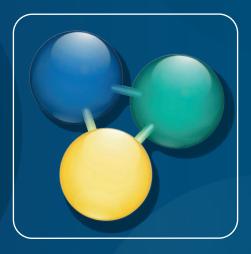
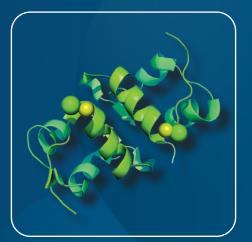


Hybrid silica-based stationary phases for prep LC **YMC-Triart Prep**

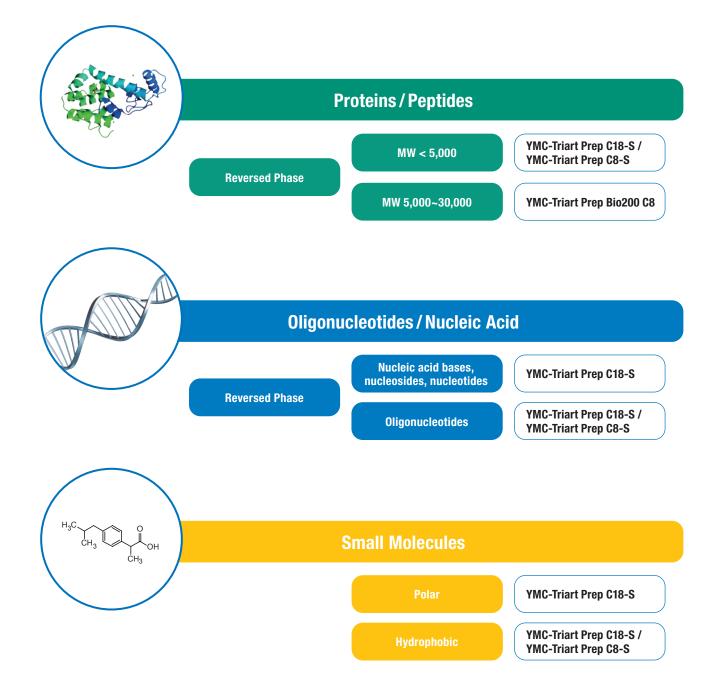












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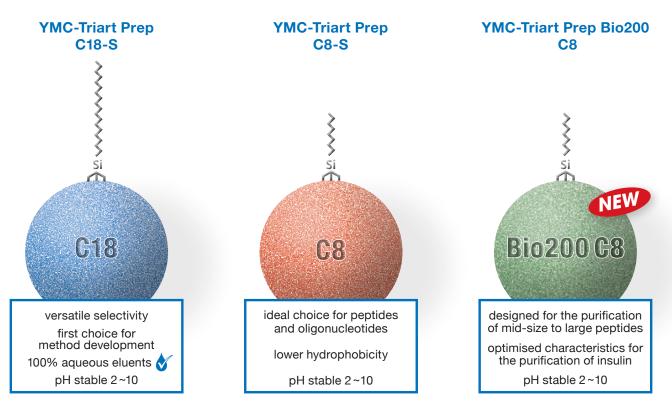
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Specifications

	YMC-Triart Prep C18-S	YMC-Triart Prep C8-S	YMC-Triart Prep Bio200 C8
Base material		inorganic/organic hybrid silica	
Particle size [µm]	7, 10, 15, 20	10, 15, 20	10
Pore size [nm]	12	12	20
Specific surface area [m²/g]	360	360	proprietary
Bonding	trifunctional C18	trifunctional C8	trifunctional C8
End-capping	yes	yes	yes
Flexible pH range	2.0 ~ 10.0	2.0 ~ 10.0	2.0 ~ 10.0
Column cleaning	common procedures up to pH 12	common procedures up to pH 12	common procedures up to pH 12

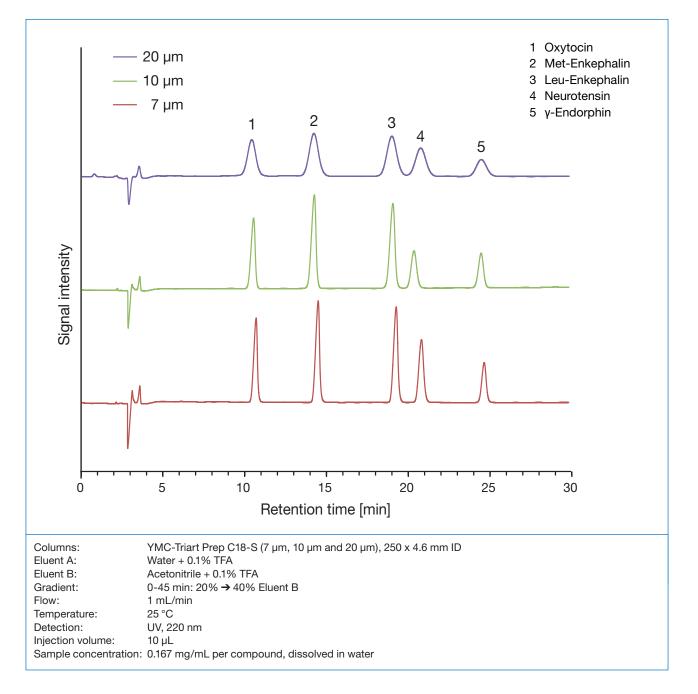
Phases Overview



Equivalent selectivity for varying particle sizes

The objectives for method developers in preparative LC are the purification and isolation of target compounds with a maximum sample recovery in the shortest cycle time within cost limitations. Whereas the isolation in R&D focuses on the scale up of mg- to g-units, the production scale needs to achieve up to kilograms or even tons.

In order to address the specific demands for the preparative LC processes in R&D and industrial scale production, YMC offers different particle sizes with equivalent selectivity. The separation behaviour of all particle sizes is the same. Depending on the actual purification, the ideal particle size can be selected to isolate the target compounds efficiently and with high productivity.



