

Your first choice.

Purospher® STAR RP-18 endcapped HPLC columns.



Designed for universal use.

Purospher® STAR RP-18 endcapped HPLC columns
are designed for universal use.

It doesn't matter if your samples are basic, neutral, metal chelating or indeed any other format. You can be sure that Purospher® STAR RP-18 endcapped can do it, naturally without peak tailing! Thanks to its outstanding performance and stability, Purospher® STAR RP-18 endcapped allows a maximum flexibility in method development. Robust methods can be developed across the entire pH spectrum from 1.5 to 10.5 enabling the use of the complete range of mobile phases and temperatures.



Experience the performance

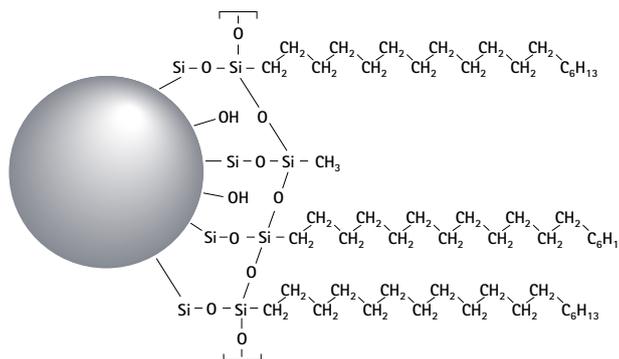
- High separation efficiency
- Outstanding robustness and excellent pH-stability
- Providing absolutely reproducible results
- Working with all sorts of samples without peak tailing
- Extremely wide application range
- Long column life

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Surface modification of Purospher® STAR RP-18 endcapped

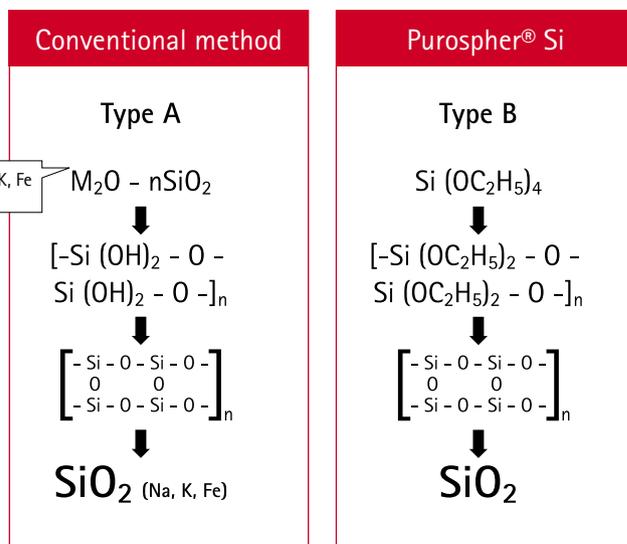
The polymeric surface modification of Purospher® STAR RP-18 endcapped provides a nearly perfect coverage of the surface. This prevents silanophilic interactions.



Highest purity silica – Enhanced performance for best results

The prerequisite for a modern RP-sorbent is a highly purified silica as a starting material. Purospher® HPLC columns are based upon a high purity, metal free silica for excellent separations with very good peak symmetry. Purospher® STAR is a type of silica manufactured in a sol-gel process from tetraalkoxysilane with a purity of 99.999 %.

4



Specifications

Purospher® STAR RP-18 endcapped

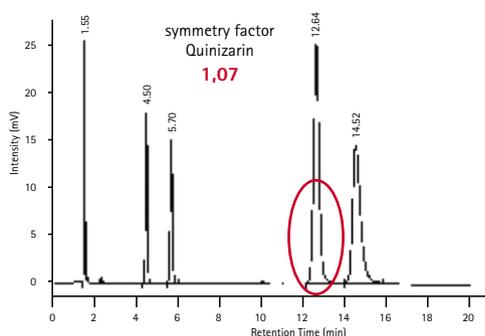
Sorbent:	High-purity, silica gel
Particle size:	3 µm and 5 µm
Surface modification:	C-18, endcapped
Pore diameter:	120 Å
Specific surface:	330 m ² /g
Pore volume:	1.1 mL/g
Carbon load:	17 %
Coverage of the surface	3 µmol/m ²
Performance	5 µm: > 85,000 N/m 3 µm: > 130,000 N/m
pH range:	1.5 - 10.5

Metal content in ppm

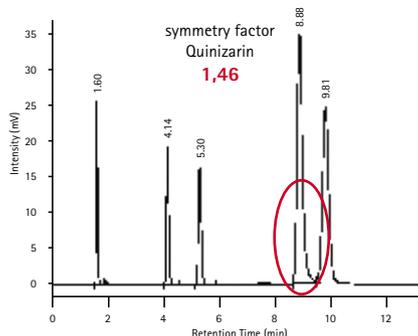
	Sodium	Calcium	Magnesium	Iron	Aluminium
LiChrosorb®	340-400	1300	160-220	20-25	15-20
LiChrospher®	150-250	6-10	4-6	20-40	75-140
Purospher® Star	1	1	1	3	1

Due to the absence of metals in the silica matrix in combination with a complete coverage of the silica surface, this stationary phase enables tailing-free chromatography of acidic, basic and chelating compounds. There are differences in quality of so-called "high purity" HPLC column materials. The peak shape of the complexing agent Quinizarin is one of the best indicators for the purity of silica.

Purospher® STAR RP-18 endcapped



Column L



Mobile phase: Methanol/
Buffer pH 7.0 80/20
(5 mmol KH_2PO_4 and
5 mmol K_2HPO_4);

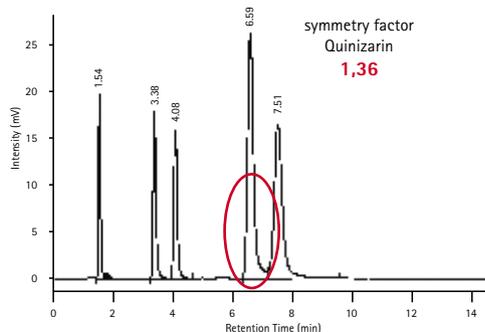
Flow rate: 1.0 mL/min;

Detection: UV 254 nm;

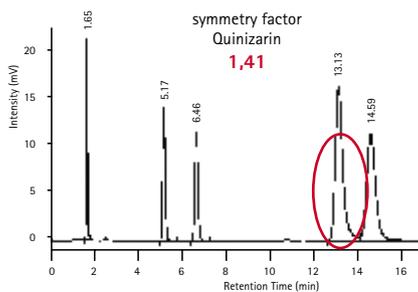
Temperature: 22 °C

Sample:
1. Uracil
2. Toluene
3. Ethylbenzene
4. Quinizarin
5. Amitriptyline

Column X



Column I



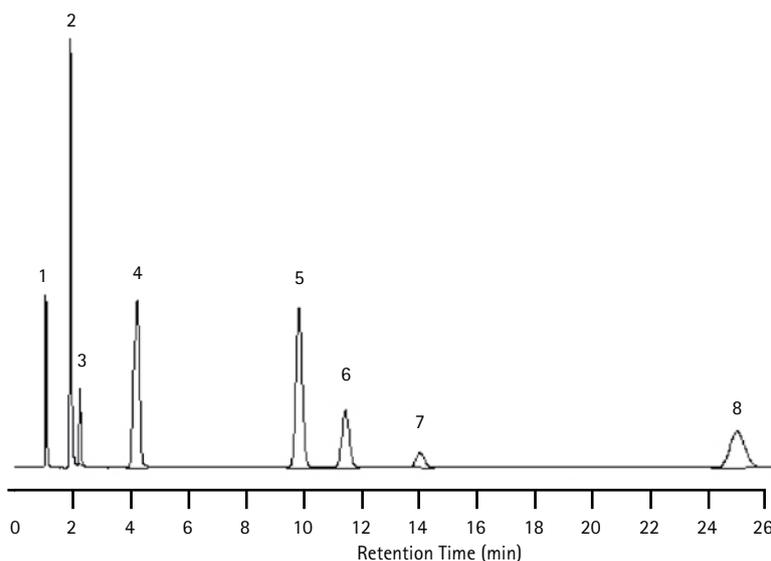
The column comparison demonstrates Purospher® STAR RP-18 endcapped with the best peak symmetry for quinizarin and the silica of highest purity.

