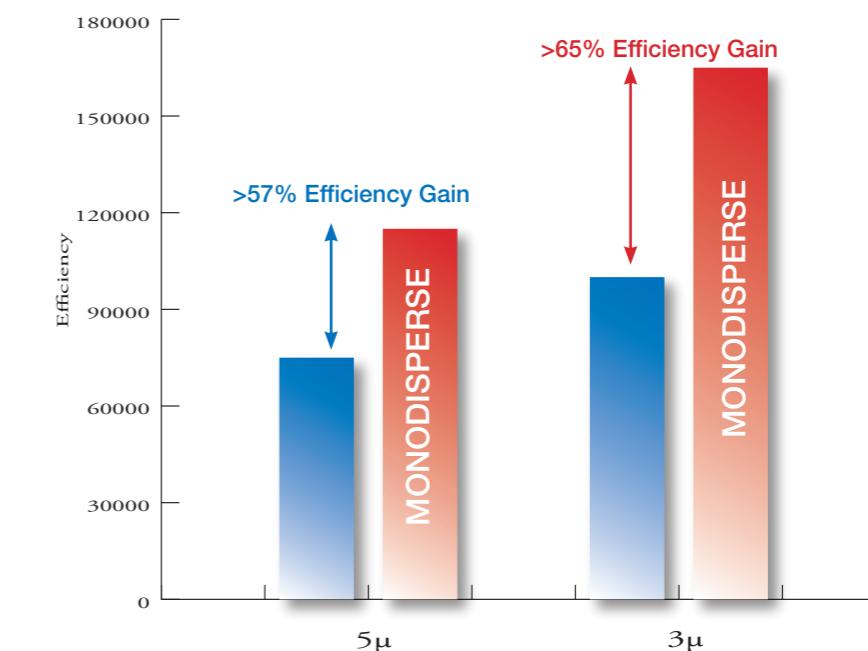
# Efficiency Gains

The monodisperse nature of Evosphere coupled with our ability to pack the column more efficiently allows for a significant increase in efficiency and therefore resolution over traditional silica particles.