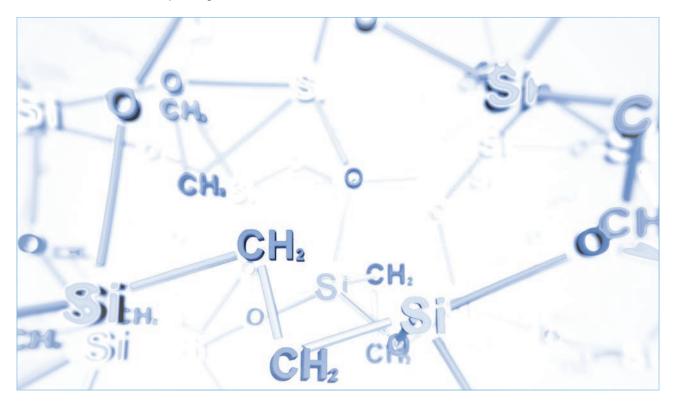
Hybrid silica particle technology for high pH stability

The innovative preparative stationary phase YMC-Triart Prep combines mechanical strength and pH stability. It is stable up to pH 10 which allows more flexibility for process development. Moreover, efficient cleaning-in-place (CIP) procedures can be applied!

From real-life process development examples, YMC-Triart Prep has been shown to outperform traditional silicabased materials in terms of stability up to 4-fold. Longer column lifetimes lead to greater amounts of product being produced per kilogram of stationary phase. The results mean improved production procedures and reduced overall costs.

Mechanical Strength and Chemical Robustness

To overcome the limitations of traditional silica-based stationary phases regarding the chemical stability, hybrid silicabased phases have been developed. Hybrid silica contains organic groups within the silicon dioxide network, making it more resistant to a wider pH range.



In addition to this, YMC-Triart Prep phases are designed to provide:

- Balanced selectivity
- Optimal batch-to-batch reproducibility
- Improved peak shape
- High loadability

Developed to meet the highest demands in preparative LC

Maintaining an excellent chromatographic performance, YMC-Triart Prep phases are fully compatible with alkaline CIP procedures. The next-generation hybrid silica base has been reinforced with ethylene cross-links. This enhances the mechanical strength and enables the particles to resist hydrolysis at elevated pH. In addition, the polymeric modification results in ligands being well protected under acidic conditions. The overall result is a chemical and mechanical stability that guarantees a long lifetime and constant results.

VS

Hybrid-silica

- Extended pH stability
- Hybrid-particles are stable under alkaline conditions
- Polymeric modification maintains retention at low pH
- Alkaline CIP procedures possible for more effective cleaning

Classical silica

- Limited pH stability
- Silica matrix is hydrolysed under alkaline conditions
- Monomeric modification: loss of ligands and retention at low pH
- Limited options for CIP procedures

With YMC-Triart, challenging pH and high temperature conditions are no longer a limitation to the day-to-day work. Most importantly, due to its unique particle composition, a balanced hydrophobicity and silanol activity are achieved which makes YMC-Triart the "First Choice" phase in process development.

Important to know: An advantage of YMC-Triart materials is the effortless method transfer from the analytical scale to preparative / process scale. The reason for this is an identical separation across all particle sizes!

Almac has been working with YMC and APEX Scientific on various projects for over 5 years – from mg impurity isolation to multi-kg purification and have found the level of technical support and guidance exemplary when using their products. This has ranged from advice on packing large DAC columns to the best media for a particular separation. Furthermore, YMC has presented on a number of different topics at Almac, which has increased our toolbox of purification technologies now regularly utilised in-house.

Steve McIntyre, Almac Group Ltd. (UK)



1. Mechanical strength: YMC-Triart Prep

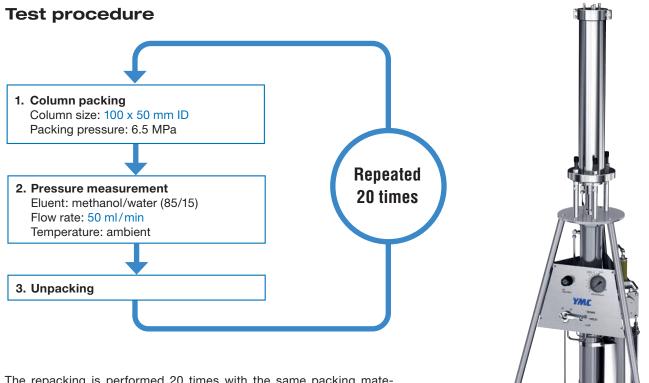
The use of mechanically stable stationary phases is an important economic aspect in a chromatographic process and also a matter of phase lifetime. A rigid material can be used longer and repacked more often before it requires replacement.

The mechanical robustness directly determines the lifetime of the packed column bed. With conventional silica materials, there are particles that are damaged by pressure or shear forces over the course of time which results in the formation of fines. These fines not only clog the column frits, they also block the flow channels of the pack-ing materi-als, resulting in a constant increase in backpressure. This effect is even more pronounced during the repacking of stationary phases.

The specifically developed hybrid-silica particle technology of YMC-Triart Prep offers a great improvement in mechanical stability. Even frequent repacking of columns is possible.



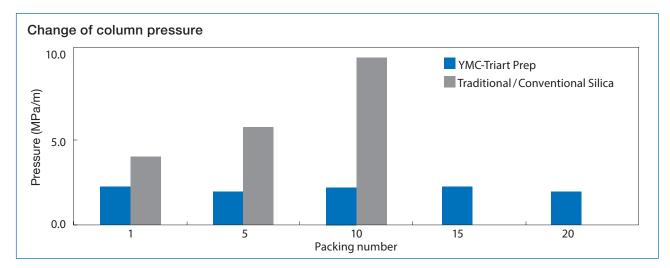
Stationary phase: Hybrid silica-based phase YMC-Triart Prep C8 (10 µm, 20 nm) Dimension of the packed bed: 100 mm x 50 mm ID Column type: Dynamic Axial Compression Column DAU-50-700S



The repacking is performed 20 times with the same packing material. After each unpacking, potential fines were not removed so that any possible degradation of particles can be observed. The materials were analysed by SEM.