Characterization

Although it is very important to control the physical and chemical properties, a consistently high level of reproducibility can only be ensured by a comprehensive chromatographic characterisation. With respect to consistent selectivity we apply different approaches of leading scientists in HPLC.

Engelhardt Test

Purospher® STAR RP-18 endcapped shows perfect co-elution of p-, m- and o-ethyl aniline indicating no polar interactions. The anilines are eluting as symmetric peaks which shows the very good suitability for separation of strong bases.



Column: LiChroCART® 125-4
Purospher® STAR
RP-18 endcapped

Mobile Phase: Methanol/Water
55/45 (v/v)

Flow rate: 1.0 mL/min

Temperature: ambient

Detection: UV 254 nm

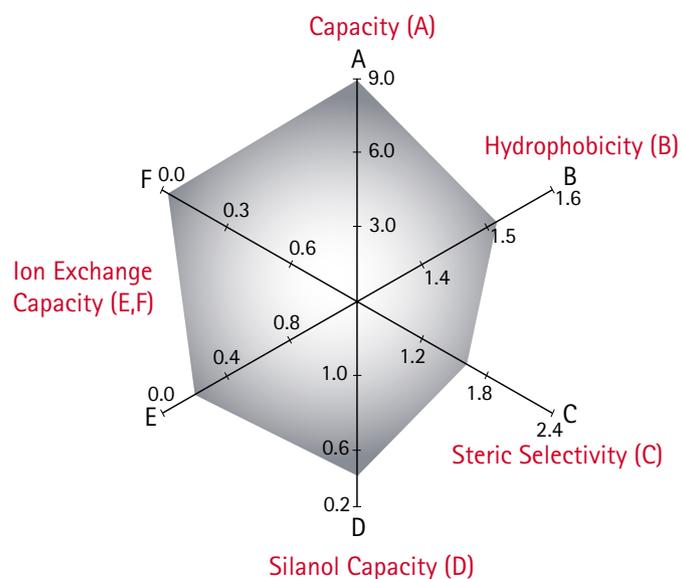
1. Thiourea	t_0
2. Aniline	basic
3. Phenol	acidic
4. p-, m-, o-ethylaniline	basic
5. N, N - Dimethylaniline	basic
6. Ethyl benzoate	neutral
7. Toluene	neutral
8. Ethylbenzene	neutral

Excellently balanced – Tanaka Test

The Tanaka test is established worldwide as the best method of comparing the selectivity and performance of HPLC columns. This test summarizes and visualizes all the most important parameters required when choosing the right HPLC column and allows easy comparisons to be made.

A set of seven selected substances is used to describe capacity, hydrophobicity, steric selectivity and silanophilic properties. To facilitate the illustration and to recognize the quality of a sorbent at one glance, the values of these parameters are outlined on the six axes of a hexagon.

The more symmetrical the hexagon appears and the larger its area, the more balanced the stationary phase is in the sum of its chromatographic properties.



A:	k' (Pentyl benzene)	9.59
B:	α (Pentyl-/Butyl benzene)	1.51
C:	α (Triphenylene/o-Terphenyl)	1.63
D:	α (Caffeine/Phenol)	0.44
E:	α (Benzylamine/Phenol; pH 7.6)	0.23
F:	α (Benzylamine/Phenol; pH 2.7)	0.02

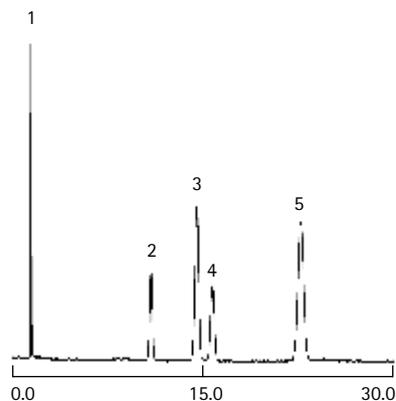
Parameters	Property of the stationary phase	Factors in preparation of the stationary phase
Capacity (A):	number of alkyl chains	silica surface; surface coverage
Hydrophobicity (B):	CH ₂ group selectivity	surface coverage
Steric selectivity (C):	differentiation according to the shape of compounds	silane functionality surface coverage
Silanol capacity (D):	content and type of silanol groups	residual silanols endcapping; surface coverage
Ion exchange capacity (E):	at high pH	residual silanols; active sites pH 7
Ion exchange capacity (F):	at low pH	metal impurities

Literature: *J. Chromato. Sci.* 27, 125, 1989.



Tanaka 1

The Tanaka 1 Test describes the retention capacity, the hydrophobicity and the steric selectivity of RP-phases.



Column: LiChroCART® 150-4.6
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile phase: Methanol/Water 80 : 20

Flow rate: 1.0 mL/min

Detection: UV 254 nm

Temperature: 30 °C

Inj. Volume: 10 µL

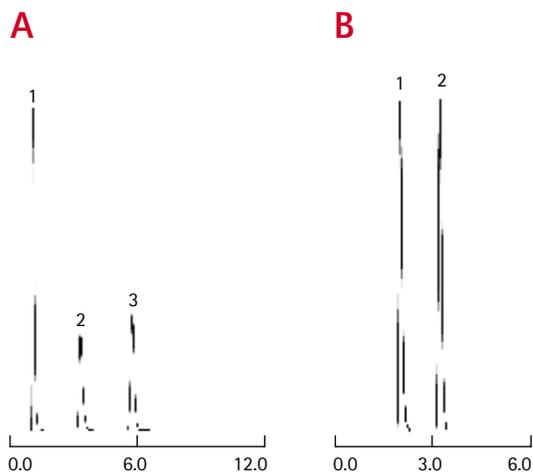
Sample:

1. Uracil
2. Butylbenzene
3. *o*-Terphenyl
4. Pentylbenzene
5. Triphenylene

7

Tanaka 2

The Tanaka 2 Test (Chromatogram A) illustrates the silanophilic properties of stationary phases. Chromatogram B is not part of Tanaka 2 Test. The Phenol/Pyridine test measures Silanol activities.



Column: LiChroCART® 125-4
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile phase A: Methanol/Water 30 : 70 (v/v)

Mobile phase B: Acetonitrile/Water 30 : 70 (v/v)

Flow rate: 1.0 mL/min

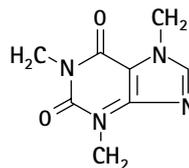
Detection: UV 254 nm

Sample A:

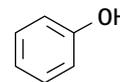
1. Uracil
2. Caffeine
3. Phenol

Sample B:

1. Pyridine
2. Phenol



Caffeine



Phenol

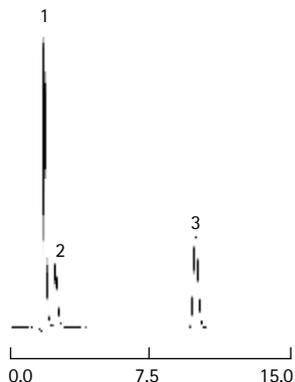


Pyridine

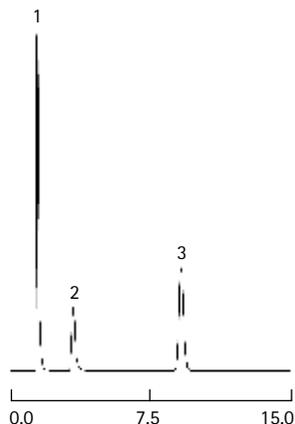
Tanaka 3 + 4

The result obtained from Tanaka 3 shows the deactivation of the residual silanol groups is complete. Tanaka 4 indicates the essential absence of metal in the sorbent.