## 3 $\mu$ Monodisperse vs 3 $\mu$ Traditional



## Efficiency



# Principle Component Analysis (PCA)

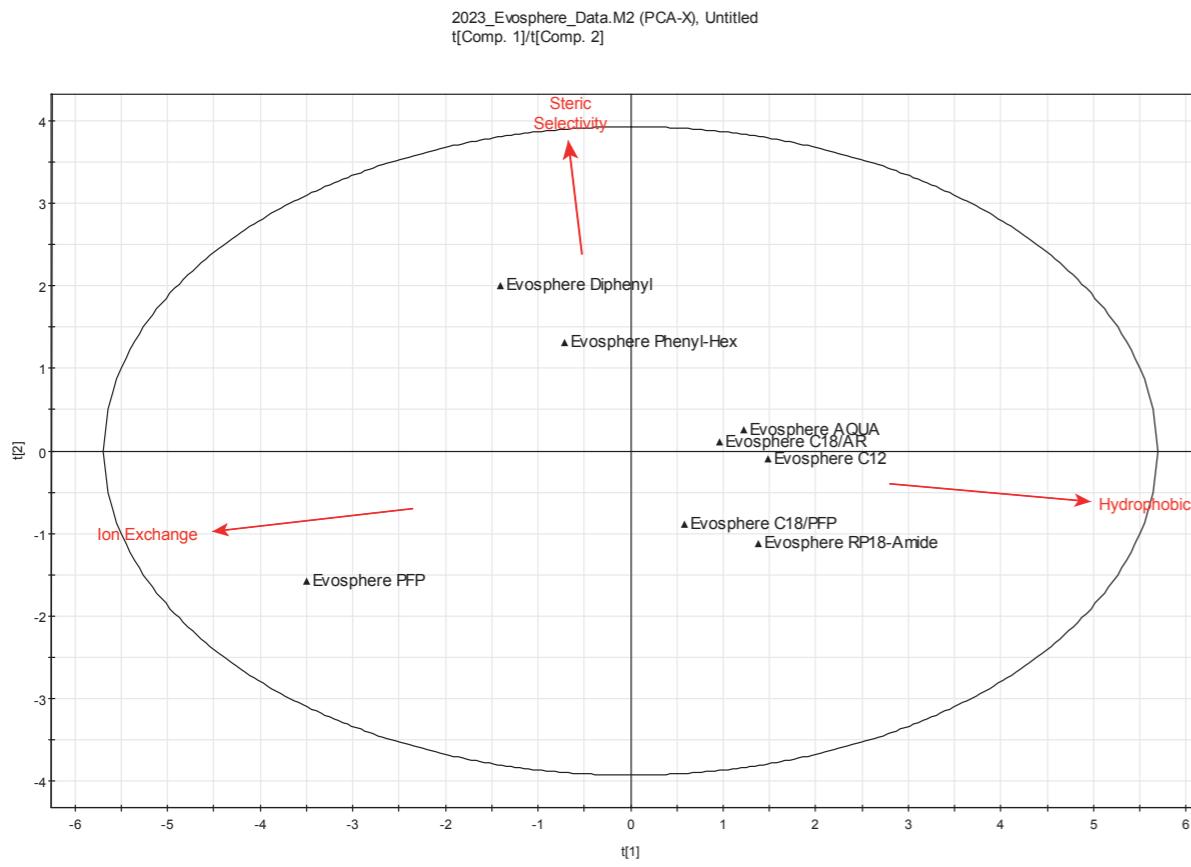


## PCA analysis

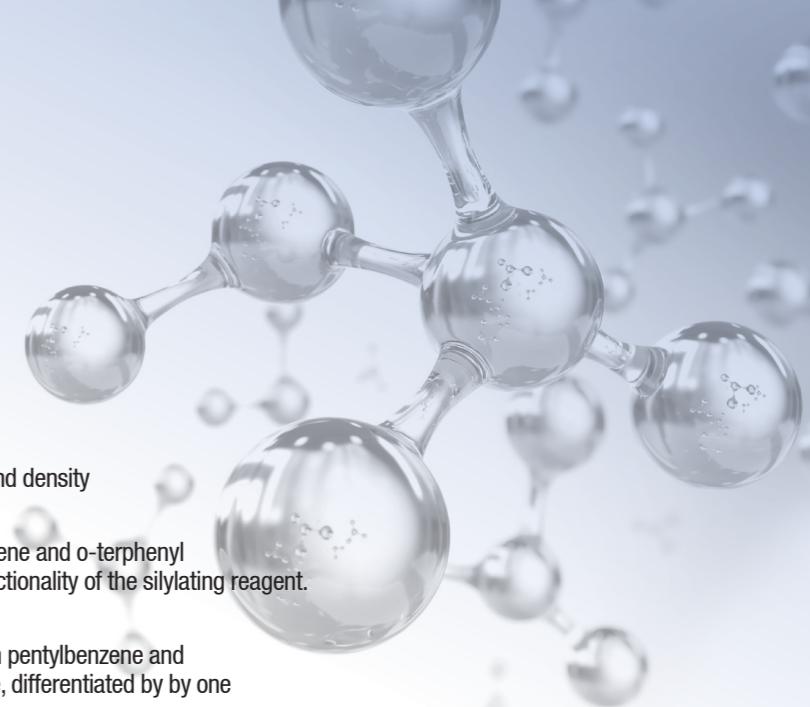
Principle component analysis has been used to differentiate columns for use in Chromatography for a number of years,\* first developed by Euerby and Petersson based around tests suggested by Tanaka. A systematic approach to column characterisation allows the analyst to choose from a diverse (or similar) range of columns.

\* M.Euerby, P.Petersson, LC-GC Europe (Sept 2000) 665-677

PCA analysis has been shown below to highlight the diversity of the stationary phases in the EvoSphere range.  
Choose a phase based on orthogonal selectivity to your current column or by mechanism to match the analytes



## EvoSphere PCA

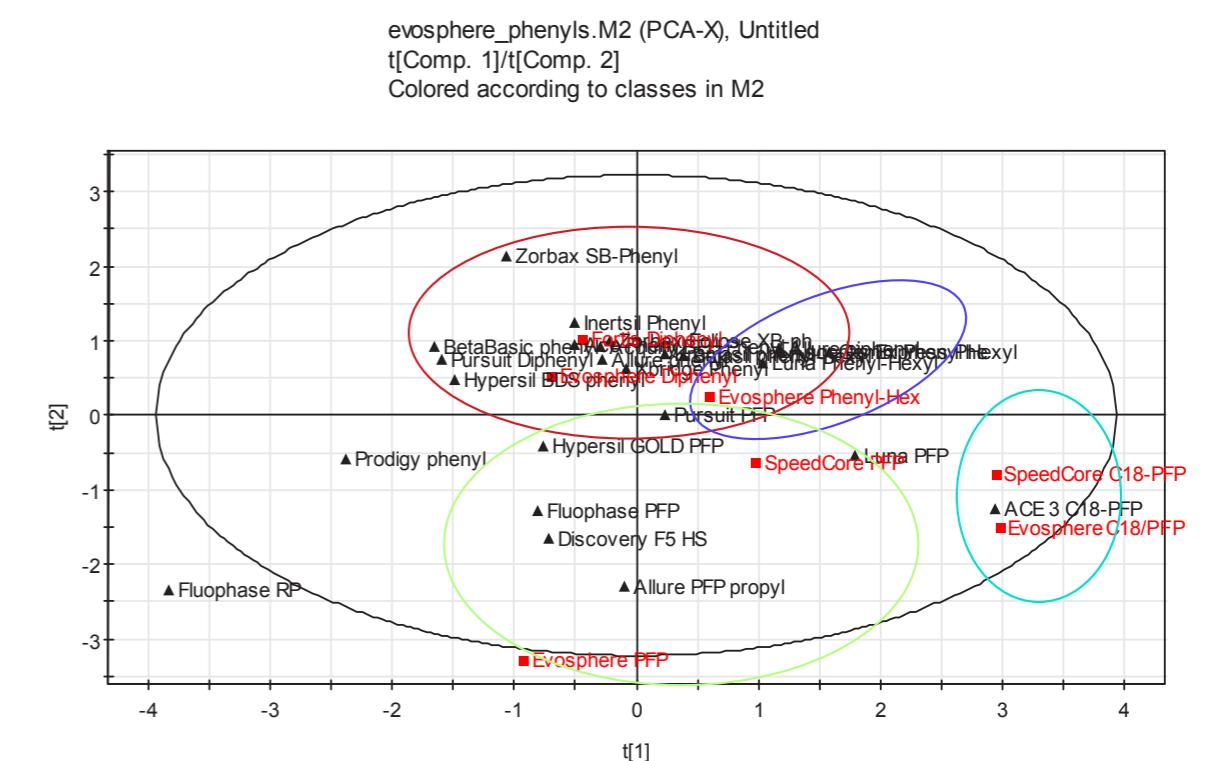


### Factors characterised:

- **$k'$  PB** Retention factor of Pentylbenzene, an indicator of ligand density
- **Steric selectivity** Retention factor ratio between triphenylene and o-terphenyl  $\alpha T/O = k_1/k_0$  this is a measure of the shape selectivity and functionality of the silylating reagent.
- **Hydrophobic selectivity**  $\alpha_{CH_2}$  Retention factor between pentylbenzene and butylbenzene. A measure of the surface coverage of the phase, differentiated by one methylene group is dependant upon the ligand density.
- **Hydrogen bonding capacity**  $\alpha_{C/P}$  retention factor between caffeine and phenol. An indicator of the degree of endcapping
- **Ion-exchange capacity**  $\alpha_{k_p/k_o}$  Retention factor between benzylamine and phenol at both pH 2.7 (acidic activity of silanols) and pH 7.6 an estimate of total silanol activity.