Practical result during use: a constantly low backpressure!

The observed backpressure for the hybrid silica-based YMC-Triart Prep remains unaffected over the repacking procedure which proves the mechanical stability of the particles. This allows for extended usage, especially in cases where the material needs to be repacked frequently. In addition, the backpressure is significantly lower compared to silica-based phases under the same conditions. As a result, higher flow rates and significantly longer lifetimes of the packed column can be achieved.



SEM results after DAC repacking study

By using a scanning electron microscope (SEM), the defects of the particles after usage can be seen.

Conventional silica



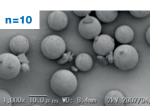
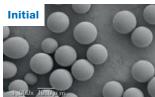


Figure 1: SEM pictures of the particles of conventional silica material before the first packing and after the 10th packing

For conventional silica, a high number of fragments of collapsed particles can be seen. These so-called fines cause clogging of the frits and the flow channels of the packed column bed. This results in a constant increase in backpressure.

YMC-Triart Prep



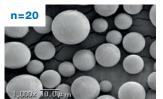
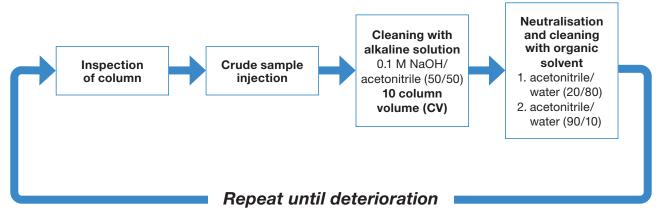


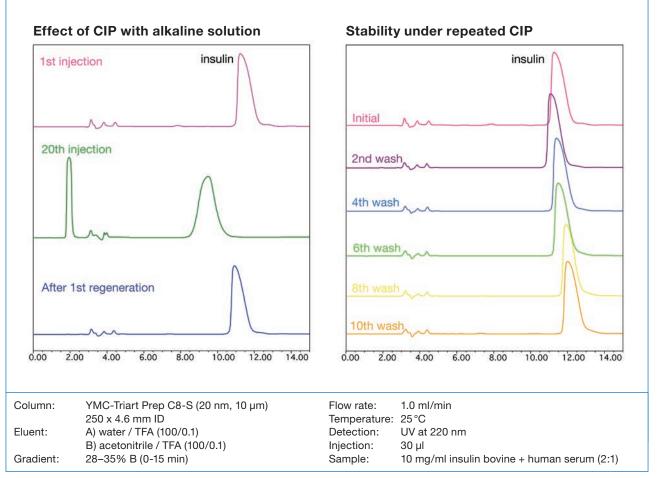
Figure 2: SEM pictures of the hybrid silica-based YMC-Triart Prep before the first packing and after the 20th packing

The particle shape of the YMC-Triart Prep material is highly regular. Fines are shown to be absent even after 20 packing cycles. The improved particle size distribution allows an evenly packed column bed resulting in better separation characteristics and lower backpressures.

2. Compatibility with alkaline CIP procedures

Challenge: Silica materials are unsuitable for alkaline wash conditions due to their limited stability at high pH. **Solution:** Hybrid silica-based YMC-Triart Prep has excellent stability at high pH. It is fully compatible with alkaline cleaning conditions. This lowers consumption of packing material and requires less downtime due to column repacking which in turn reduces production costs. An extension of column lifetime by a factor of more than three has been achieved in CIP studies.

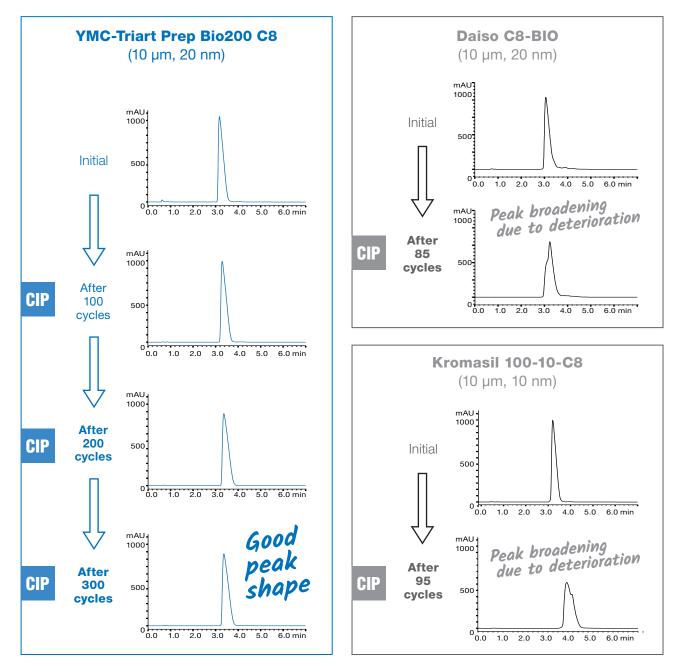




* courtesy of YMC Co., Ltd.

Lifetime comparison study – Number of cleaning cycles

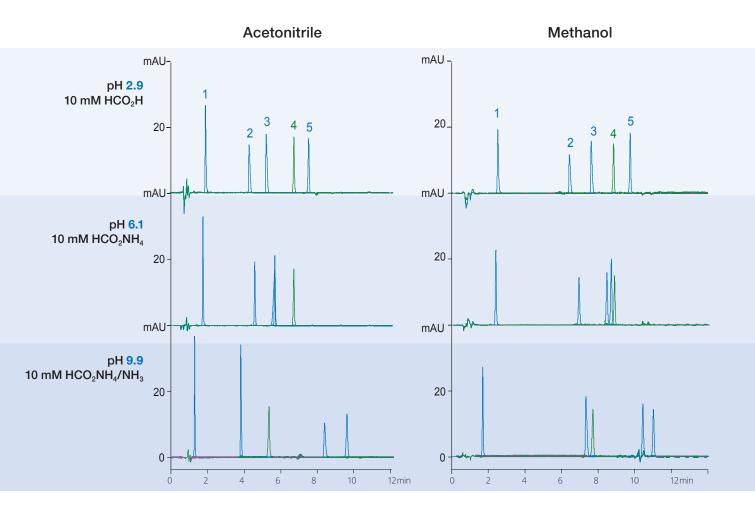
In order to evaluate the effective lifetime of the packing material for the isolation of insulin, a CIP study was carried out. Different stationary phases were tested using the CIP protocol with 0.1 M NaOH. It shows the superior chemical stability of the YMC-Triart Prep phases compared to other stationary phases. This increased life-time improves the cost efficiency of the related processes.