Ion exchange < pH 3



Ion exchange > pH 7



Column: LiChroCART® 125-4
Purospher® STAR
RP-18 endcapped, 5 µm

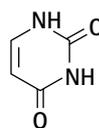
Mobile phase Tanaka 3: 0.02 M Phosphate buffer pH 7.6
Methanol/Buffer 30/70

Mobile phase Tanaka 4: 0.02 M Phosphate buffer pH 2.7
Methanol/Buffer 30/70

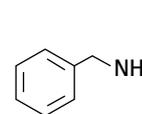
Flow rate: 0.6 mL/min

Detection: UV 254 nm

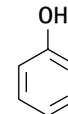
Sample: 1. Uracil
2. Benzylamine
3. Phenol



Uracil



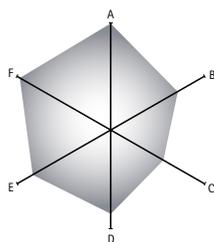
Benzylamine



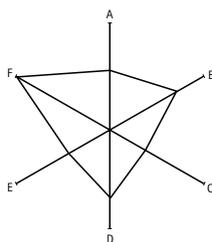
Phenol

In comparison to other “high purity” RP-18 endcapped columns, Purospher® STAR RP-18 endcapped shows the best over-all selectivity and due to this, the best chance for a successful separation.

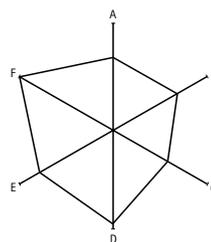
Purospher® STAR RP-18 endcapped, 5 µm



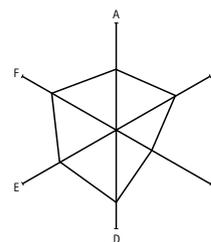
Column Z



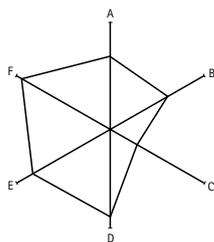
Column S



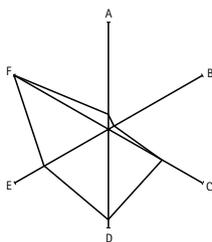
Column P



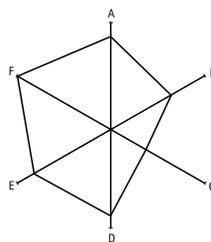
Column L



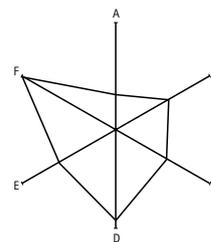
Column N



Column I



Column D

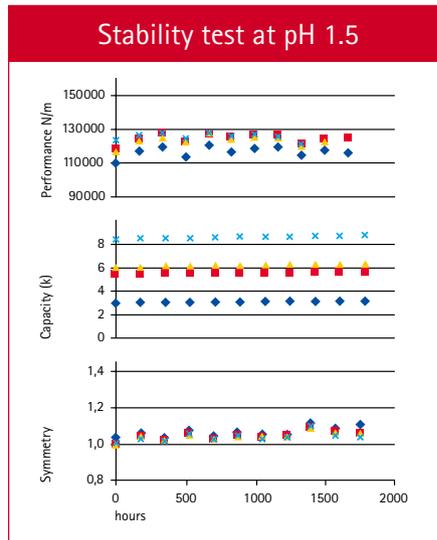


Outstanding pH-stability

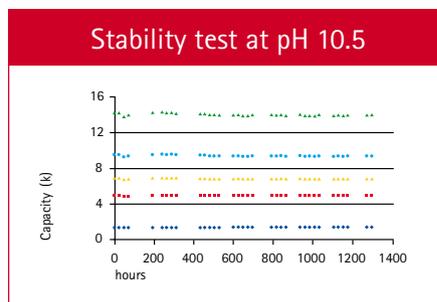
Of course you need a column that is robust, stable in a range of eluent conditions and has a very long column life. Based on the metal-free alkoxysilane production process, Purospher® STAR RP-18 endcapped is outstandingly robust and has excellent pH-stability. Naturally all Purospher® STAR columns, regardless of batch, provide absolutely reproducible results.

pH-stability

Purospher® STAR RP-18 endcapped has outstanding pH-stability. Various studies have shown that Purospher® STAR RP-18 endcapped is stable and reproducible in a pH range of 1.5 to 10.5. This provides the required pH-stability for 99 % of all common applications.



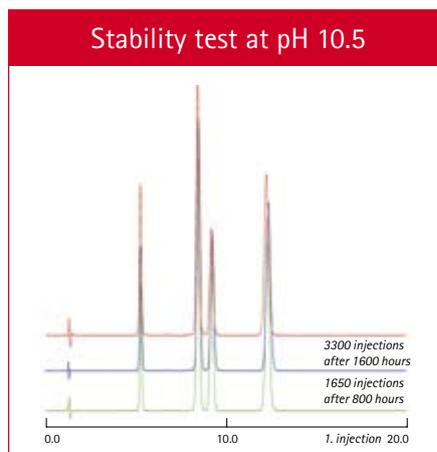
Column: LiChroCART® 150-4.6, Purospher® STAR RP-18 endcapped, 5 µm
 Eluent: Acetonitrile/Water (0.01 % TFA; 50 : 50)
 Flow rate: 1.0 mL/min
 Detection: UV 254 nm
 Temperature: RT
 Inj. volume: 10 µL
 Sample: Ketoprofen (blue diamond), Fenoprofen (red square), Flurbiprofen (yellow triangle), Ibuprofen (cyan cross)



Column: LiChroCART® 150-4.6, Purospher® STAR RP-18 endcapped, 5 µm
 Eluent: Acetonitrile/Water (0.1 % NH₃; [25 %]; 60 : 40)
 Flow rate: 1.0 mL/min
 Detection: UV 254 nm
 Temperature: RT
 Inj. volume: 10 µL
 Sample: 1. Thiourea (blue diamond), 2. Benzene (red square), 3. Toluene (yellow triangle), 4. Propylbenzene (cyan circle), 5. Butylbenzene (green triangle)

Stable retention times

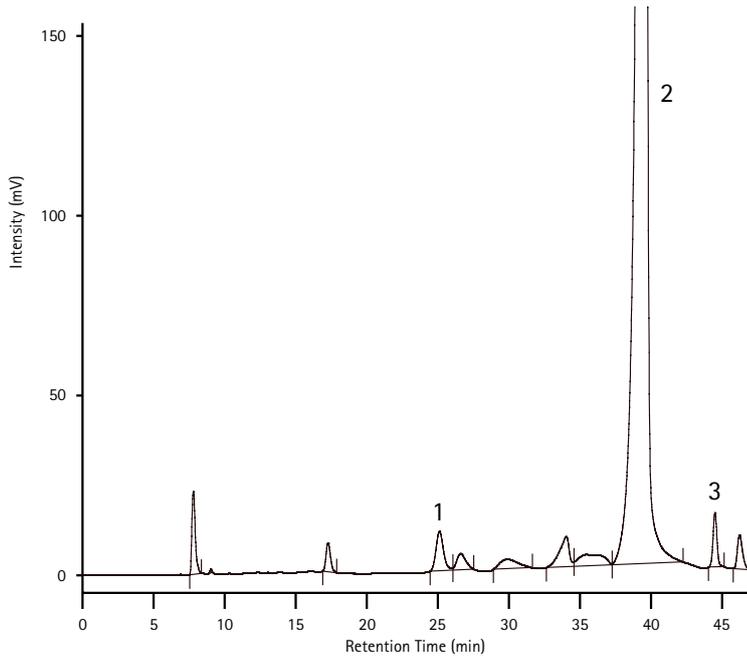
A special derivatization and endcapping process have been developed to ensure the long term chemical stability of Purospher® STAR RP-18 endcapped HPLC columns. This is proved by constant retention times even after 3000 injections. The user gets excellent value for money.