In the example below PCA analysis can be used to show the differences between several commercial Phenyl type phases. It can be seen how the new C18-PFP phase adds a new dimension being predominantly hydrophobic in nature but offering alternate selectivity due to the mixed C18 and PFP ligands.

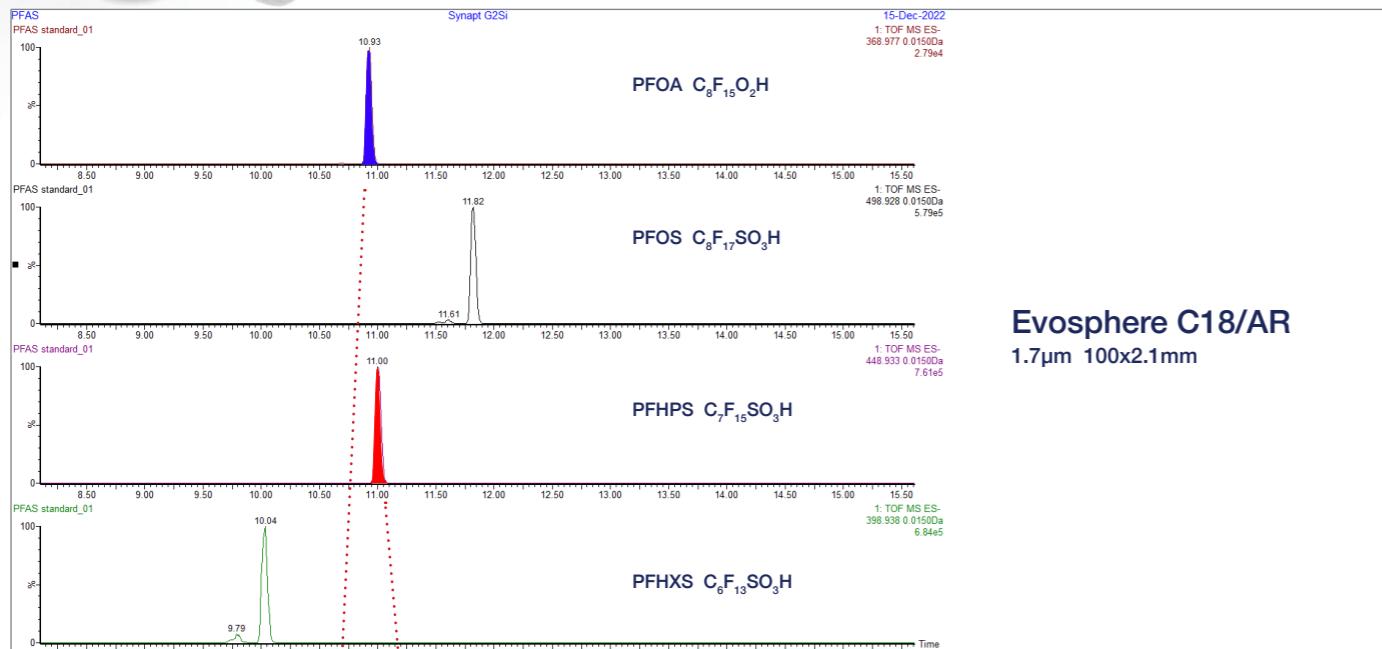
EvoSphere Phenyl-Hex also offers a new selectivity to the range combining hydrophobicity and pi-pi interactions together.



# Polyfluoroalkyl substances (PFAS)



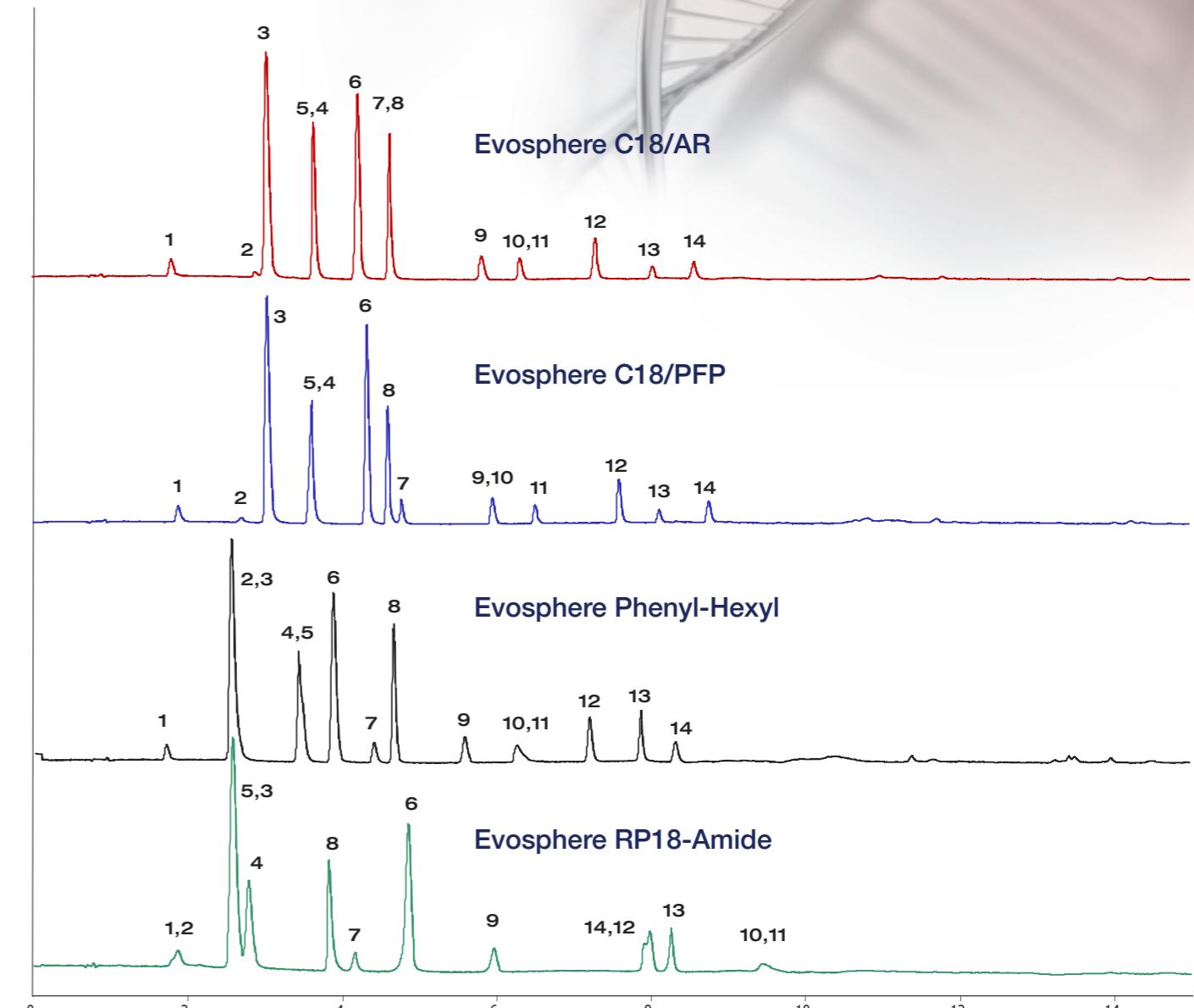
There is a lot of interest in PFAS analysis, if diverse selectivity of stationary phase's can be employed then closely related analytes can be separated with ease, leading to better quantitatively and qualitatively confirmation.