- YMC-Triart Prep maintains good peak shape even after 300 alkaline cleaning cycles.
- Competitors show peak shape deterioration after < 100 cycles.

Improving the separation by pH modification

Equally important, **YMC-Triart C18** can be used within a pH range of 2 to 10. This allows a process development with greater flexibility. Optimal retention and resolution can be achieved by adjusting the eluent pH.



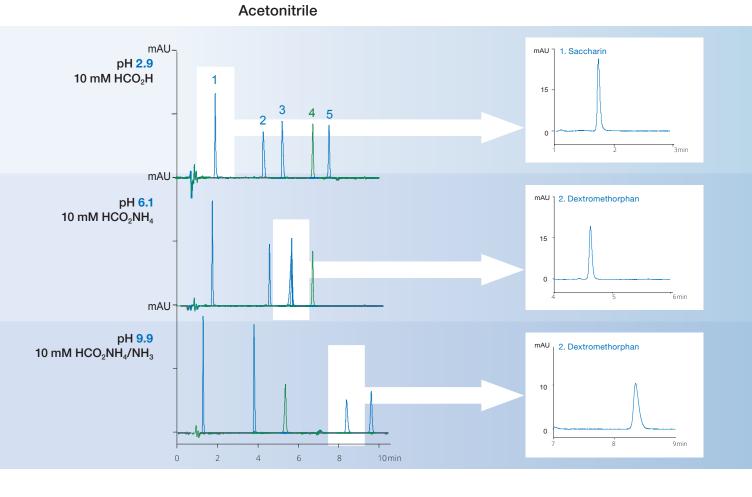
Column: Eluent:	YMC-Triart C18 (5 µm) 50 x 2.0 mm ID A) 10 mM ammonium formate buffer B) organic solvent
Gradient: Flow rate: Temperature: Detection:	5–90% B (0–10 min), 90%B (10–15 min) 0.2 ml/min

- 1. Saccharin
- 2. Dextromethorphan
- Amitriptyline
 n-Butylparaben
- 5. Ibuprofen

The key to a successful separation is typically a combination of pH modification and the most suitable organic modifier.

Improved Peak Shape

YMC-Triart C18 delivers excellent peak shapes and superior resolution for acids, bases, and neutral compounds. It also provides the method development chemist with the confidence of batch-to-batch and column-to-column reproducibility and long column lifetimes.



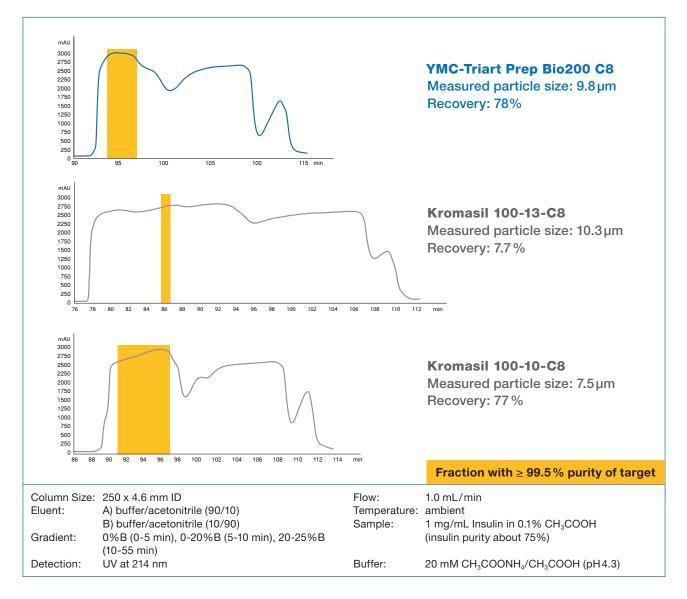
Column: Eluent:	YMC-Triart C18 (5 μm) 50 x 2.0 mm ID A) 10 mM ammonium formate buffer	1. Saccharin 2. Dextromethorphan
	B) organic solvent	3. Amitriptyline
Gradient:	5–90% B (0–10 min), 90%B (10–15 min)	4. <i>n</i> -Butylparaben
Flow rate:	0.2 ml/min	5. Ibuprofen
Temperature:	25°C	
Detection:	UV at 230 nm	

Excellent peak shapes.

YMC-Triart Prep

3. Loadability and efficiency

In a joint project with an insulin manufacturer, a comprehensive screening was performed to identify the most suitable stationary phase for their existing process. Loadability studies were done based on the requirement to achieve a maximum amount of insulin with a purity of 99.5 %. All experiments were carried out with insulin samples representing the feed of the production processes in place.



The best recovery was achieved with the **YMC-Triart Prep Bio200 C8** phase which is based on 9.8µm particles. Also the volume of the fraction was lower using the YMC material which has the advantage of requiring a simplified post-separation treatment of the more concentrated fractions. This also increases the production efficiency of the overall process.

	YMC-Triart Prep Bio200 C8	Kromasil 100-10-C8
Fraction volume (\geq 99.5%)	3.5 mL	5.5 mL
Insulin concentration in recovered fraction (\geq 99.5%)	11.2 mg/mL	7.1 mg/mL





Productivity evaluation by the required stationary phase amount

Typically, the loadability and productivity are tested for the different stationary phases using the same column dimensions. However, the required amount of stationary phase can be very different when packed in this column dimension.