Column: LiChroCART® 150-4.6, Purospher® STAR RP-18 endcapped, 5 µm
 Eluent: Acetonitrile/Water (0.01 % TFA; 50 : 50)
 Flow rate: 1.0 mL/min
 Detection: UV 254 nm
 Temperature: RT
 Inj. volume: 10 µL
 Sample: 1. Ketoprofen, 2. Fenoprofen, 3. Flurbiprofen, 4. Ibuprofen

Determination of Betabion at pH 10.1

Betabion is a medicine for preventing vitamin B1 deficiency. During the HPLC method development for Betabion, the pH of the mobile phase was found to have a big effect on the selectivity, and optimum selectivity was obtained at pH 10.1. This is a good example how the excellent pH stability of Purospher® STAR RP-18 endcapped from pH 1.5 - 10.5 is ideal to developing good separations and rugged HPLC methods.

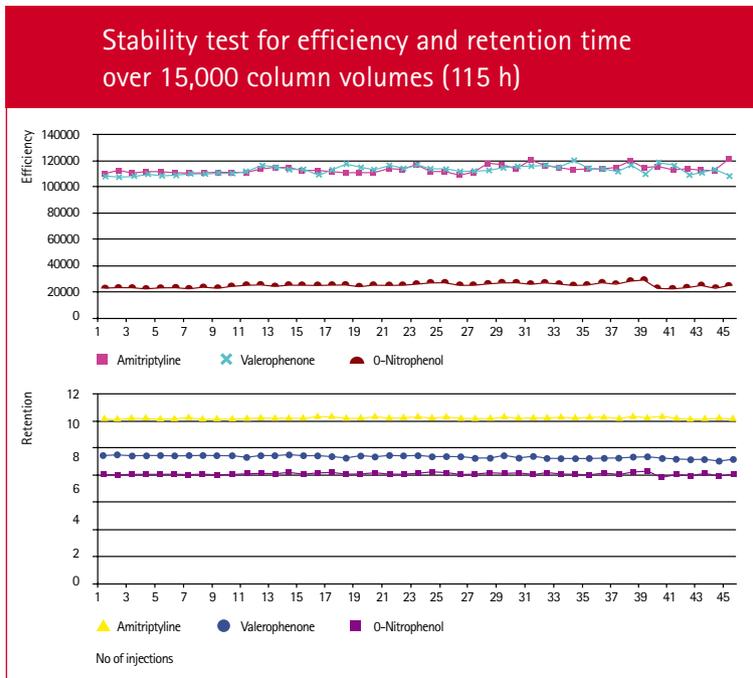


Column: LiChroCART® 250-4.6
Purospher® STAR RP-18 endcapped, 5 µm

Mobile phase gradient:

Time [min]	20 mM/L Tetra-borate-Buffer pH 10.1 [%]	Acetonitrile [%]	Flow [mL/min]
0	96	4	0.3
34	96	4	0.3
35	96	4	0.3
38	94	6	1.75
40	94	6	1.75
41	94	6	1.25
50	94	6	1.25

Detection: UV 240 nm
 Temperatur: 22 °C
 Injektion: 10 µL
 Sample: 1. Toxopyrimidine
 2. Thiamin
 3. 4-Methyl-5-thiazolethanol



Column: LiChroCART® 55-4
Purospher® STAR RP-18 endcapped, 3 µm
 Mobile phase: 0.1 v/v % H₃PO₄ in Water
 Acetonitrile

Gradient:

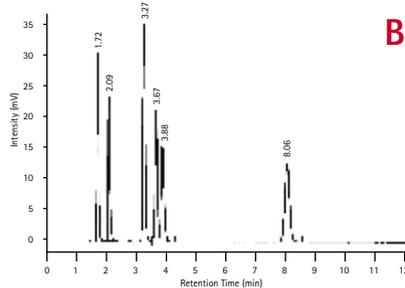
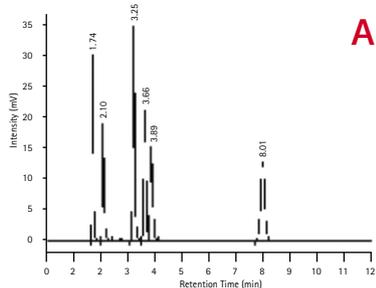
Time [min]	Buffer	Acetonitrile
0	95	5
2	95	5
12	20	80
15	20	80
15.1	95	5
20	95	5

Temperature: 60 °C
 Flow rate: 1.5 mL/min

Use with aqueous mobile phases

Standard reversed phase columns, particularly RP-18 columns often suffer from phase collapse when used in combination with highly aqueous mobile phases. The outstanding performance of Purospher® STAR RP-18 endcapped enables the use with 100 % aqueous mobile phases in combination with selectivity of a classical RP-18 stationary phase.

Purospher® Star RP-18 endcapped

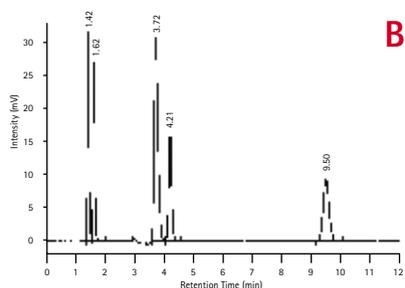
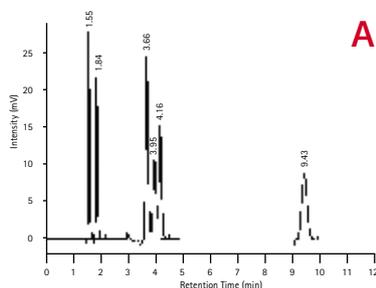


Chromatogram **A** shows the first separation with 1% Acetic acid as mobile phase.

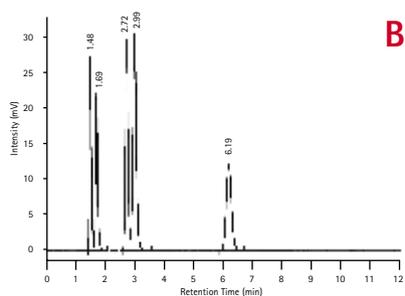
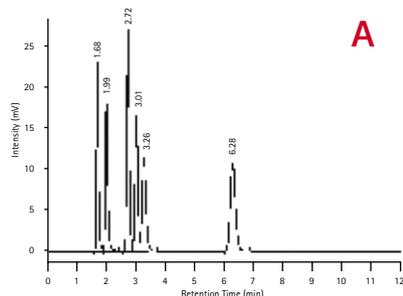
Chromatogram **B** shows the same separation after 3 hours.

Only Purospher® STAR RP-18 endcapped shows the same separation in Chromatogram B.

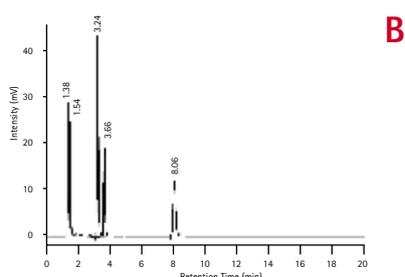
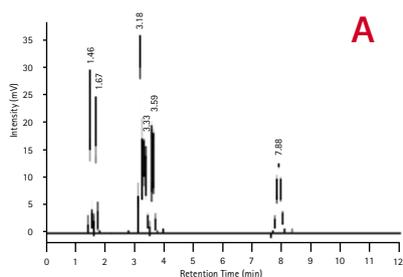
Column I



Column X



Column L



The combination of extremely high purity silica, best all-round retention characteristics, outstanding pH stability up to pH 10.5 and suitability for use with 100 % aqueous mobile phases makes Purospher® STAR RP-18 endcapped an all-round top performance column, almost universal in its range of applications.

