

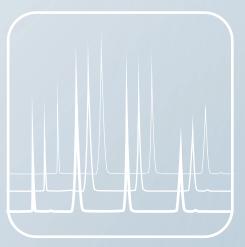
# Preparative columns for HPLC and SFC

## YMC-ACTUS





Throughput
Yield
Long Lasting



#### Fast (semi-)preparative chromatography

Semi-preparative chromatography is the link between analytical HPLC and preparative LC. Even though the chromatographic systems used for (semi-)preparative LC are not as large as preparative LC systems, the objectives remain the same

- · Purification and isolation of maximum sample quantity
- Savings in time and costs.

YMC-Actus packed with YMC's innovative resins

- Yield
- Throughput
- · Long column lifetime

are easily achieved!

# With YMC-Actus, time is on your side!



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## **Specifications**

	YMC-Actus
Stationary phases	YMC-Triart RP Classics CHIRAL ART
Particle size / μm	5, 7*, 10*, 15*, 20*
Internal diameter / mm	20, 30, 50
Length / mm	50, 75, 100, 150, 250
Pressure limit	20-30 mm ID: 30 MPa (4,351 psi) 50 mm ID: 20 MPa (2,900 psi)

\*not all combinations of stationary phase and particle size are available

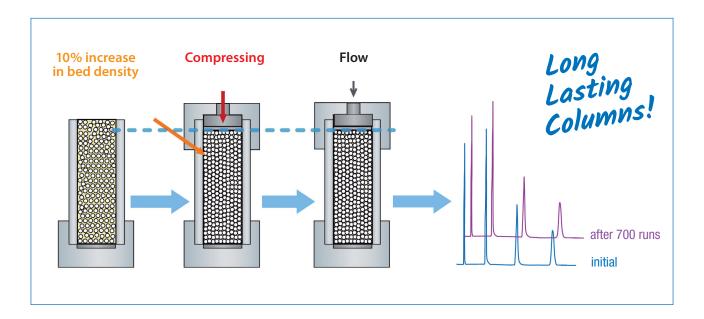


#### High performance purifications with YMC-Actus (semi-)preparative columns

#### How to obtain long lasting columns!

YMC-Actus series columns are semi-preparative HPLC columns that have excellent column stability and efficiency as a result of applying axial compression technology.

YMC-Actus series columns show high stability under high flow rate or steep gradient conditions which are desirable for milligram scale preparative HPLC of various compounds.



Uniformly high density packing is necessary for highly efficient and stable HPLC columns.

DAC (Dynamic Axial Compression) columns are widely used for preparative separation in pilot or production scale. This allows uniformly high density packing and prevents formation of voids.

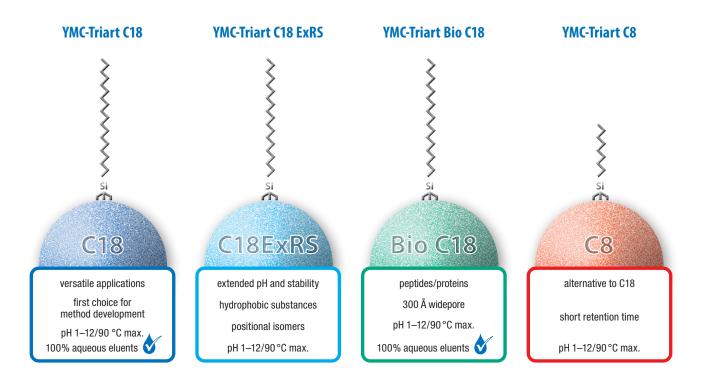
YMC-Actus series columns have been developed by applying this Axial Compression Technology to semiprep column production. The column bed is compressed appropriately when attaching the inlet end assembly of the newly designed YMC-Actus hardware. It provides increased bed density (10% higher than conventional columns) and bed uniformity.

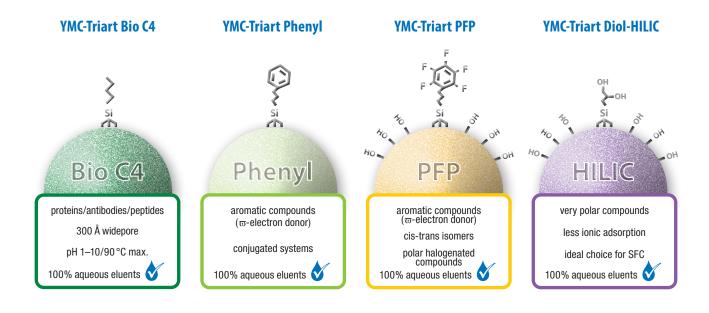
#### YMC-Triart and Actus Technology: A perfect match!

YMC-Triart is a versatile material with exceptional narrow particle and pore size distributions. YMC-Triart allows challenging pH and high temperature conditions in work day-to-day in laboratories. Most importantly,

due to its unique particle composition, a balanced hydrophobicity and silanol activity are achieved which makes YMC-Triart a "First Choice" column in method development.