Therefore, the honest way to evaluate the productivity is to identify the amount of purified product per amount of stationary phase.

For the described comparison, the packing densities are very different. For the **YMC-Triart Prep Bio200 C8**, the packing density is 0.50 g/mL whereas the two Kromasil phases have a packing density of 0.60 g/mL.

As an example, the amount of stationary phase needed to pack a 250 mm x 300 mm ID DAC column is 8.85 kg of the YMC stationary phase whereas 10.62 kg of the Kromasil materials would be needed. This means 20 % more material is needed to pack the same column dimension. Consequently, the productivity in terms of the amount of purified target compounds per kg of used stationary phase is about 20% higher for the YMC material.





YMC-Triart Prep Bio200 C8 = 8.85 kg







Kromasil 100-10-C8 = 10.62 kg



YMC-Triart Prep

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The loadability of the **YMC-Triart Prep Bio200 C8** phase is significantly higher compared to the alternative stationary phases. Comparing the same particle sizes, the separation is much better for the YMC material. The productivity is more than 10 times higher. Due to the reduced packing density, less YMC stationary phase is required for the column packing.

YMC-Triart Bio200 C8 reduces the consumption of stationary phase and improves the productivity and cost-efficiency of the downstream process.

	YMC-Triart Prep Bio200 C8	Kromasil 100-10-C8	Kromasil 100-13-C8
Stated particle size	10 µm	10 µm	13 µm
Measured particle size	9.8 µm	7.5 µm	10.3 µm
Recovery of target purity (≥ 99.5%)	78%	77%	7.7%
Bulk density	0.50 g/mL	0.60 g/mL	0.60 g/mL
Required stationary phase 250 x 300 mm ID column	8.85 kg	10.62 kg	10.62 kg
Productivity (Target/kg gel)	18.8 g	15.5 g	1.6 g
	*****	·	••••



More than 10 x higher



4. Economic aspects of choosing stationary phases

What is the relationship between stationary phase properties and process economics?

The efficiency-related parameters of a preparative LC process are directly connected to the stationary phase. The phase has the strongest influence on how much product can be purified in a given amount of time. An example of further, highly important traits is the ongoing and worldwide availability of a constant product quality. This enables a full reproducibility of the optimised method on all sites of globally active enterprises.

Cost-Efficiency

- Stability
- Lifetime
- Loadability

Robustness

- Mechanical stability
- Chemical stability
- 100 % aqueous applications

Availability

- All-round selectivities
- Multi-ton scale
- Worldwide support

Reliability

- Lot to lot
- Year to year
- Lab to lab and site to site

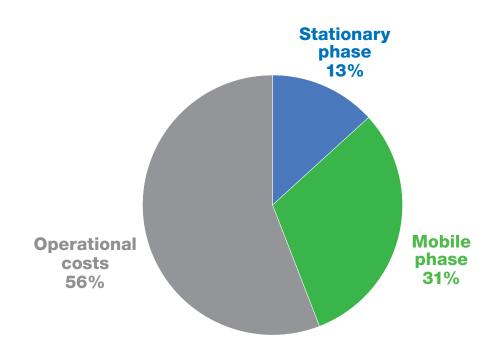
Case Study – How to reduce the costs for preparative LC processes

Abstract

The use of preparative chromatography for the isolation of compounds such as active pharmaceutical ingredients will ensure the highest possible purities. While the costs for the purification of target compounds in high purity via preparative chromatography are frequently considered to be very high, these costs are mainly driven by the costs of the solvents and the running costs of the operation itself. There are huge opportunities for cost savings for virtually all existing and new preparative processes.

The potential to reduce the costs and increase the lifetime of the stationary phase is the most obvious area for cost reductions.

In this case study, all relevant method parameters were evaluated for the isolation of insulin. A comprehensive screening was performed for different stationary phases. Based on the actual results obtained, cost calculations showed that savings of up 40% in the overall product costs were possible!

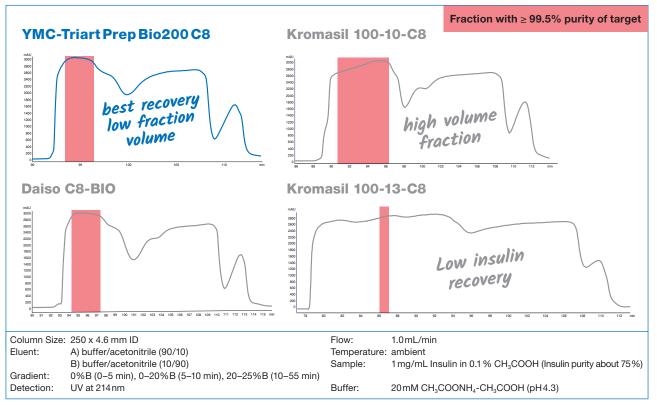


Typical cost structure for preparative LC processes

The main cost drivers are the operational costs such as work force and the solvents used as mobile phase. The stationary phase dictates the cost efficiency of a process and defines the required amounts of solvents and operational parameters such as runtimes and the lifetime of the process. Therefore, the choice of the stationary phase is the most important step during the process development. However, the actual cost of the preparative stationary phase itself represents only a small proportion of the overall costs of preparative processes. Based on the cost calculation of this case study, the cost for the stationary phase represents only 13% of the overall costs.

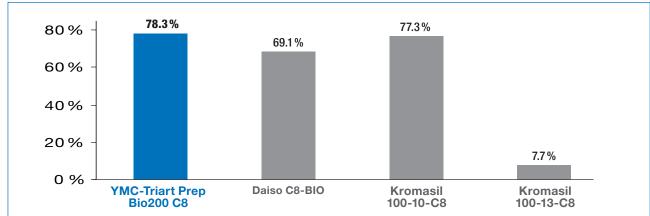
Case Study - Purification of insulin

For the purification of insulin, a detailed cost calculation and an analysis of possible savings was carried out by YMC. In co-operation with an insulin manufacturer, real insulin samples were used to perform a comprehensive screening and process optimisation study. Different stationary phases were used for the screening.