In contrast to competitive classical RP-18 columns Purospher® STAR RP-18 endcapped shows a high stability in 100 % aqueous mobile phases.

One problem frequently encountered in LC / MS is the appearance of mass peaks, which show up totally unrelated to the samples run – “ghost” mass peaks. It is impossible to differentiate whether these signals come from an unknown component in the sample co-eluting with a known peak, or from an impurity in the mobile phase or from some residual contaminations “bleeding” from the column.

To solve this problem, a three-step procedure is proposed for washing columns and choosing solvents for use in LC / MS measurements. This procedure also increases ionization efficiency and thereby increases both sensitivity and reproducibility of LC / MS measurements.

A simple 3-step procedure optimizes performance in LC / MS

1. Start with the column – the column must be washed with a special solvent to remove trace impurities.

The recommended LC / MS wash solvent is iso-propanol with 0.1 % formic acid at a flow rate of 0.5 mL/min. (for 3 mm id columns).

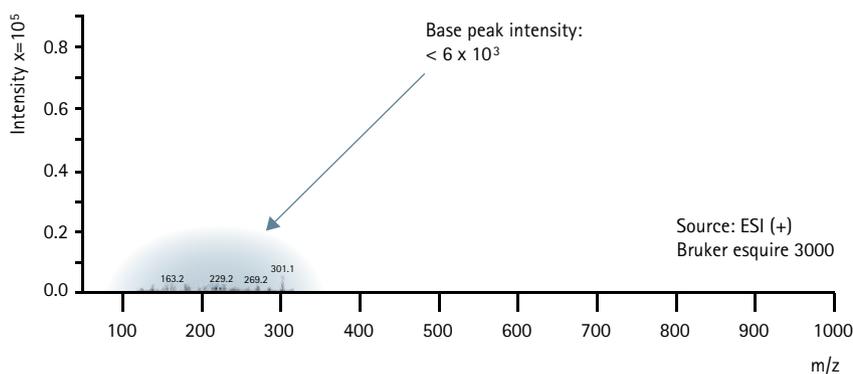
Wash the column for 60 minutes.

2. To reduce the LC / MS background signal, it is essential to work with extremely high purified solvents. The recommended solvent is **LiChrosolv® Hypergrade** with special MS specifications.

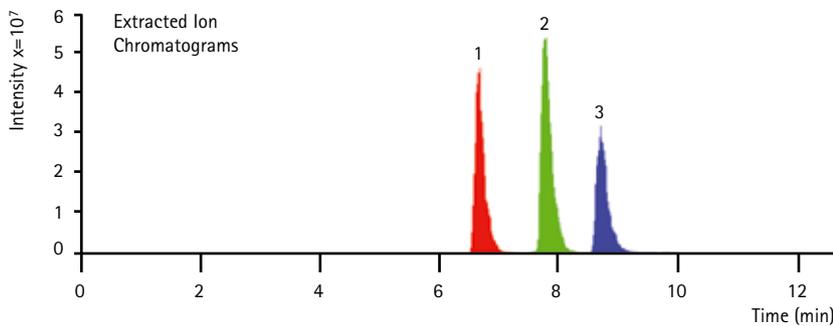
3. **Equilibrating the HPLC column**

Finally the column must be re-equilibrated with the mobile phase. Best results are obtained when two blind gradients (without sample injection) are run prior to analysing samples.

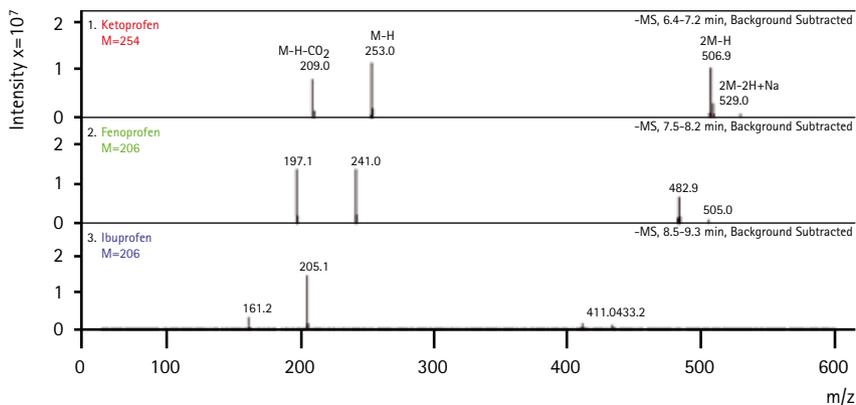
Purospher® STAR RP-18 endcapped HPLC columns give ideal low and very stable background signal in LC / MS **after simple washing with iso-propanol/ 0.1 % formic acid.**



Extracted Ion Chromatograms of Profens in negative ion mode separated on Purospher® STAR RP-18 endcapped



EIC 209; 253; 507 – All MS
 EIC 161; 205; 411 – All MS
 EIC 197; 241; 483 – All MS



Ketoprofen, Fenoprofen and Ibuprofen (100 ng) give clear MS spectra with no noise or spikes using LiChrosolv® Acetonitrile hypergrade and Purospher® STAR RP-18 endcapped columns

Chromatographic conditions:

Column: LiChroCART® 55-2
 Purospher® STAR
 RP-18 endcapped, 3 µm

Mobile Phase A: 0.1% Acetic acid
 in Acetonitrile

Mobile Phase B: 0.1% Acetic acid
 in Water

Gradient: From 25 % to 50 % A
 in 3 minutes, then
 isocratic

Flow rate: 300 µL/min
 without split

Detection: UV 220 nm and
 Ion Trap MS

Temperature: ambient

Inj. volume: 1 µL

Sample: 3 Profens, 0.1 µg/µL

MS Conditions:

Ionisation: ESI (-)

Nebulizer: 36 psi

Dry Gas: 8.5 L/min

Dry Temperature: 330 °C

Smart Mode

Optimisation: Target Mass 205

Ion Charge

Control: Target 50,000,
 max. 50 ms

Scan Mode: Standard/Normal

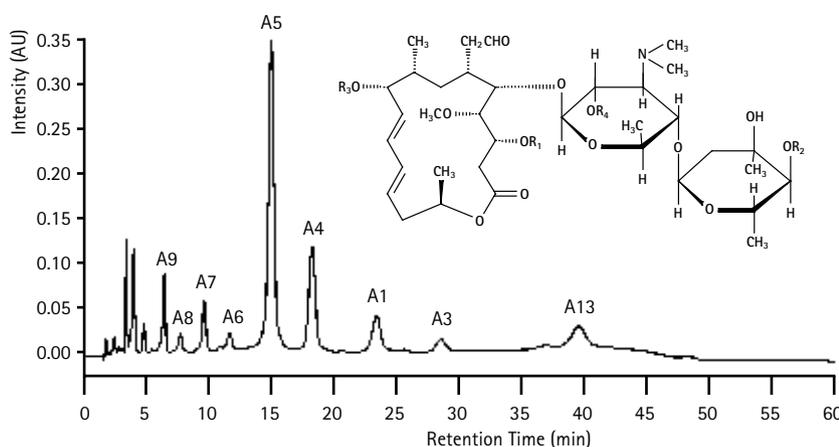
Scan Range: 50 – 600 m/z



In Pharmaceutical Analytics, antibiotics are one of most difficult classes of substance for HPLC separation. Many antibiotics are chelating compounds and the separation on conventional HPLC materials often shows peak tailing. The following applications of commonly used antibiotics show generally the excellent performance of Purospher® STAR RP-18 endcapped and qualifies the column for separation of pharmaceuticals.