A: 95:5 Water+2mm ammonium acetate: MeOH    B: MeOH+ 2mm ammonium acetate    Flow 0.3ml/min    Temp 35°C    MS ESI-

## Evosphere Selectivity

When developing a new method in chromatography having a diverse range of selectivities to choose from can help in deciding how peaks resolve and which is the best starting point. In this example a gradient run across several stationary phase shows orthogonality selectivity for many of the peaks.



1. Hydroquinone
2. Theobromine
3. Paracetamol
4. Theophylline
5. Paraxanthine
6. 4-Hydroxybenzoic acid
7. 2-Acetamidophenol
8. Caffeine
9. Phenol
10. Aspirin
11. 2-hydroxybenzoic acid
12. 4-nitrophenol
13. 4-Chloracetanilide
14. 2-nitrophenol

Mobile phase A:

10mM ammonium formate pH3.0

Mobile phase B:

10mM ammonium formate pH3.0 in ACN

Flow rate : 0.4ml/min

Wavelength : 254nm

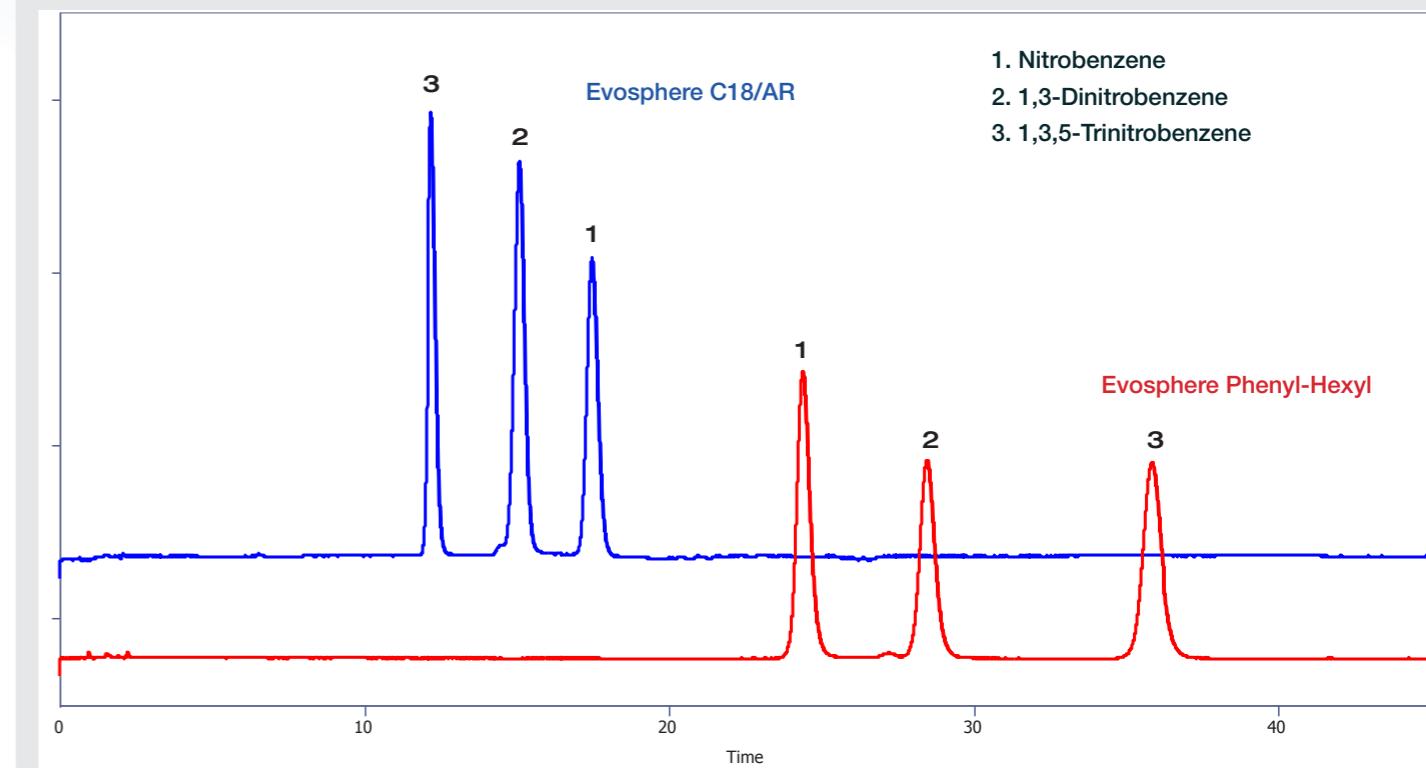
Temperature : 40°C

\* All columns 3µm 100x2.1mm

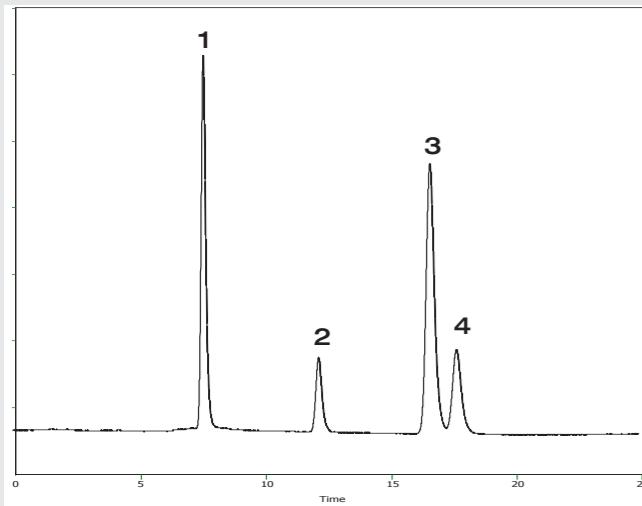
# Applications



## SELECTIVITY COMPARISON - EXPLOSIVES



### HALOGENATED POSITIONAL ISOMERS



Column: 5µm Evosphere C18/PFP 150x4.6mm

Mobile Phase: 50:50 Water:MeOH

Flow: 1.0ml/min

Temp: 20°C

Wavelength: 254nm

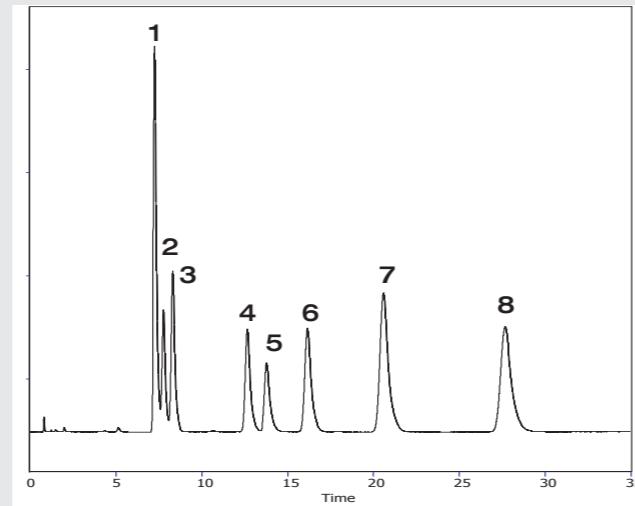
1. Acetophenone

2. 2-Chloroacetophenone

3. 4-Chloroacetophenone

4. 3-Chloroacetophenone

### SUBSTITUTED BENZENES



Column: 5µm Evosphere C18/PFP 150x4.6mm

Mobile Phase: 50:50 Water:MeOH

Flow: 1.0ml/min

Temp: 20°C

Wavelength: 210nm

1. 1,2,3-Trimethoxybenzene

2. 1,2-Dimethoxybenzene

3. 1,2,4-Trimethoxybenzene

4. 1,4-Dimethoxybenzene

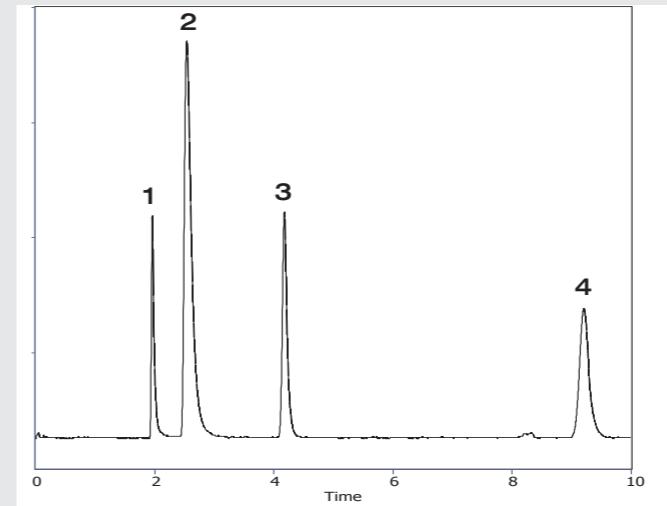
5. Anisole

6. 1,3-Dimethoxybenzene

7. 1,3,5-Trimethoxybenzene

8. Toluene

### NUCLEOSIDES



Column: 3µm Evosphere AQUA 150x4.6mm

Mobile Phase: 98:2 25mM NH4OAc : ACN

Flow: 1.0ml/min

Temp: 20°C

Wavelength: 254nm

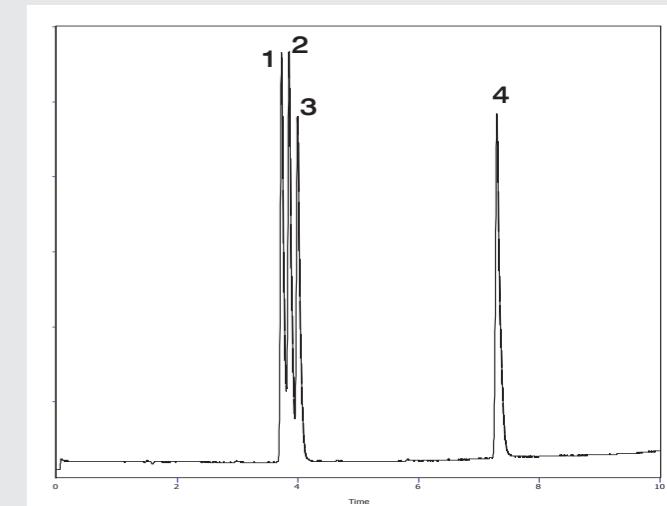
1. Uracil

2. Uridine

3. Cytosine

4. Guanosine

### STEROIDS



Column: 3µm Evosphere AQUA 150x4.6mm