Screening results for a loading of 50 mg insulin

It was obvious that the Kromasil 100-13-C8 is not suitable for the purification. The separation is insufficient to recover insulin with the purity of 99.5% in reasonable quantities. The fraction volume of the Kromasil 100-10-C8 is larger compared to the YMC-Triart Prep Bio200 C8 phase. The smaller fraction volume with the YMC phase simplifies the post-chromatography steps. The chromatograms obtained with YMC and Daiso seem to deliver similar results. Both phases are able to purify in small fraction volumes although the actual recovery amounts show the outstanding performance of YMC-Triart Prep Bio200 C8.



Recovery of insulin at 99.5% purity

Case Study - Cost estimation for the isolation of 100kg purified insulin

Based on the obtained data, a cost estimation was carried out for the isolation of 100 kg purified insulin. A linear scale up to a 60 cm ID column was chosen as a realistic scenario. The target for the process is to produce insulin with a purity of 99.5 %. The following conditions were set for the calculation.

	Common conditions
Target	100 kg of purified insulin
Target purity	> 99.5%
Material	 YMC-Triart Prep Bio200 C8 Daiso C8-BIO Kromasil 100-10-C8
Column	600 mm ID x 250 mm length
Sample	Crude insulin (75% purity)
Loading per run	850g of crude insulin
Purification cycle time	120 min/run
Operation	24 hours/10 cycles per day (20 hours operation + 4 hours CIP)
Condensation capacity	500 L/day
Lyophilisation	10 days

Item	Unit cost in €
Packing material/kg	3,000
Mobile phase / 1000 L	3,000
Operational costs (incl. work force, equipment) / day	10,000



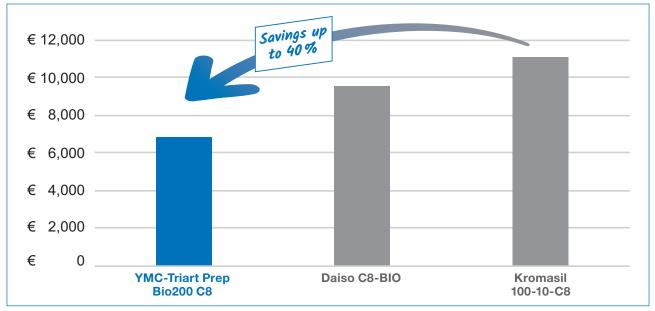


Case Study -Potential for savings up to 40%

In summary, the YMC-Triart Prep Bio200 C8 phase shows the highest performance, resulting in the greatest cost efficiency and productivity. The isolation of 100 kg insulin with a purity of 99.5% is achieved after 35 days with the lowest number of injections. Moreover, the smallest amount of crude is needed with the YMC material which additionally improves the cost efficiency of the overall process.

	YMC-Triart Prep Bio200 C8	Daiso C8-BIO	Kromasil 100-10-C8
Packing material amount (bulk density)	35.3 kg (0.5 kg/L)	35.3 kg (0.5 kg/L)	42.2 kg (0.6 kg/L)
Lifetime of packing material	> 200 runs	80 runs	100 runs
Recovery	78%	69%	77%
Required crude	170 kg	192 kg	172 kg
Required purification cycle	200 runs	225 runs	202 runs
Fraction volume per run	60 L	60 L	95 L
Campaign period	35 days	38 days	50 days
Total solvent required	75,600L	85,100 L	76,400 L

Purification costs per kg insulin



Conclusions

This case study proves that the **YMC-TriartPrepBio200C8** phase allows savings up to 40% to be achieved for the isolation of insulin. High loadability combined with the perfectly matched selectivity and long lifetimes result in a substantial reduction in operational costs. With **YMC-TriartPrepBio200C8** the best possible process is possible. This phase outperforms all other phases screened in all disciplines: it is the fastest, the most economic, the most ecologic as well as the most efficient phase.

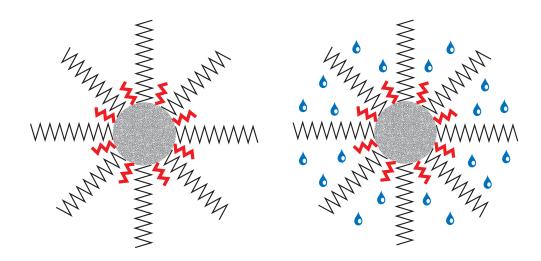
Contact YMC for your free sample today and discover the qualities of YMC-Triart Prep!



5. Aqueous stability

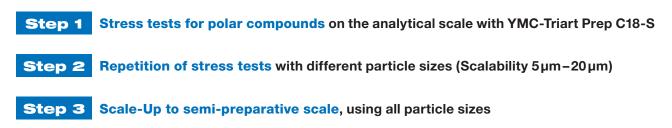
The ability to use 100% aqueous conditions opens the door to an extended purification range that includes polar compounds efficiently and economically. Still, it is a challenging task: the use of 100% water as an eluent in RP-LC! Even today, many C18 materials suffer from unacceptable short lifetimes, as a result of the C18 chains collapsing which drastically reduces the separation performance.

Using YMC-Triart Prep overcomes this hurdle. It is fully compatible with 100% aqueous conditions!



YMC provided the "original" silica based reversed phase media stable in 100% aqueous conditions. YMC-Triart Prep C18-S continues this 100% aqueous compatibility. Benefit from one of many YMC developments and enjoy boundless freedom for process development.

In the following project, the task was to provide an efficient separation of polar compounds. Various conditions, columns dimensions and phases were tested to show the extraordinary stability of YMC-Triart Prep C18-S under aqueous conditions.



Based on these results this customer project was successfully developed to an industrial-scale process, using aqueous conditions for the purification and re-salting.