#### **Phase overview**

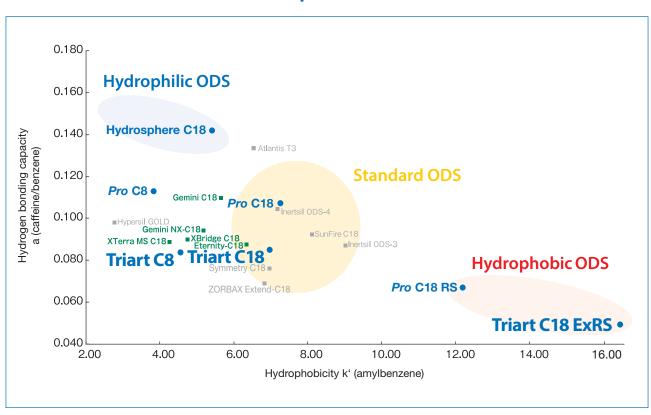


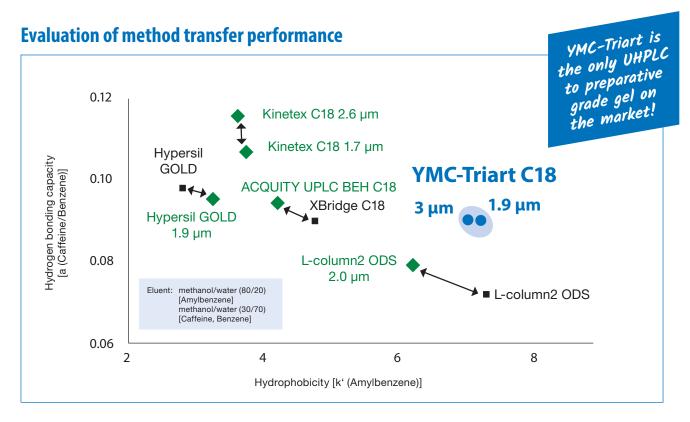


#### **Specification YMC-Triart**

	C18	C18 ExRS	Bio C18	C8	Bio C4	Phenyl	PFP	Diol-HILIC
Base		•	•	organic/ino	rganic silica			
Stationary phase	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)	Phenyl (USP L11)	Penta- fluorophenyl (USP L43)	Diol (USP L20)
Particle size				1.9, 3 a	nd 5 µm			
Pore size	12 nm	8 nm	30 nm	12 nm	30 nm	12 nm	12 nm	12 nm
Specific surface	360 m²/g	430 m²/g	_	360 m²/g	_	360 m²/g	360 m²/g	360 m²/g
Carbon content	20%	25%	_	17 %	_	17 %	15 %	_
Bonding				trifun	ctional			
Endcapping	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	none	none
pH range	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 10	1 ~ 10	1 ~ 8	2 ~ 10
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	50°C	50°C	50°C
Pressure limit	20-30 mm ID: 30 MPa (4,351 psi) 50 mm ID: 20 MPa (2,900 psi)							
100% aqueous eluents	1	×	<b>/</b>	×	<b>✓</b>	<b>√</b>	<b>✓</b>	✓

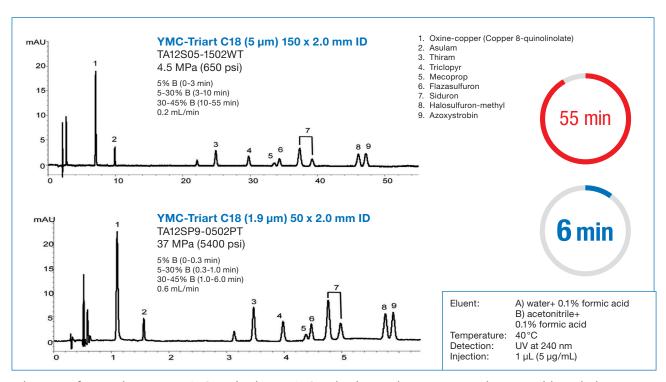
## "First choice" column for method development





With the introduction of UHPLC, sub-2-µm particles became neccessary. Therefore smaller particles have been added to existing column lines. Consequently, sub-2-µm particles may exhibit differences in chromatographic performance. By introducing YMC-Triart, YMC provides matching chromatographic behaviour for all particles sizes!

#### Method transfer HPLC ↔ UHPLC

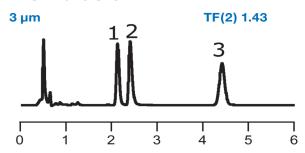


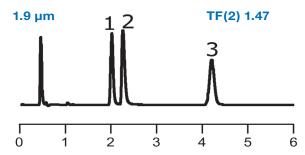
When transferring the 55 min HPLC method to UHPLC scale, the resolution remains the same although the separation time is reduced to only 6 min.

#### **Secure your method transfer!**

Differences in selectivity, retention time, and also peak shapes between different particle sizes of commercially available C18 phases in the same brand (or an alternative as recommended by its manufacturer) have been observed.

#### **YMC-Triart C18**

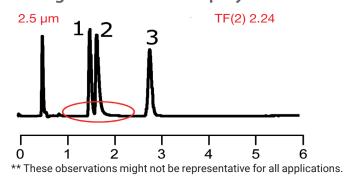


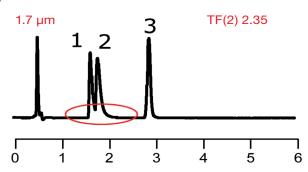


YMC has addressed this issue of method transfer. YMC-Triart columns show identical selectivity and excellent peak shapes for basic compounds for all 3.0 µm to 1.9 µm particle sizes. It allows predictable scale up from UHPLC to conventional HPLC and even to semi-preparative LC, and vice versa.

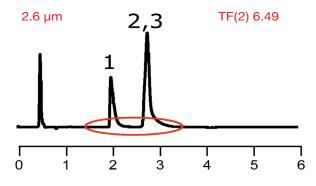
#### **Case Studies**

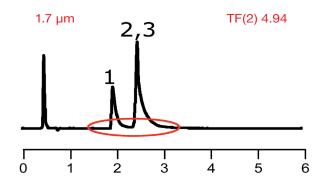
#### X-Bridge BEH C18 and Acquity UPLC BEH C18





#### Kinetex<sup>™</sup> C18





KinetexTM C18 columns show significant peak tailing and have limited scalability due to lack of larger particle sizes.