Analysis of Kitasamycin compositions

Kitasamycin is a macrolide antibiotic produced by streptomyces kitasatoensis. The drug has antimicrobial activity against a wide spectrum of pathogens. Kitasamycin comprises several components: Leucomycin A1, A3-A9 and A13.



Column: LiChroCART® 125-4
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile Phase: 0.1 mol/L Ammonium acetate
solution-Methanol-Acetonitrile
(40 : 55 : 5)

Flow rate: 0.7 mL/min

Detection: UV 231 nm

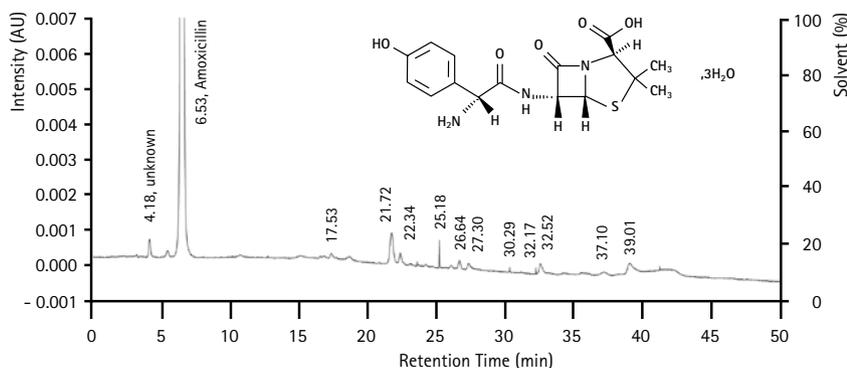
Temperature: 60 °C

Inject. volume: 10 µL

Sample: Kitasamycin
A9; A8; A7; A6; A5; A4; A1; A3; A13

Determination of Amoxicillin sodium sterile for injection

Amoxicillin is a moderate-spectrum β-lactam antibiotic used to treat bacterial infections caused by susceptible micro-organisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other beta-lactam antibiotics.



Column: LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile Phase A: Solution of 99 volumes of 0.05 mol/L
Phosphate buffer (produce 0.05 mol/L
Potassium dihydrogen phosphate, adjust to
pH 5.0 with 2 mol/L Sodium hydroxide
solution) and 1 volumes of Acetonitrile

Mobile Phase B: Solution of 0.05 mol/L Phosphate buffer
adjust to pH 5.0 and 20 volumes of
Acetonitrile.

Gradient: 0 min 8 % B; 25 min 100 % B;
40 min 100 % B; 41 min 8 % B;
55 min 8 % B

Flow rate: 1 mL/min

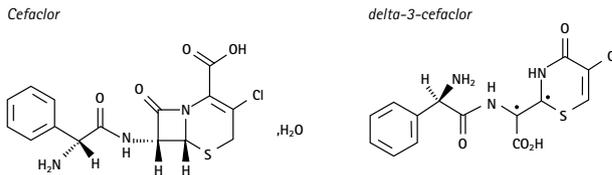
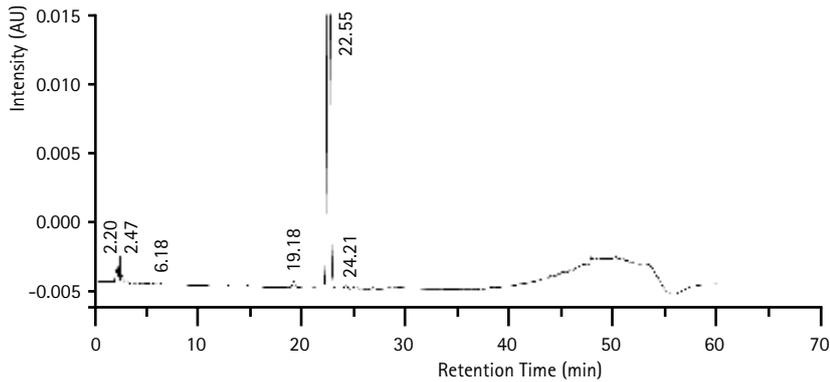
Detection: UV 254 nm

Inject. volume: 20 µL

Sample: 6.53 min Amoxicillin

Determination of Cefaclor

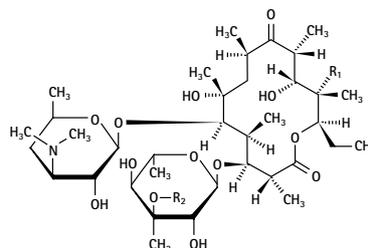
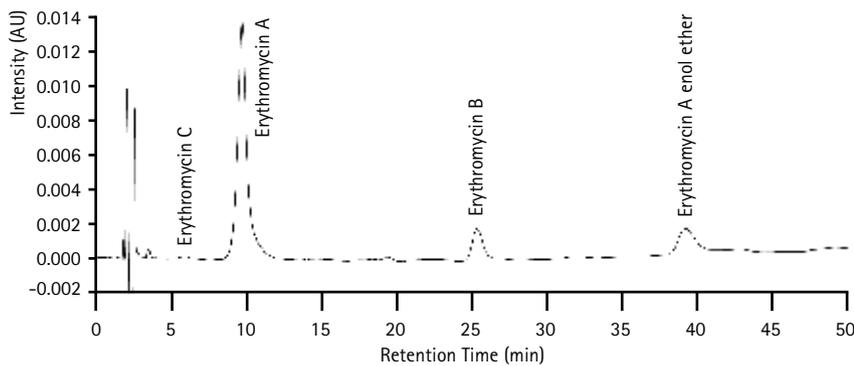
Cefaclor is a second-generation cephalosporin antibiotic used to treat certain infections caused by bacteria such as pneumonia and ear, lung, skin, throat, and urinary tract infections.



- Column:** LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm
- Mobile Phase A:** Solution of 0.78 % Sodium dihydrogen phosphate, Sodium dihydrogen phosphate 7.8 g, dilute to 1000 mL with water and adjust to pH 4.0 with dilute phosphoric acid
- Mobile Phase B:** Solution of 55 volumes of 0.78 % Sodium dihydrogen phosphate pH 4.0 and 45 volumes of Acetonitrile
- Gradient:** 0 min 5 % B; 30 min 25 % B; 45 min 100 % B; 50 min 100 % B; 51 min 5 % B; 61 min 5 % B
- Flow rate:** 1.0 mL/min
- Detection:** UV 220 nm
- Inject. volume:** 20 µL
- Sample:** Cefaclor; delta-3-cefaclor

Determination of Erythromycin

Erythromycin is a macrolide antibiotic which has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often used for people who have an allergy to penicillins.

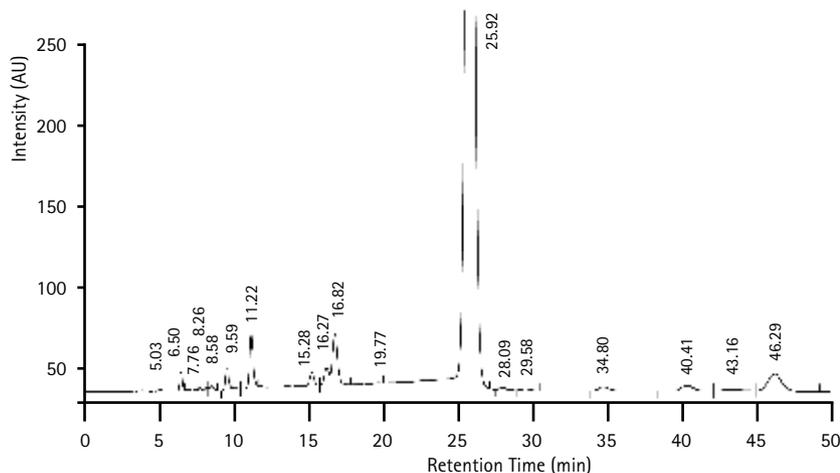


- Column:** LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm
- Mobile Phase:** 0.2 mol/L Ammonium phosphate buffer weigh Ammonium dihydrogen phosphate 1.15 g, dissolve with 50 mL water and adjust to pH 6.5 with Triethylamine- 0.2 mol/L Tetramethyl ammonium hydroxide (measure 25 % Tetramethyl ammonium hydroxide 14.6 mL, dissolve with 100 mL Water and adjust to pH 6.5 with Phosphoric acid, then dilute to 200 mL with Water) – Acetonitrile – Water (5 : 20 : 30 : 45)
- Detection:** UV 215 nm
- Inject. volume:** 10 µL
- Sample:** 5.99 min Erythromycin C;
9.68 min Erythromycin A;
25.32 min Erythromycin B;
39.19 min Erythromycin A enol ether