Mobile Phase: 30-100% B in 10mins

A: 0.1% Formic acid in Water

B: 0.1% Formic acid in ACN

Flow: 1.0ml/min

Wavelength: 254nm

1. Prednisolone

2. Prednisone

3. Cortisone

4. 17 $\alpha$  Hydroxyprogesterone

# Capillaries & Prep Scaling



Evosphere capillaries are available in 75 $\mu\text{m}$ , 200 $\mu\text{m}$ , 0.5mm, 1mm i.d. with any phase chemistry and any particle size from the Evosphere range. Request a quote from your local distributor.

## Evosphere Prep

- **10mm and 21.2mm**
- **High Loadability**
- **Optimised Packing Efficiency**
- **Narrow peak profile, High Efficiency and Resolution**

Evosphere Prep columns are designed for high sample loading, high throughput applications. The optimised packed bed (OPB) process ensures excellent peak shapes and efficiency, whilst the lifetime of the column is increased.



## 1.7 $\mu\text{m}$ EVOSPHERE® part numbers

1.7 $\mu\text{m}$ EVOSPHERE C12	Column Length			
	30	50	100	150
2.1	EV012-020201	EV012-020301	EV012-020501	EV012-020701
Column Diameter	3.0	EV012-030201	EV012-030301	EV012-030501
	4.6	EV012-050201	EV012-050301	EV012-050501
				EV012-050701

1.7 $\mu\text{m}$ EVOSPHERE C18/AR	Column Length			
	30	50	100	150
2.1	EV018AR-020201	EV018AR-020301	EV018AR-020501	EV018AR-020701
Column Diameter	3.0	EV018AR-030201	EV018AR-030301	EV018AR-030501
	4.6	EV018AR-050201	EV018AR-050301	EV018AR-050501
				EV018AR-050701

1.7 $\mu\text{m}$ EVOSPHERE C18/PFP	Column Length			
	30	50	100	150
2.1	EV018FP-020201	EV018FP-020301	EV018FP-020501	EV018FP-020701
Column Diameter	3.0	EV018FP-030201	EV018FP-030301	EV018FP-030501
	4.6	EV018FP-050201	EV018FP-050301	EV018FP-050501
				EV018FP-050701

1.7 $\mu\text{m}$ EVOSPHERE RP18-AMIDE	Column Length			
	30	50	100	150
2.1	EVORP18-020201	EVORP18-020301	EVORP18-020501	EVORP18-020701
Column Diameter	3.0	EVORP18-030201	EVORP18-030301	EVORP18-030501
	4.6	EVORP18-050201	EVORP18-050301	EVORP18-050501
				EVORP18-050701