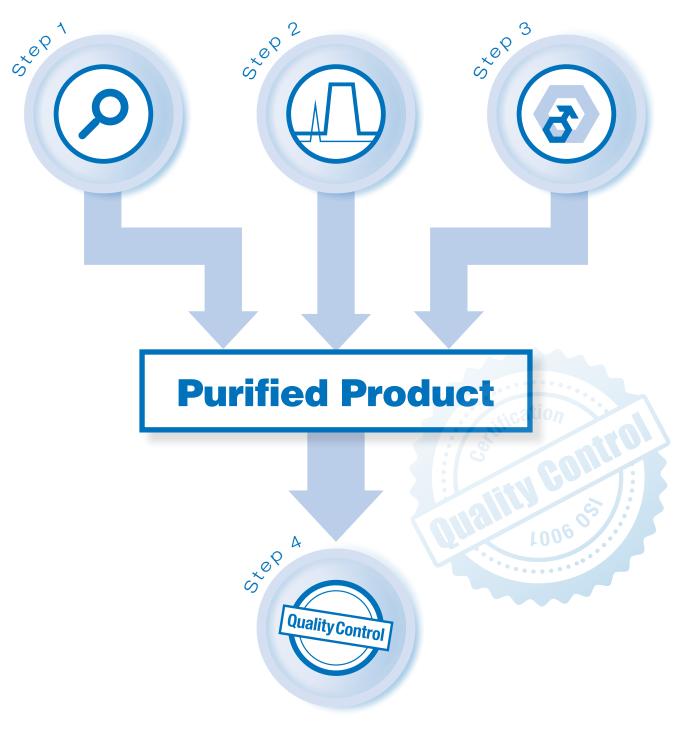
Column: 50 x 2 0 mm ID or 2 1 mm ID

Eluent: 20 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (pH 6.9) / acetonitrile (65/35) Temperature: Flow rate: 40 °C

Detection: UV at 235 nm

- 1. Chlorpheniramine (basic)
- 2. Dextromethorphan (basic)
- 3. Propyl paraben (internal standard)

#### **Effective purification method development!**



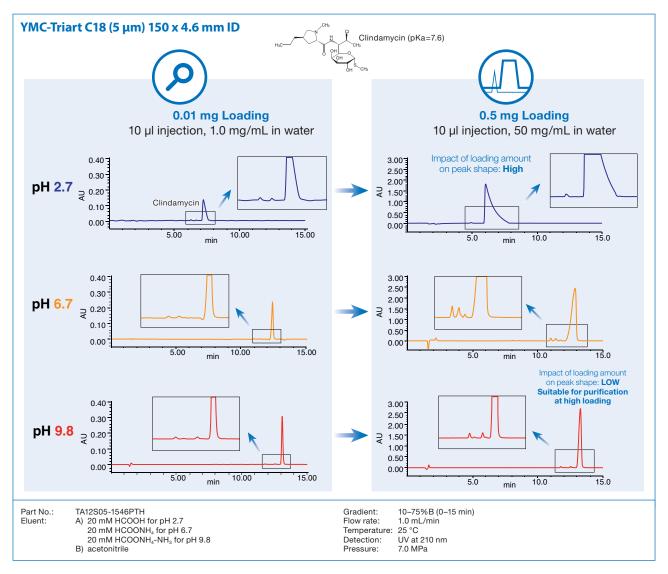




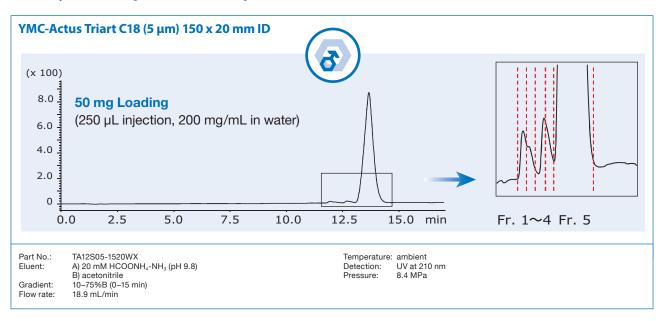




#### **Development of the analytical method**



#### **Quality control/proof of concept**



In order to develop a successful semi-preparative method it is beneficial to carry out the following steps:



Step 1 Developing a well optimised separation on an analytical scale

Determine separation conditions by using analytical columns packed with different stationary phases and various conditions.



Step 2 Loading study on an analytical scale

Select the particle size of the packing material and the inner diameter of column appropriate for the sample volume. Optimise the separation conditions and perform loadability studies using analytical columns with inner diameter of 4.6 mm or 6.0 mm packed with the packing material selected for the preparative separation (scout column). If the particle size of the packing material is the same as in step 1, this process can be omitted. If the preparative column is more than 100 mm ID, it is advisable to insert another step with a scout column of 20 mm ID in order to more accurately predict loadability and calculations of the running costs.



Step 3 Scale-up to semi-preparative column dimensions

Proceed with the preparative separation by scaling up the chromatographic parameters such as flow rate, column ID and sample load by the required factor. Of all the steps in this process, the most demanding step will be the scale-up of the chromatographic parameters in order to meet the preparative demands.

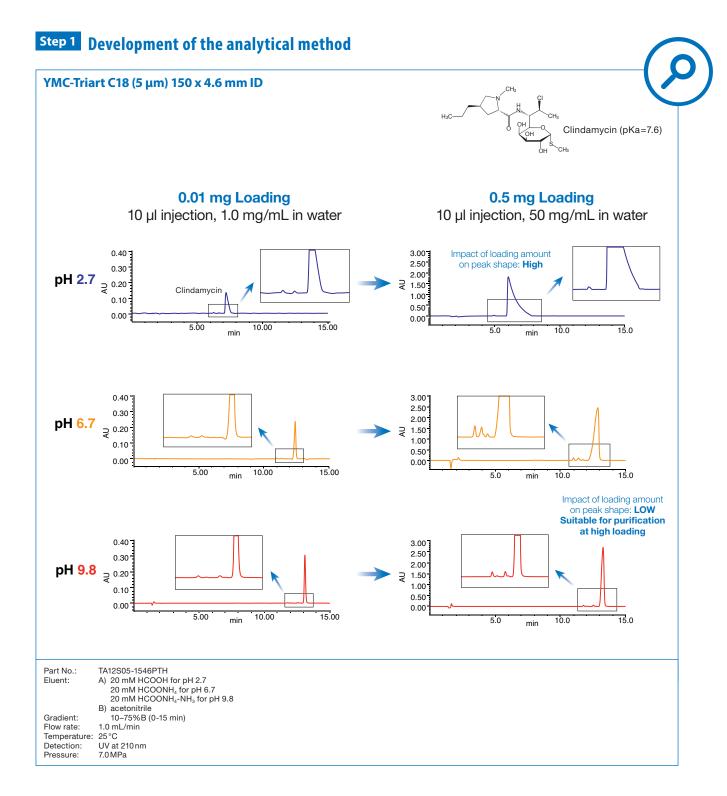
There are a number of scalable parameters which must be included: flow rate, column ID, sample load, tubing ID, sample injection concentration, volume of sample loop, consumption of solvent, dead volume, fraction mass, size of the detector cell.