Determination of Meleumycin

Meleumycin is a sixteen-acrolides antibiotic produced from *Streptomyces mycarofaciens* and is a multi-component compound of medemycin A1 and kitasamycin A6. It is antibacterial of Gram-positive, Gram-negative such as *Staphylococcus aureus*, *epiderhylococcus aureus*, *Corynebacterium diphtheriae*.



Column: LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm

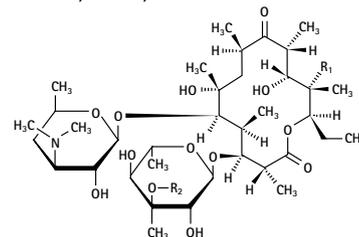
Mobile Phase: 0.2 mol/L Ammonium formate
(adjust to pH 7.3 with
Triethylamine) - Acetonitrile
(62 : 38)

Detection: UV 232 nm

Temperature: 30 °C

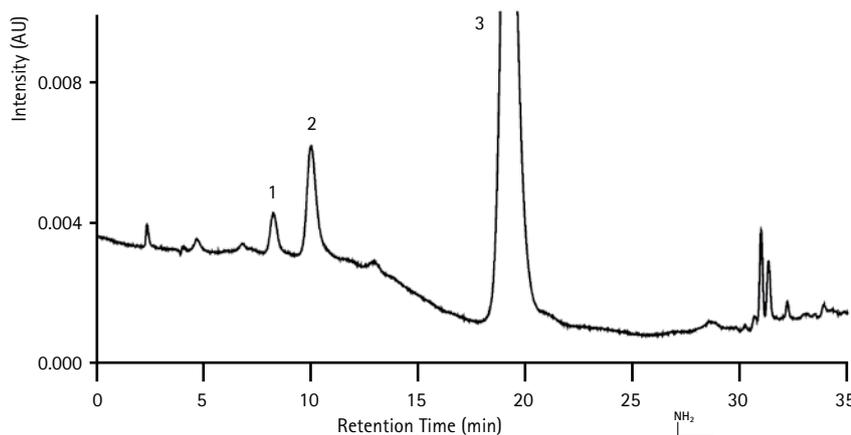
Inject. volume: 20 µL

Sample: Mydecamycin



Determination of Norvancomycin Hydrochloride

Norvancomycin is a new glycopeptide antibiotic, which has the same anti-microbial spectrum as vancomycin. It is used extensively in the clinic to treat methicillin-resistant *Staphylococcus aureus* infections.



Column: LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile Phase A: Triethylamine buffer (measure
6 mL triethylamine, dilute to
2000 mL with water and adjust
to pH 3.2 with dilute phosphoric
acid) - Acetonitrile - Tetrahydro-
furan (96 : 3 : 1)

Mobile Phase B: Triethylamine buffer -
Acetonitrile - Tetrahydrofuran
(70 : 29 : 1)

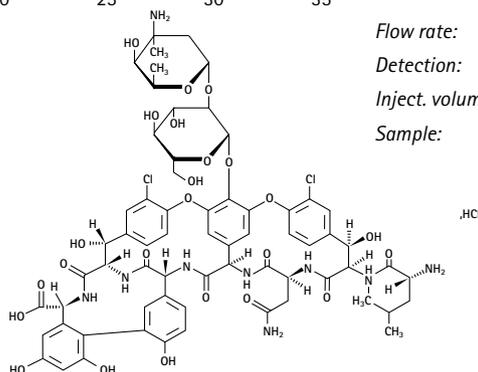
Gradient: 0 min 0 % B; 23 min 0 % B;
38 min 100 % B; 40 min 100 % B;
41 min 0 % B; 50 min 0 % B

Flow rate: 1 mL/min

Detection: UV 280 nm

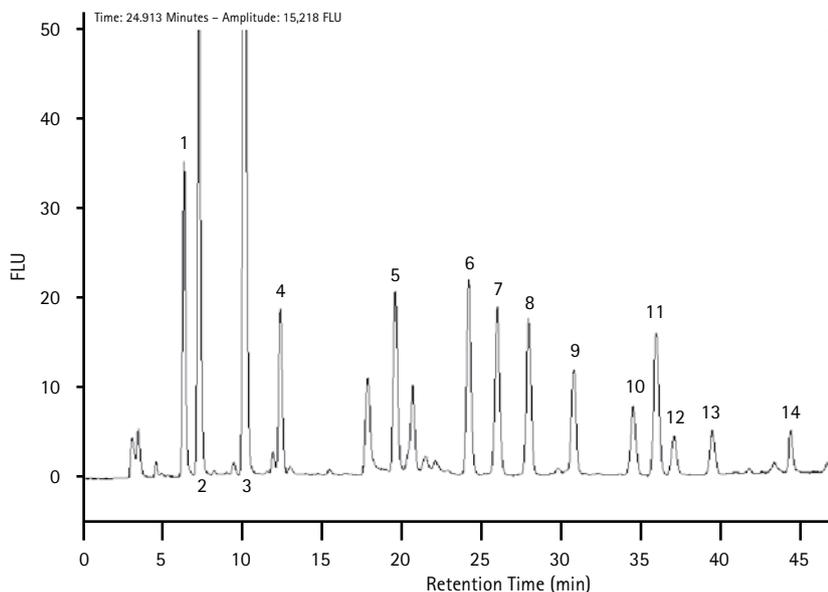
Inject. volume: 20 µL

Sample: Norvancomycin HCl



Sulfonamides in honey

Sulfonamide antibiotics are commonly used in veterinary medicine. Prevention and treatment of bacterial bee diseases with sulfonamides can lead to residues of these compounds in honey. Residues of these antibacterial drugs in honey are of major concern because of possible allergic reactions in sensitive or sensitised people, but principally because of their contribution in development of antibiotic-resistant pathogenic bacteria. Considering this risk and the status of honey as pure natural product the European Union has forbidden the use of antibiotics in agriculture. The limit of quantitation (LOQ) differ between 20 and 50 ng/g depending on the country.