1.7 $\mu\text{m}$ EVOSPHERE PHENYL-HEXYL	Column Length			
	30	50	100	150
2.1	EVOHEX-020201	EVOHEX-020301	EVOHEX-020501	EVOHEX-020701
Column Diameter	3.0	EVOHEX-030201	EVOHEX-030301	EVOHEX-030501
	4.6	EVOHEX-050201	EVOHEX-050301	EVOHEX-050501
				EVOHEX-050701

1.7 $\mu\text{m}$ EVOSPHERE DIPHENYL	Column Length			
	30	50	100	150
2.1	EVOPH-020201	EVOPH-020301	EVOPH-020501	EVOPH-020701
Column Diameter	3.0	EVOPH-030201	EVOPH-030301	EVOPH-030501
	4.6	EVOPH-050201	EVOPH-050301	EVOPH-050501
				EVOPH-050701

1.7 $\mu\text{m}$ EVOSPHERE PFP	Column Length			
	30	50	100	150
2.1	EVOPFP-020201	EVOPFP-020301	EVOPFP-020501	EVOPFP-020701
Column Diameter	3.0	EVOPFP-030201	EVOPFP-030301	EVOPFP-030501
	4.6	EVOPFP-050201	EVOPFP-050301	EVOPFP-050501
				EVOPFP-050701

1.7 $\mu\text{m}$ EVOSPHERE AQUA	Column Length			
	30	50	100	150
2.1	EVOAQ-020201	EVOAQ-020301	EVOAQ-020501	EVOAQ-020701
Column Diameter	3.0	EVOAQ-030201	EVOAQ-030301	EVOAQ-030501
	4.6	EVOAQ-050201	EVOAQ-050301	EVOAQ-050501
				EVOAQ-050701

## Evosphere Sample Filters



- Low volume in-line filter for all core-shell/UHPLC columns
- Increase lifetime of columns
- Change over time seconds not minutes
- Pressure rated to 1000bar

High pressure In-line Filters	
UHPSAV2	UHPLC In-line filter pk 2
UHPSAV4	UHPLC In-line filter pk 4
UHPSAV2-w	UHPLC In-line filter pk 2 Acquity® Compatible
UHPSAV4-w	UHPLC In-line filter pk 4 Acquity® Compatible

## 3µm EVOSPHERE® part numbers

3µm EVOSPHERE C12		Column Length			
		30	50	100	150
	2.1	EV012-020203	EV012-020303	EV012-020503	EV012-020703
Column Diameter	3.0	EV012-030203	EV012-030303	EV012-030503	EV012-030703
	4.6	EV012-050203	EV012-050303	EV012-050503	EV012-050703

3µm EVOSPHERE C18/AR		Column Length			
		30	50	100	150
	2.1	EV018AR-020203	EV018AR-020303	EV018AR-020503	EV018AR-020703
Column Diameter	3.0	EV018AR-030203	EV018AR-030303	EV018AR-030503	EV018AR-030703
	4.6	EV018AR-050203	EV018AR-050303	EV018AR-050503	EV018AR-050703