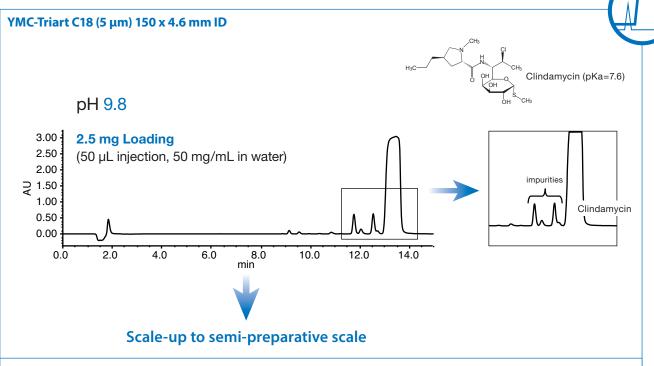
Step4 Quality control/proof of concept

Analyse the fractions from your purification.

#### **Example: Separation of Clindamycin as basic drug**



#### Step 2 Loading study on an analytical scale

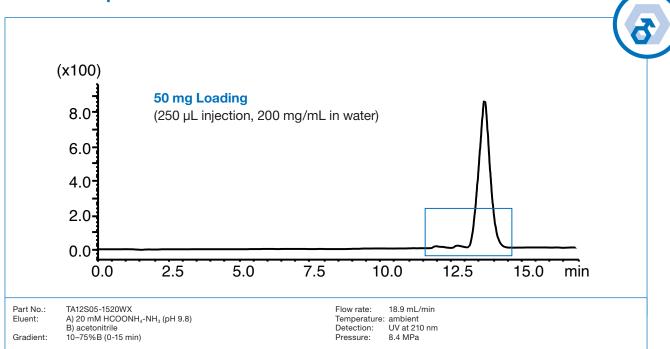


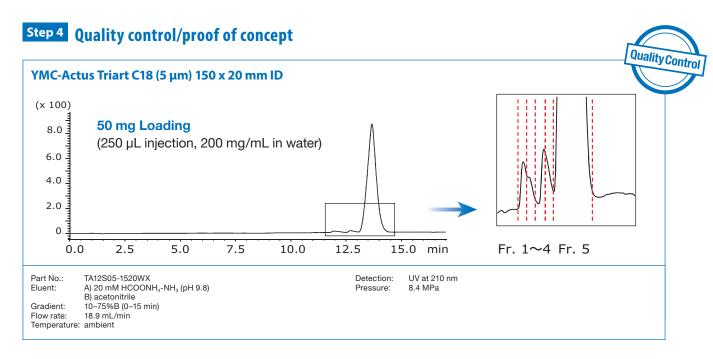
Part No.: Eluent:

TA12S05-1546PTH A) 20 mM HCOONH<sub>4</sub>-NH<sub>3</sub> for pH 9.8

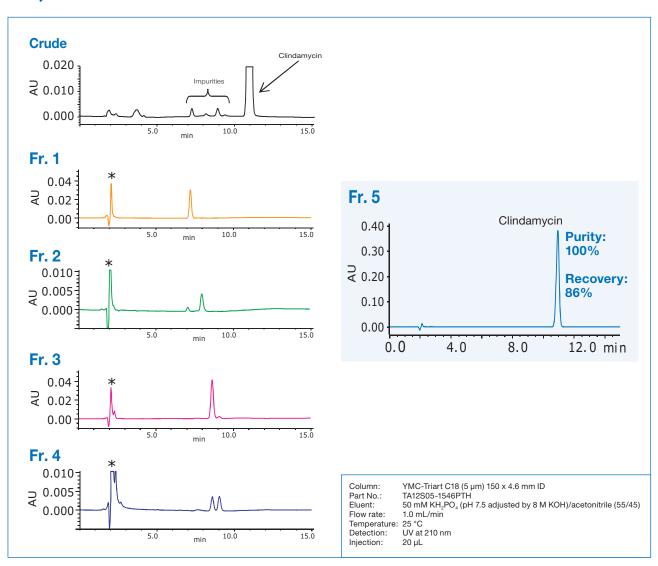
B) acetonitrile 10-75%B (0-15 min) Gradient: Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV at 210 nm
Pressure: 7.0 MPa Detection: Pressure:

#### Step 3 Scale-up





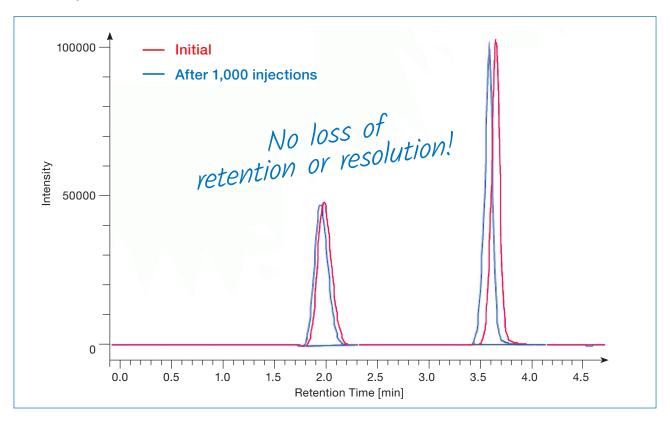
#### **Analysis of fractions**



#### **YMC-Actus delivers SFC stability!**

In general, the stability of chiral and achiral 50 mm ID SFC columns can often be reduced. Also, the inlet frit is often distorted after about 500 runs which leads to a silica leakage.