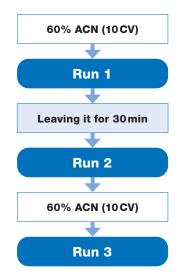


Step 1 Stress test for Polar Compounds

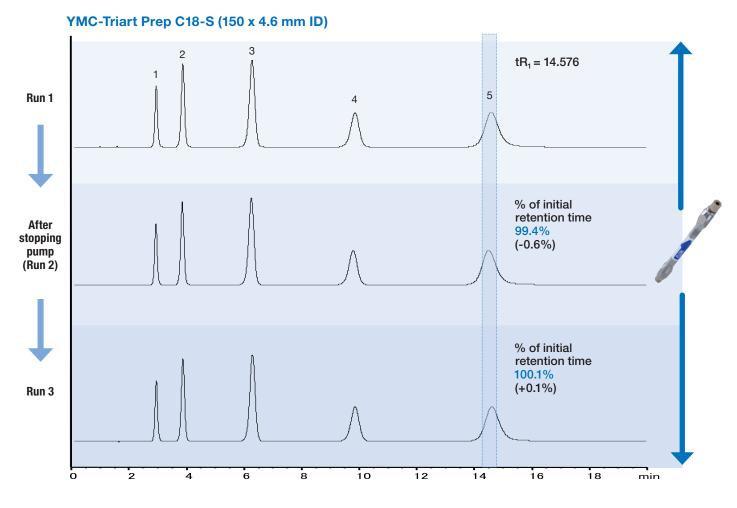
Polar compounds - Procedure

Conditions

System:	Agilent 1200
Column:	YMC-Triart Prep C18-S (10µm) 150 x 4.6 mm ID
Eluent:	1) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.9)
	2) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.9) / acetonitrile = 98/2
Flow rate:	1.0 mL/min
Temperature:	37°C
Detection:	UV at 254 nm
Injection:	8.0 mL
Sample:	1. Cytosine (0.01 mg/mL),
	2. Uracil (0.01 mg/mL),
	3. Guanine (0.02 mg/mL),
	4. Thymine (0.015 mg/mL),
	5. Adenine (0.015 mg/mL)



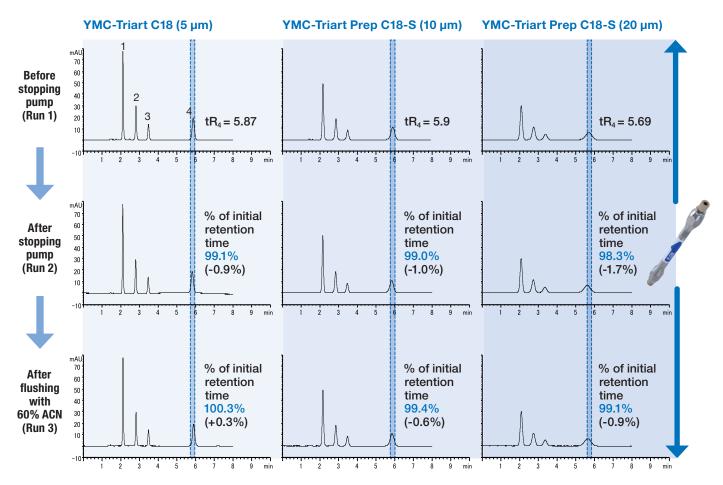
Results





Step 2 Repetition of stress tests

Scalability - Results

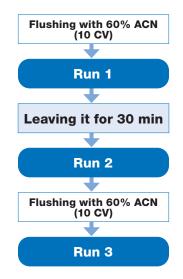


Scalability - Procedure & Conditions

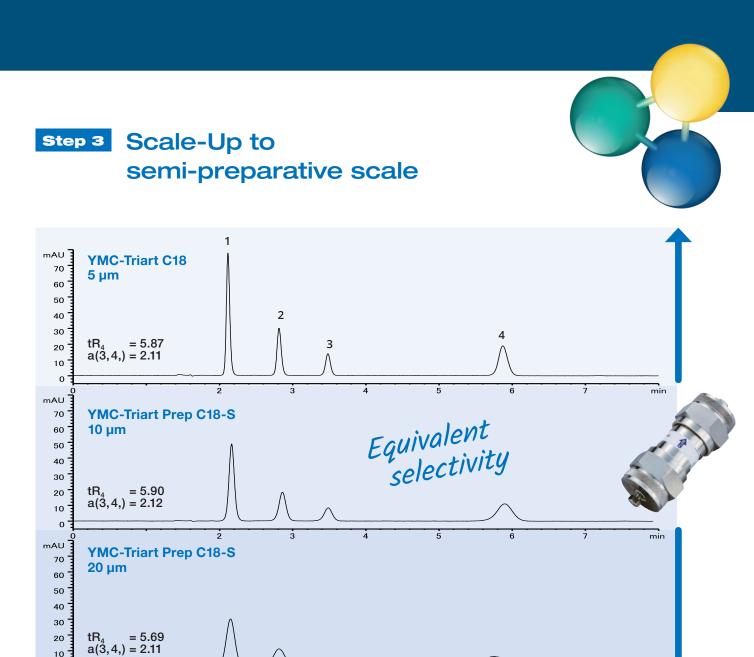
HPLC conditions

$150 \times 4.6 \text{ mm ID}$ Eluent: 20 mM H ₃ PO ₄ Flow rate: 1.0 mL/min Temperature: 37 °C Detection: UV at 220 nm Injection: 10.0 mL Sample: 1. Tartaric acid (0.5 mg/mL) 2. L-Malic acid (0.5 mg/mL) 3. Lactic acid (0.5 mg/mL) 4. Citric acid (0.5 mg/mL)	Flow rate: Temperature: Detection: Injection:	20 mM H_3PO_4 1.0 mL/min 37 °C UV at 220 nm 10.0 mL 1. Tartaric acid (0.5 mg/mL) 2. L-Malic acid (0.5 mg/mL) 3. Lactic acid (0.5 mg/mL)
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Dewetting test conditions







Column:	250 X 20 mm ID
Eluent:	20 mM H ₃ PO ₄
Flow rate:	18.9 mL/min
Temperature:	ambient
Detection:	UV at 220 nm
Injection:	400 mL
Sample:	1. Tartaric acid (0.5 mg/mL)
	2. L-Malic acid (0.5 mg/mL)
	3. Lactic acid (0.5 mg/mL)
	4. Citric acid (0.5 mg/mL)

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Methods developed on analytical YMC-Triart material are readily transferred to the preparative scale. Therefore, YMC-Triart Prep is equally suitable for industrial scale purifications and in process controls.