3µm EVOSPHERE C18/PFP		Column Length			
		30	50	100	150
	2.1	EV018FP-020203	EV018FP-020303	EV018FP-020503	EV018FP-020703
Column Diameter	3.0	EV018FP-030203	EV018FP-030303	EV018FP-030503	EV018FP-030703
	4.6	EV018FP-050203	EV018FP-050303	EV018FP-050503	EV018FP-050703

3µm EVOSPHERE RP18-AMIDE		Column Length			
		30	50	100	150
	2.1	EVORP18-020203	EVORP18-020303	EVORP18-020503	EVORP18-020703
Column Diameter	3.0	EVORP18-030203	EVORP18-030303	EVORP18-030503	EVORP18-030703
	4.6	EVORP18-050203	EVORP18-050303	EVORP18-050503	EVORP18-050703

3µm EVOSPHERE PHENYL-HEXYL		Column Length			
		30	50	100	150
	2.1	EVOHEX-020203	EVOHEX-020303	EVOHEX-020503	EVOHEX-020703
Column Diameter	3.0	EVOHEX-030203	EVOHEX-030303	EVOHEX-030503	EVOHEX-030703
	4.6	EVOHEX-050203	EVOHEX-050303	EVOHEX-050503	EVOHEX-050703

3µm EVOSPHERE DIPHENYL		Column Length			
		30	50	100	150
	2.1	EVOPH-020203	EVOPH-020303	EVOPH-020503	EVOPH-020703
Column Diameter	3.0	EVOPH-030203	EVOPH-030303	EVOPH-030503	EVOPH-030703
	4.6	EVOPH-050203	EVOPH-050303	EVOPH-050503	EVOPH-050703

3µm EVOSPHERE PFP		Column Length			
		30	50	100	150
	2.1	EVOPFP-020203	EVOPFP-020303	EVOPFP-020503	EVOPFP-020703
Column Diameter	3.0	EVOPFP-030203	EVOPFP-030303	EVOPFP-030503	EVOPFP-030703
	4.6	EVOPFP-050203	EVOPFP-050303	EVOPFP-050503	EVOPFP-050703

3µm EVOSPHERE AQUA		Column Length			
		30	50	100	150
	2.1	EVOAQ-020203	EVOAQ-020303	EVOAQ-020503	EVOAQ-020703
Column Diameter	3.0	EVOAQ-030203	EVOAQ-030303	EVOAQ-030503	EVOAQ-030703
	4.6	EVOAQ-050203	EVOAQ-050303	EVOAQ-050503	EVOAQ-050703



- Direct connect guard system for all 3µm and 5µm phases
- Quick replacement cartridges
- Highly Cost Effective

3µm Evosphere Guard Cartridges	
DCGUA-1	Guard Cartridge Holder
DCxx-040003G/2	10x4mm Evosphere 3µm Guard pk 2
DCxx-040003G/4	10x4mm Evosphere 3µm Guard pk 4
DCxx-020003G/2	10x2mm Evosphere 3µm Guard pk 2
DCxx-020003G/4	10x2mm Evosphere 3µm Guard pk 4