Using YMC-Actus for SFC long life times can be achieved as a result of the robust column packing. The following test shows the stability of YMC-Actus.



	Repeated injections	Standard test		
Mobile Phase	CO <sub>2</sub> /methanol (70/30)	CO <sub>2</sub> /methanol (70/30)		
Flow Rate	195 mL/min	195 mL/min		
Pressure	120 bar (at column head)	120 bar (at column head)		
Detection	254 nm	254 nm		
Temperature	30 °C	30 °C		
Backpressure	100 bar	100 bar		
Sample	Methanol	1. Toluene (5 μL/mL), 2. Caffeine (500 μg/mL)		
Injection Volume	1 mL (inject every 1 min)	1 mL (inject every 1 min)		
Pressure drop		40 bar		

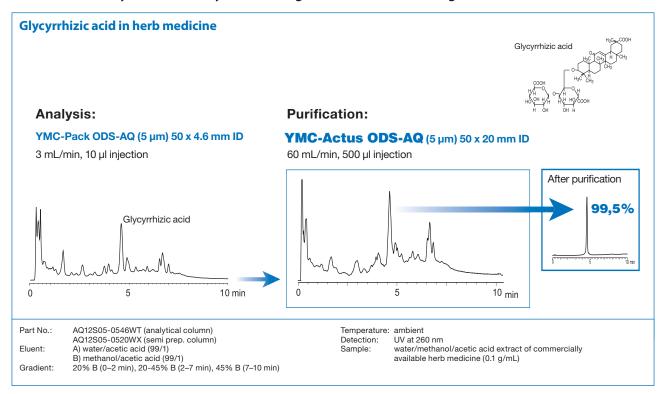
In order to monitor column stability under a customer's usual SFC working conditions not only was a pressure of 120 bar (at column head) applied but 1 mL injections were also applied every minute with a pressure drop of 40 bar. The column performance was checked after every 100 injections.

No significant change in performance could be detected after 1,000 repeated injections (corresponding to about 17 h).

Furthermore, no significant changes in retention time, theoretical plate count and tailing factor could be determined over the complete test period.

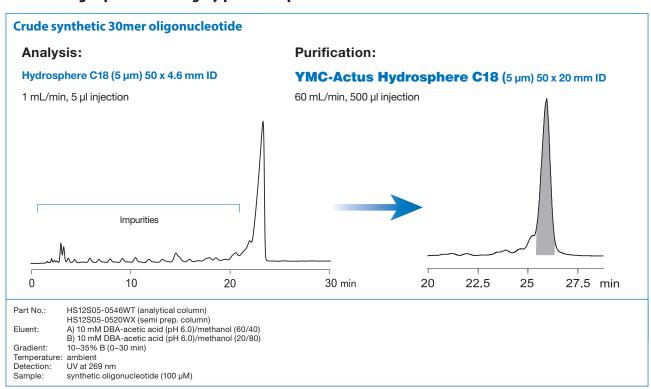
#### YMC-Actus and YMC-Pack ODS-AQ

#### Excellent stability and efficiency under fast gradient condition at high flow rate



#### **YMC-Actus and Hydrosphere C18**

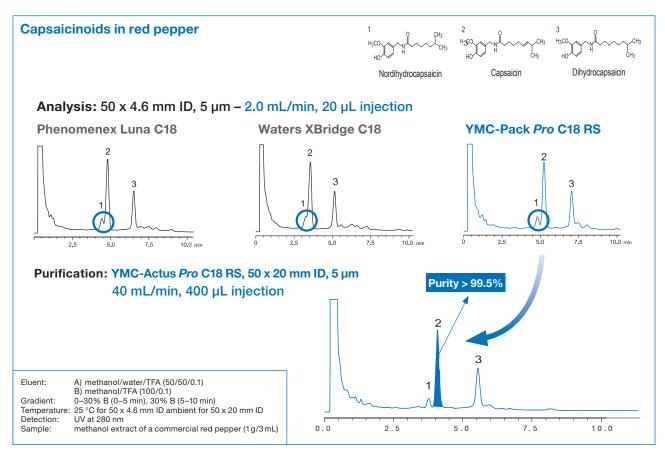
#### **Outstanding separation of highly polar compounds**



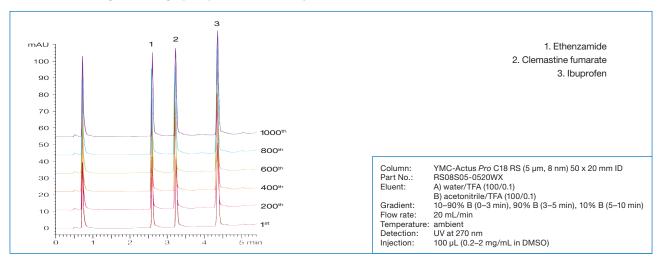
#### YMC-Actus and YMC-Pack Pro C18 RS

#### **Excellent separation of compounds with similar structures**

YMC-Actus shows the same excellent separation results as achieved with analytical columns. Even compounds with similar structures can be separated with high purities.



#### Available for high-throughput purification: Injection in DMSO



As shown in the above overlay of chromatograms, YMC-Actus columns provide outstanding stability and reproducibility for the separation of pharmaceuticals dissolved in 100% DMSO, even after 1,000 injections under the test conditions. This demonstrates that YMC-Actus columns are ideal for high-throughput purification in drug discovery.