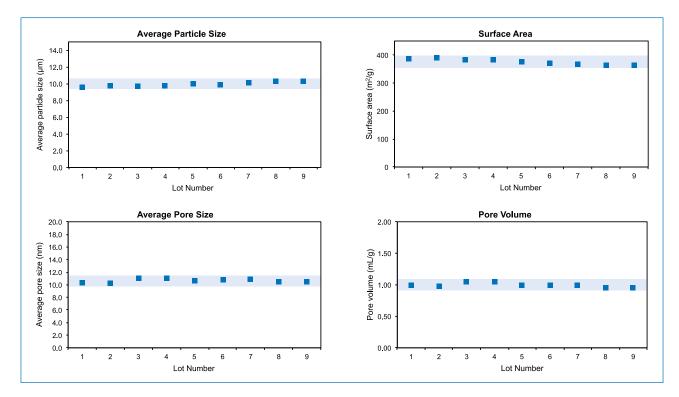




6. Quality Control

Quality control of the hybrid silica-based material

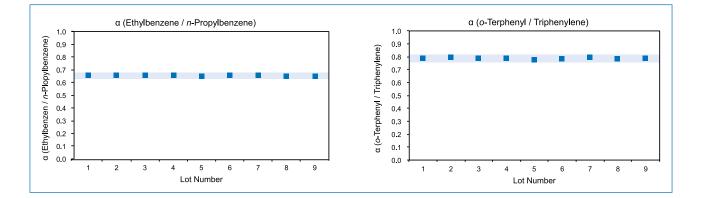
The rigorous quality control procedures set by YMC start with the hybrid base material. The hybrid base material is tested against demanding specifications, which include particle size and distribution, pore size distribution, surface area, pore volume, pH and metal content, etc. Only when the base material satisfies the strict criteria for each parameter can the lot can be allowed to proceed to the bonding processes.



Quality control of the bonded media

YMC's rigorous quality control is reflected in the reproducible separations obtained by the chromatographer. Every batch of bonded material is evaluated for reproducibility to ensure consistent performance with chromatographic tests for:

- hydrophobicity
- performance with acidic compounds
- · performance with basic compounds
- performance with coordination compounds





7. Ordering information/ regulatory compliance/ availability

YMC-Triart Prep C18-S			YMC-Triart Prep C8-S			YMC-Triart Prep Bio200 C8		
Pore size [nm]	Particle size [µm]	Product Code	Pore size [nm]	Particle size [µm]	Product Code	Pore size [nm]	Particle size [µm]	Product Code
12	7	TAS12S07	20	10	TOS20S11	20	10	TOB20S11
	10	TAS12S11		15	TOS20S16			
	15	TAS12S16						
	20	TAS12S21		20	TOS20S21			

Typical pack sizes

- Laboratory scale: smallest amount is 100 grams up to 4 kg in PE bottles
- Industrial scale: more than 4 kg in double lined PE bags inside metal drums (10 or 25 kg drums)



Regulatory support file available under non-disclosure agreement. Used in validated cGMP-manufacturing processes.

Customised material available on request. DMF registered with FDA.

Samples

Bulk samples

Features / Properties

- Stationary phase media for self-packing
- Various quantities and pack sizes available
- Available for all types of YMC RP/NP bulk media

Available pack sizes

• Flexible, customised sample formats



Packed analytical columns

Features / Properties

- Pre-packed stainless-steel scout columns
- · Analytical HPLC columns packed with preparative bulk media
- Available for all types of YMC RP/NP bulk media
- Available for all particle sizes

Exemplary dimensions

- 250 mm x 4.6 mm ID stainless-steel column
- 150 mm x 4.6 mm ID stainless-steel column
- 250 mm x 10 mm ID stainless-steel column
- 150 mm x 10 mm ID stainless-steel column





More about YMC







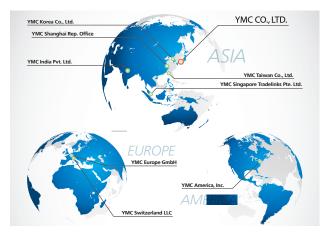




Fully Integrated Manufacturing

YMC operates a fully integrated manufacturing process for the YMC-Triart Prep phases. This process includes the manufacture of the base material and the modification for the RP functionality.

This gives YMC complete traceability and control over the entire manufacturing process. YMC can guarantee reliable product supplies for today and in the future.



Global Supplies

The YMC-Triart Prep phases are available worldwide through a dedicated support network headed by YMC operations in Japan, the US and in Europe to ensure easy, reliable method transfer between research and production sites across the world. Batch sizes up to 200 kg are available in many different packaging formats. Individual supply agreements are common for validated processes.



Quality Control

The rigorous quality control procedures set by YMC start with the manufacturing of the base material. Every batch is evaluated for compliant reproducibility to ensure consistent performance. The YMC facilities are certified according to ISO 9001. Quality agreements are common for validated processes. The manufacturing site is regularly audited successfully by numerous pharmaceutical companies all around the globe.



Regulatory Support

Since all YMC processes and working procedures are thoroughly monitored and documented, YMC always has been in perfect position to prove full compliance with the requirements. The YMC-Triart Prep phases are supplied with the full technical documentation to show compliance with all applicable regulations. The phases are registered for Drug Master Files and are already used for a number of GMP production processes for active pharmaceutical ingredients.

Your local distributor:



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