## 5µm EVOSPHERE® part numbers

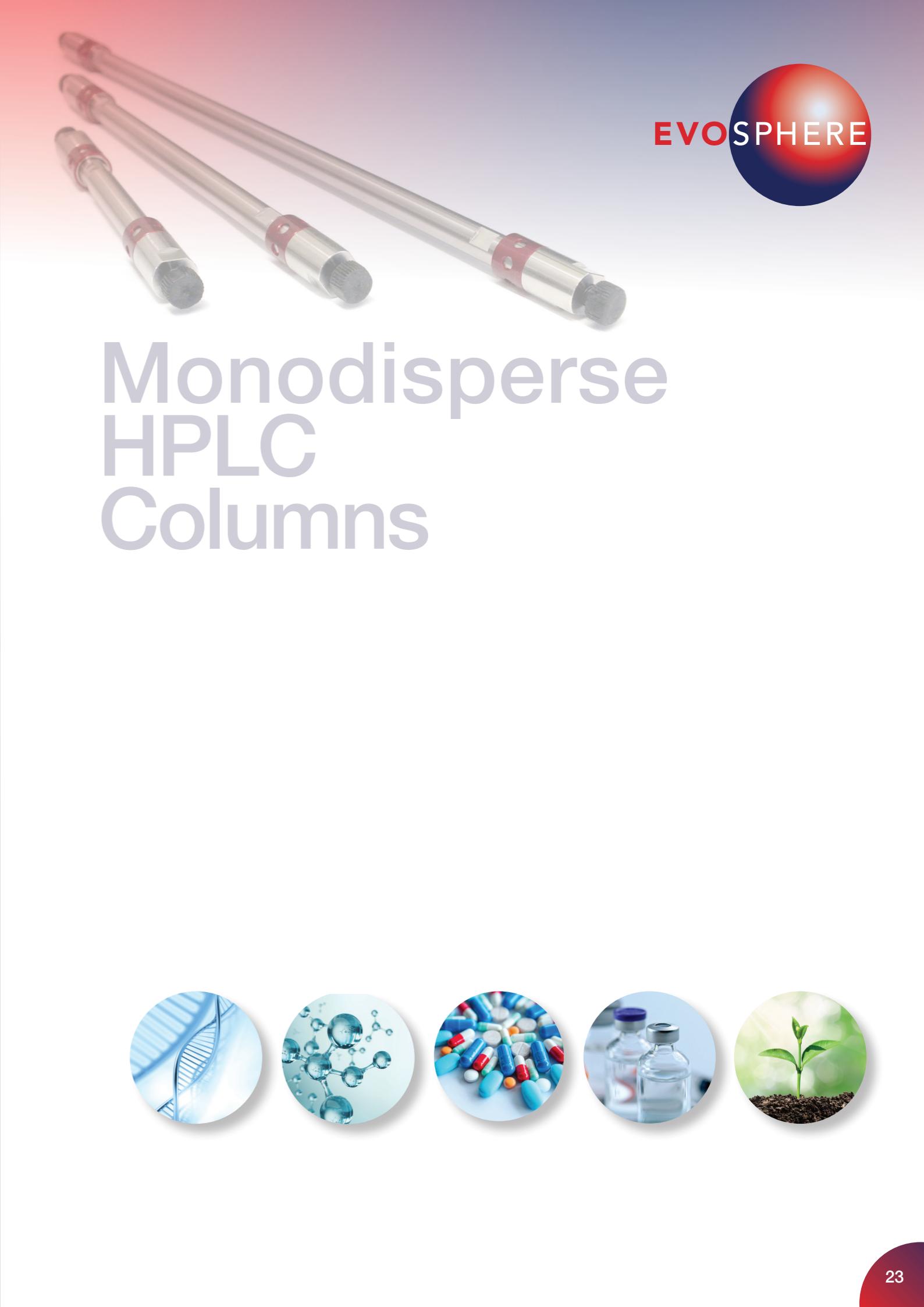
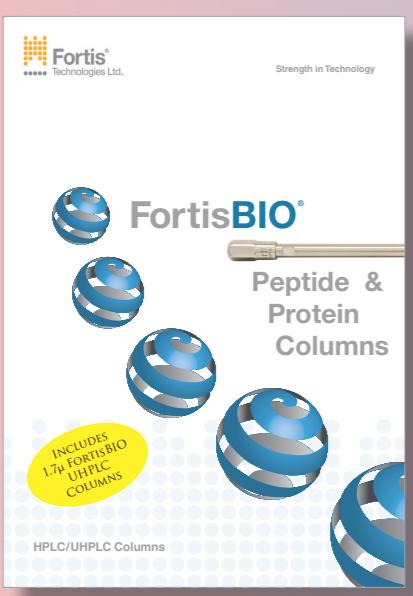
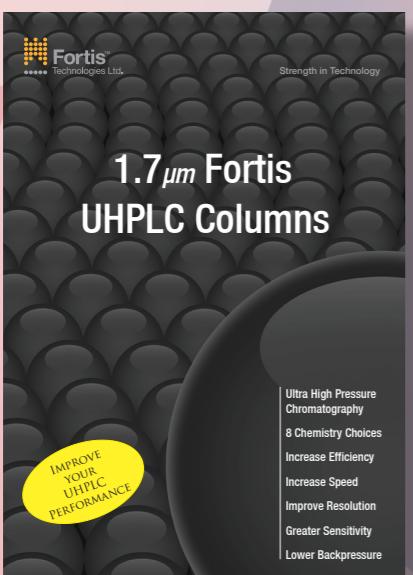
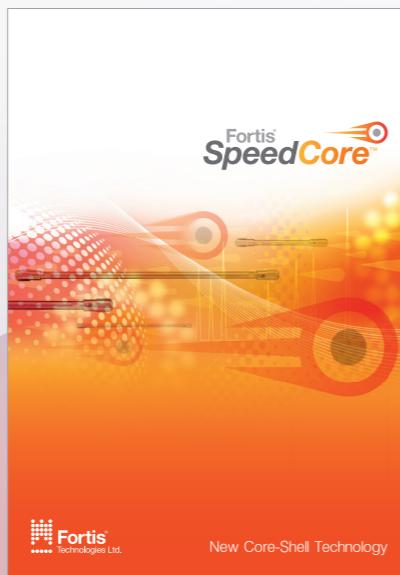
5µm EVOSPHERE C12		Column Length			
		30	50	100	150
	2.1	EV012-020205	EV012-020305	EV012-020505	EV012-020705
Column Diameter	3.0	EV012-030205	EV012-030305	EV012-030505	EV012-030705
	4.6	EV012-050205	EV012-050305	EV012-050505	EV012-050705

5µm EVOSPHERE C18/AR		Column Length			
		30	50	100	150
	2.1	EV018AR-020205	EV018AR-020305	EV018AR-020505	EV018AR-020705
Column Diameter	3.0	EV018AR-030205	EV018AR-030305	EV018AR-030505	EV018AR-030705
	4.6	EV018AR-050205	EV018AR-050305	EV018AR-050505	EV018AR-050705

5µm EVOSPHERE C18/PFP		Column Length			
		30	50	100	150
	2.1	EV018FP-020205	EV018FP-0203		



## Other Product Guides Available



# Monodisperse HPLC Columns

# WORLDWIDE AVAILABILITY



45 Coalbrookdale Road  
Clayhill Industrial Park  
Neston  
Cheshire, UK  
CH64 3UG

t: +44 151 336 2266  
f: +44 151 336 2669  
[www.fortis-technologies.com](http://www.fortis-technologies.com)  
e: [info@fortis-technologies.com](mailto:info@fortis-technologies.com)

Company No. 5449466  
VAT No. 866 8966 43

Fortis products are available worldwide. For the distributor in your country, contact Fortis international Sales Office, UK by telephone, fax or email: [info@fortis-technologies.com](mailto:info@fortis-technologies.com)

- Austria
- Hong Kong
- Poland
- Bangladesh
- Hungary
- Portugal
- Brazil
- India
- Romania
- Canada
- Ireland
- Russia
- China
- Israel
- Singapore
- Columbia
- Italy
- South Africa
- Czech Republic
- Japan
- Spain
- Ecuador
- Korea
- Sweden
- Egypt
- Malaysia
- Switzerland
- France
- Mexico
- Taiwan
- Germany
- Netherlands
- Thailand
- Greece
- Norway
- Turkey
- Holland
- Puerto Rico
- USA

**For technical support or applications contact :**  
**[technicalsupport@fortis-technologies.com](mailto:technicalsupport@fortis-technologies.com)**

**For more information VISIT :**  
**[www.fortis-technologies.com](http://www.fortis-technologies.com)**