#### **CHIRAL ART Immobilised Polysaccharide Derivatives Series**

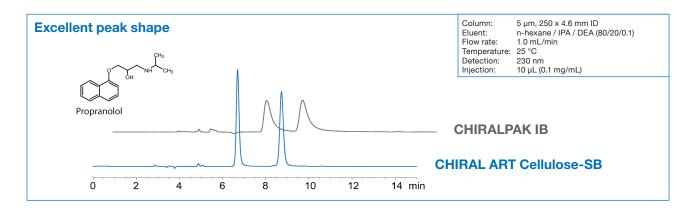
- · applicable for normal and reversed phase modes
- · unique immobilised chiral selector
- · more flexibility due to wide range of usable solvents
- · highly robust, also suitable for SFC/SMB
- HPLC columns and preparative grade bulk media with particle sizes of 3, 5, 10 or 20 µm available
- · extremely attractive pricing

#### Introduction

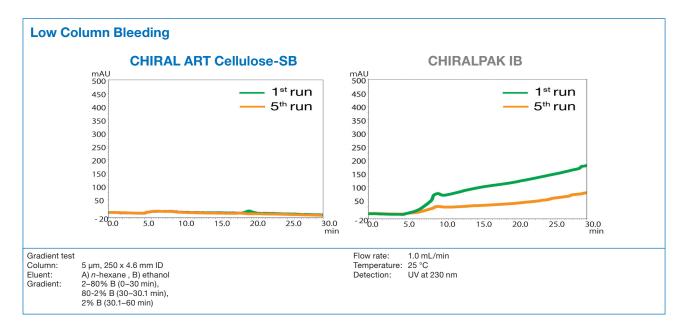
# CHIRAL ART polysaccharide derivatives are a series of chiral separation columns/packing materials with high stereo-selectivity. They are suitable for separations of a wide range of chiral compounds, cis-trans isomers and geometric isomers. The range of particle sizes and column dimensions available offer outstanding cost effectiveness for analytical to preparative separations.

#### **Immobilised Type**

CHIRAL ART immobilised polysaccharide derivatives can be used either in normal phase or in reversed phase modes. They are available in HPLC columns and in preparative grades, in large (multi kg) quantities.



CHIRAL ART polysaccharide derivatives provide excellent peak shapes for ionic and metal coordinating compounds.



#### **Immobilised Polysaccharide Derivatives Series**

	CHIRAL ART Amylose-SA	CHIRAL ART Cellulose-SB	CHIRAL ART Cellulose-SC	CHIRAL ART Cellulose-SJ				
Particle size		3, 5, 10	, 20 µm					
Chiral selector	Amylose tris (3,5-dimethylphenyl- carbamate) (3,5		Cellulose tris (3,5-dichlorophenyl- carbamate)	Cellulose tris (4-methylbenzoat)				
USP	L99	L99 — –		_				
Туре		Immobili	sed type					
Separation mode		Normal Phase / Rev	versed Phase / SFC					
Shipping solvent		n-hexane / 2-propanol (90/10)						
Usable pH-range		2.0-9.0						
Temperature	0-40°C							
Pressure limit	20–30 mm ID: 30 MPa (4,351 psi) 50 mm ID: 20 MPa (2,900 psi)							

#### **Product Line-up**

Product name	Particle size	CHIRAL selector	Туре	Competitive product
CHIRAL ART Amylose-SA	3 µm	Amylose tris (3,5-dimethylphenylcarbamate)		CHIRALPAK® IA, IA-3
CHIRAL ART Cellulose-SB	5 μm	Cellulose tris (3,5-dimethylphenylcarbamate)		CHIRALPAK® IB, IB-3
CHIRAL ART Cellulose-SC	10 μm	Cellulose tris (3,5-dichlorophenylcarbamate)	Immobilised	CHIRALPAK® IC, IC-3
CHIRAL ART Cellulose-SJ	20 μm	Cellulose tris (4-methylbenzoat)		same chiral selector as CHIRALPAK® OJ (coated)

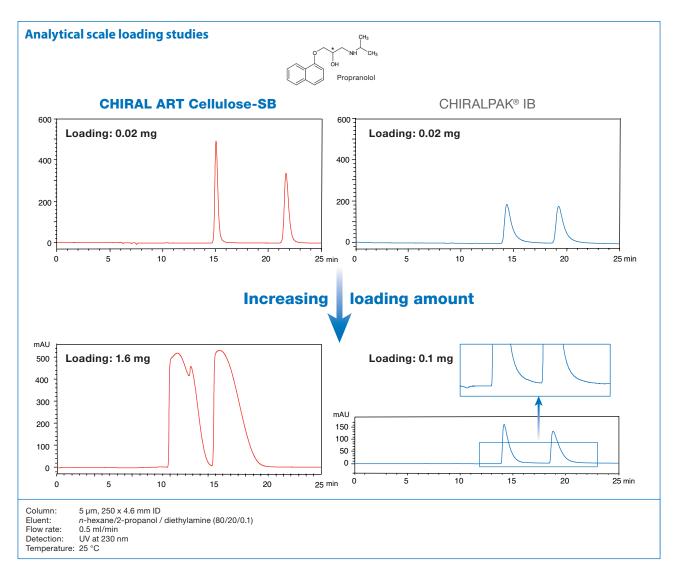
#### **Column Care**

The recommended pH range for using CHIRAL ART immobilised polysaccharide columns is 2.0-9.0. Remove acid and buffer salts before storage. Store the column in n-hexane/2-propanol = 90/10 (NP) or methanol/water = 50/50 (RP).

If columns are affected by undesired contaminants or clogged inlet frits which cause back pressure increases, flush the column (in the reversed direction) with ethanol.

For detailed information please refer to the "Column Care and Use Instructions" which can be downloaded from www.ymc.de/support-documentation.html.

#### **Efficient purification using YMC-Actus CHIRAL ART**



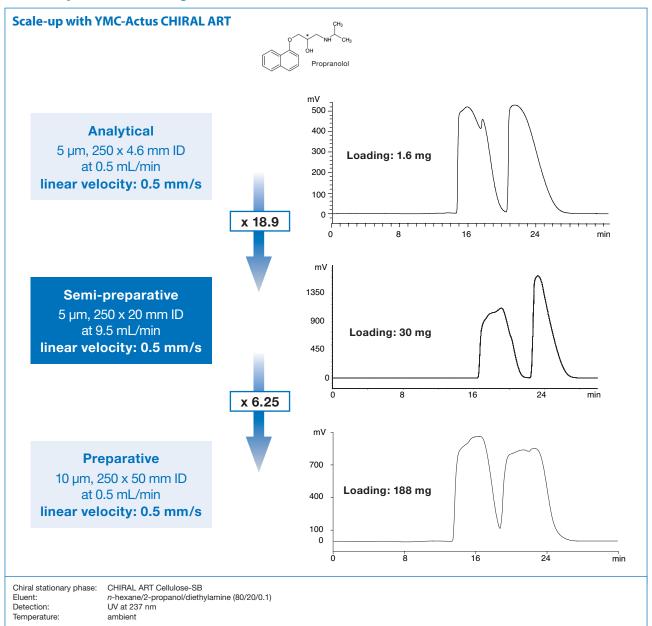
With the competitor's product, loading amounts of more than 0.1 mg was not possible because the enantiomeric excess of the 2nd peak was already less than 98%ee with a loading amount of 0.1 mg.

	CHIRAL ART	Cellulose-SB	CHIRALPAK® IB		
	1 <sup>st</sup> peak 2 <sup>nd</sup> peak		1 <sup>st</sup> peak	2 <sup>nd</sup> peak	
Enantiomeric excess	>99.9%ee	99.3%ee	>99.9%ee	97.9%ee	
Recovery	99%	99%	99%	97%	
Productivity (mg/h)*	3.1	3.3	0.3	0.3	

\*Calculated for repeated injections every 15 minutes (CHIRAL ART Cellulose-SB) and every 10 minutes (CHIRALPAK® IB).

The calculated maximum loading amount on CHIRAL ART Cellulose-SB of 1.6 mg was 10 times larger than that obtained for the competitor's product due to the large differences in the peak shapes, even though the interval between repeat injections was longer!

#### **Efficient purification using YMC-Actus CHIRAL ART**



	Analytical 250 x 4.6 mm ID		Semi-pre	YMC-Actus Semi-preparative 250 x 20 mm ID		Self-packed DAC Preparative 250 x 50 mm ID	
	1 <sup>st</sup> peak	2 <sup>nd</sup> peak	1 <sup>st</sup> peak 2 <sup>nd</sup> peak		1 <sup>st</sup> peak	2 <sup>nd</sup> peak	
Enantiomeric excess	>99.9%ee	99.3%ee	99.9%ee	99.8%ee	99.1%ee	99.3%ee	
Recovery	99%	99%	97%	99%	99%	94%	
Productivity (mg/h)	3.1	3.3	58.6	62.4	366	390	

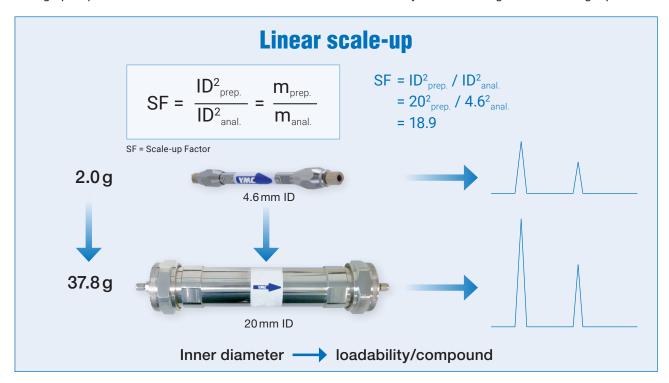
Linear scale-up was performed using the appropriate scaling factors. The Dynamic Axial Compression Column self-packed with CHIRAL ART Cellulose-SB 10  $\mu$ m can be easily and linearly scaled-up for an even greater purification scale. The final productivity is 366 and 390 mg/h respectively for peaks 1 and 2.

#### **Linear scale-up**

In order to simplify the scale-up process the three most important scale-up factors are summarised below.

Scalable factor SF	ID "Linear scale-up"	Column length	Column length and ID "Volume"
	$SF = \frac{ID^2_{prep.}}{ID^2_{anal.}}$	$SF = \frac{L_{prep.}}{L_{anal.}}$	$SF = \frac{ID_{\text{prep.}}^2}{ID_{\text{anal.}}^2} / \frac{L_{\text{prep.}}^2}{L_{\text{anal.}}^2}$
Impact	Flow rate Eluent composition	Retention time Cycle time Plate number	Amount of adsorbent

In most cases it is beneficial to start development of a semi-preparative method using an analytical scale column. The analytical separation carried out on a  $150 \times 4.6 \text{ mm}$  ID column has to be scaled up to  $150 \times 20 \text{ mm}$  ID. Therefore the chromatographic parameters such as flow rate and column load have to be adjusted according to the following equation:



#### **Guideline for Sample Load according to column ID**

Column ID (mm)	Scale-up factor	Loadability (mg)
4.6	1	1–4
10	4.7	5–20
20	18.9	20–80
30	42.5	40–160
50	118	80–350
75	266	270-980
100	472	470–1,900
150	1,060	1,000-4,200

#### **Optimisation of preparative chromatography**

The main task for a preparative chromatographer is to find the suitable system. In order to simplify the decisions that have to be made YMC have developed a "Preparative Column Selection Guide".

				Lab sca	ile						Productio	n scale		
Column inner diameter [mm ID]			r [mm ID]	4.6	10	20	30	50	100	200	500	1,000		
Cross s	section	nal are	a ratio	1.0	4.7	19	42	118	473	1,890	11,800	47,300		
		FI	low rate	0.5	2.4	9.5	21	60	235	950	6,000 (6 L)	24,000 (24 L)		
Example of calcula	cample [ml/min]		ml/min]	1.0	4.7	19	42	120	470	1,900	12,000 (12 L)	47,000 (47 L)		
		Loa	iding [mg]	5	25	100	220	600	2,500	10,000	60,000 (60 g)	240,000 (240 g)		
HIGH					5	+++	+++	+++	+++	++	+	+		
			10	++	+++	+++	+++	+++	++	++	++	++		
Column efficiency, Pressure, Costs	Part si: [μι	ze	10–20	+	++	++	++	+++	+++	+++	++	++		
Low		15–30		+	+	+	++	+++	+++	+++	++			
			50~					+	++	++	+++	+++		

+++ Most appropriate, ++ Appropriate, + Depending on purpose

Flow rate equation (Use the same equation to calculate the sample load)

 $F' = Fx(Dc'/Dc)^2$ 

F': Analytical column flow rate [mL/min]

F: Preparative column flow rate [mL/min]

Dc: Analytical column diameter [mm ID]

Dc': Preparative column diameter [mm ID]

The "Preparative Column Selection Guide" will help selection of:

- 1. the column ID for the required sample loading
- 2. the particle size for optimum efficiency
- 3. the column length for the necessary resolution

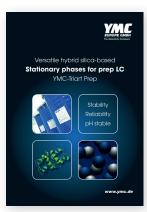
#### **R&D Kits**

To minimise the problems that the process of scale-up may introduce, YMC offers YMC R&D kits, which consist of one analytical column and one YMC-Actus column packed with exactly the same packing material from the same production batch. Therefore, no further method development is needed, just a simple linear scale-up calculation.

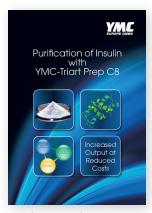
#### **Other Catalogues/Brochures Available**



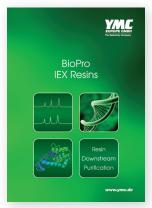
(U)HPLC columns YMC-Triart



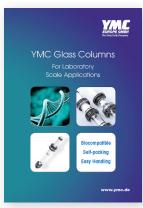
**YMC-Triart Prep** 



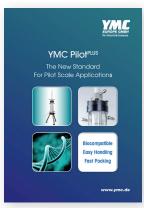
Purification of Insulin with YMC-Triart Prep C8



**BioPro IEX Resins** 



YMC Glass Columns



YMC PilotPlus

#### Application data mainly by courtesy of YMC Co., Ltd.

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## **Ordering Information**

**Product code:** 

XXXXXXX-

Phase (code)

#### **Available Phases**

Phase	Particle size [µm]	Pore size [nm]	Phase code
YMC-Triart C18	5	12	TA12S05-
YMC-Triart C18 ExRS	5	8	TAR08S05-
YMC-Triart Bio C18	5	30	TA30S05-
YMC-Triart C8	5	12	TO12S05-
YMC-Triart Bio C4	5	30	TB30S05-
YMC-Triart Phenyl	5	12	TPH12S05-
YMC-Triart PFP	5	12	TPF12S05-
YMC-Pack Pro C18	5	12	AS12S05-
Hydrosphere C18	5	12	HS12S05-
YMC-Pack Pro C18 RS	5	8	RS08S05-
YMC-Pack Pro C8	5	12	OS12S05-
YMC-Pack Pro C4	5	12	BS12S05-
YMC-Pack ODS-A	10	12	AA12S05-
YMC-Pack ODS-AQ	15	12	AQ12S05-
YMCbasic	20	20	BA99S05-
	7	12	TAS12S07-
V440 T: + B	10	12	TAS12S11-
YMC-Triart Prep C18-S	15	12	TAS12S16-
	20	12	TAS12S21-
	10	20	TOS20S11-
YMC-Triart Prep C8-S	15	20	TOS20S16-
	20	20	TOS20S21-
YMC Omega	10	_	OMG99S11-
	5	_	KAN99S05-
CHIRAL ART Amylose-C	10	_	KAN99S11-
	20	-	KAN99S21-
CUIDAL ARTA L. CAL	5	_	KBN99S05-
CHIRAL ART Amylose-C Neo	10 20	_	KBN99S11-
	5		KBN99S21- KCN99S05-
CHIRAL ART Cellulose-C	10	_	KCN99S11-
O'min Le / ii ii oonalooo o	20	_	KCN99S21-
	5	_	KSA99S05-
CHIRAL ART Amylose-SA	10	_	KSA99S11-
	20	_	KSA99S21-
	5	_	KSB99S05-
CHIRAL ART Cellulose-SB	10	_	KSB99S11-
	20	-	KSB99S21-
	5	-	KSC99S05-
CHIRAL ART Cellulose-SC	10	_	KSC99S11-
	20	_	KSC99S21-
CHIRAL ART Cellulose-SJ	5 10	_	KSJ99S05- KSJ99S11-
Offinal Arti Ociulose-33	20		KSJ99S11- KSJ99S21-
	20	_	V9199951-

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Hardware/Dimensions (code)

Example: YMC-Triart C18 5  $\mu$ m, 100 x 20 mm ID

Phase code: TA12S05-Hardware code: -1020WX → Product code: TA12S05-1020WX

#### **Available Dimensions**

Column ID [mm]	Column length (mm)					Guard cartridges* with 10 mm length
	50	75	100	150	250	(pack of 2)
20	-0520WX	-	-1020WX	-1520WX	-2520WX	-0120CCN
30	-0530WX	-L530WX	-1030WX	-1530WX	-2530WX	-0130CCN
50	-	-	-1053DX	-1553DX	-2553DX	(-0553DXG)**

\*holder required: 20 mm guard column ID: XPGHFSP20ID; 30 mm guard column ID: XPGHFSP30ID \*\*no holder required for 50 x 50 mm ID guard columns (no cartridge)

YMC-Actus CHIRAL ART columns are available with 100 mm, 150 mm and 250 mm length.

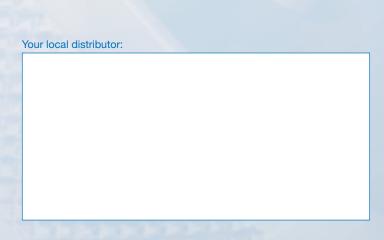
50 mm ID columns are also available with a 1/16" connection (AX) while the standard is a 1/8" connection (DX).

Adapter for 1/8" Connection: DX



Adapter for 1/16" Connection: AX





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