Column: LiChroCART® 150-4.6
Purospher® STAR
RP-18 endcapped, 5 µm

Precolumn: LiChroCART® 4-4
Purospher® STAR
RP-18 endcapped, 5 µm

Oven temperature: 45 °C

Injection volume: 50 µL

Flow Rate: 0.7 mL/min

Mobile phase A: 0.020 M Acetate buffer pH 4.75
MeCN (98 : 2, v/v)

Mobile phase B: 0.020 M Acetate buffer pH 4.75
MeCN (68 : 32, v/v)

Gradient:

Time [min]	Eluent A	Eluent B	Flow Rate [mL/min]
0.0	98	2	0.7
31	65	35	0.7
41	25	75	0.7
41.1	5	95	0.7
48	5	95	0.7
48.1	98	2	0.7
60	98	2	0.7

Sample: Honey sample spiked with 20 ppb for all sulfonamides + PABA (3) and internal standard (9)

- Peaks:**
1. sulfaguandinine,
 2. sulfanilamide, (internal standard)
 3. p-aminobenzoic acid
 4. sulfacetamide
 5. sulfadiazine
 6. sulfathiazole
 7. sulfapyridine
 8. sulfamerazine
 9. sulfamethizole
 10. sulfamether
 11. sulfamethazine
 12. sulfamethoxyipyridazine
 13. sulfachloropyridazine
 14. sulfadoxine

Detection: Fluorescence

Wavelength: Ex 420
Em 485

Sampling Rate: 200 ms

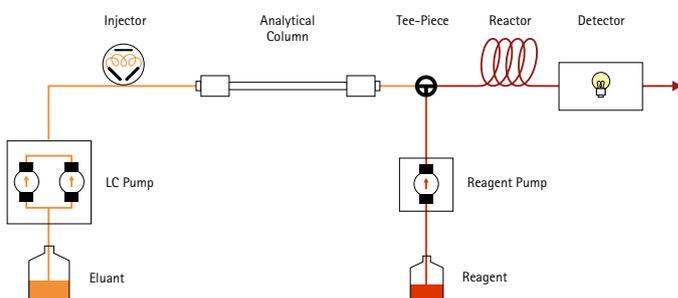
Time Constant: 1sec

Post-column derivatization

The post-column reagent, a mixture of fluorescamine: 2-mercapto-ethanol : acetonitrile : phosphate buffer 0.021 M (0.276 % sodium dihydrogen phosphate monohydrate in water, w/v, adjusted to pH 3.0 with ortho-phosphoric acid 85 %) (0.025 : 0.2 : 25 : 75, w/v/v/v) was stored in the dark at 4 °C

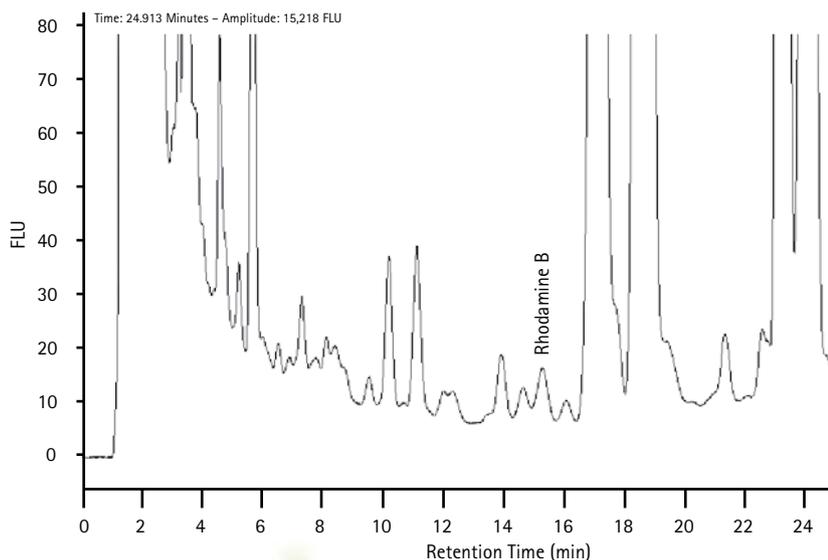
Coil: 10 m x 0.25 mm i.d. thermostated within the column oven

Reaction pump flow: 0.2 mL/min



Rhodamine B in chili extract

The red chili extract (red paprika oleoresin) has high stability to light and heat and is soluble in oil, acetone, chloroform, and n-hexane and alcohol. After emulsification, red chili extract can be made into a water-soluble or water-separate product. The group of the sudan dyes belong to the class of synthetic azo dyes. The metabolites are considered to be carcinogens and teratogens. Due to this fact, the EU and the US do not permit the use of these colors as food additives. However, in some countries, these dyes are still occasionally used in order to intensify the color of bell pepper and chili powders.



Column: LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm

Precolumn: LiChroCART® 4-4
Purospher® STAR
RP-18 endcapped, 5 µm

Temperature Oven: 40 °C

Flow Rate: 1 mL/min

Eluent A: Phosphoric acid
(1 mL 85 % Phosphoric acid in to 1 L of Water)

Eluent B: Acetonitrile

Gradient:

Time [min]	Eluent A	Eluent B	Flow Rate [mL/min]
0.0	58	42	1
0.0	58	42	1
15.0	20	80	1
20.0	20	80	1
21.0	58	42	1
35.0	58	42	1

Sample: 0.6248 g Oleoresin oil in 5 mL eluent (Oleoresin sample contaminated with 140 ppb Rhodamine B)

Injection volume: 10 µL

Detection: Fluorescence

Sampling Rate: 400 ms, Time Constant = 1sec

PMT Voltage: Medium

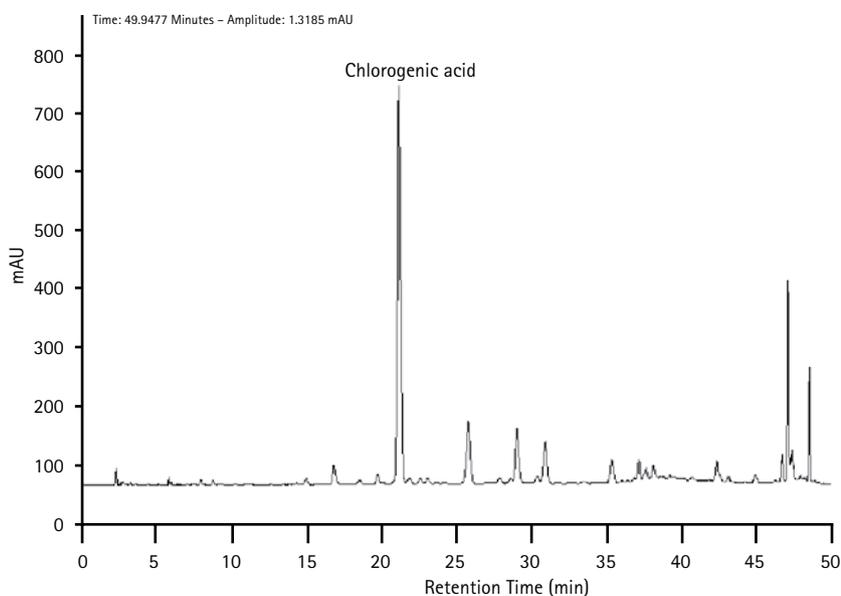
Wavelengths: EX 261/EM 575



Chlorogenic acid in apple juice

Phenolics are an important class of compounds that are derived from phenylalanine via the shikimate and phenylpropanoid pathways and are widely found in apple. The major classes of phenolics in apple include phenolic acids, flavonoids, flavonols and anthocyanins. The most important polyphenolic compound is chlorogenic acid, a derivative of the hydroxycinnamic acid.

Because of the increased interest in apple phenolics in the diet, it is important to obtain reliable data on the identity, concentration and fate of these compounds in various cultivars. In addition, many apples eaten by consumers have been stored for up to 9 months and there is little information on the effect of storage on the apple phenolics.



Column: LiChroCART® 250-4
Purospher® STAR
RP-18 endcapped, 5 µm

Precolumn: LiChroCART® 4-4
Purospher® STAR
RP-18 endcapped, 5 µm

Temperature: 40 °C

Flow Rate: 1.0 mL/min

Mobile Phase A: 0.1 % Phosphoric acid
(1mL 85 % Phosphoric acid
filled up to 1000 mL with
HPLC Water)

Mobile Phase B: Acetonitrile

Gradient:

Time [min]	A: 0.1 % Phosphoric acid	B: Acetonitrile
0.0	100	0
1.4	94	6
30.5	88	12
33.5	83	17
41.8	81	19
51.9	37	63
55.0	37	63
57.0	0	100
60.0	0	100
65	100	0
85.1	100	0

Detection: DAD 278 nm

Sample: Fresh apple juice

Peak: Chlorogenic acid

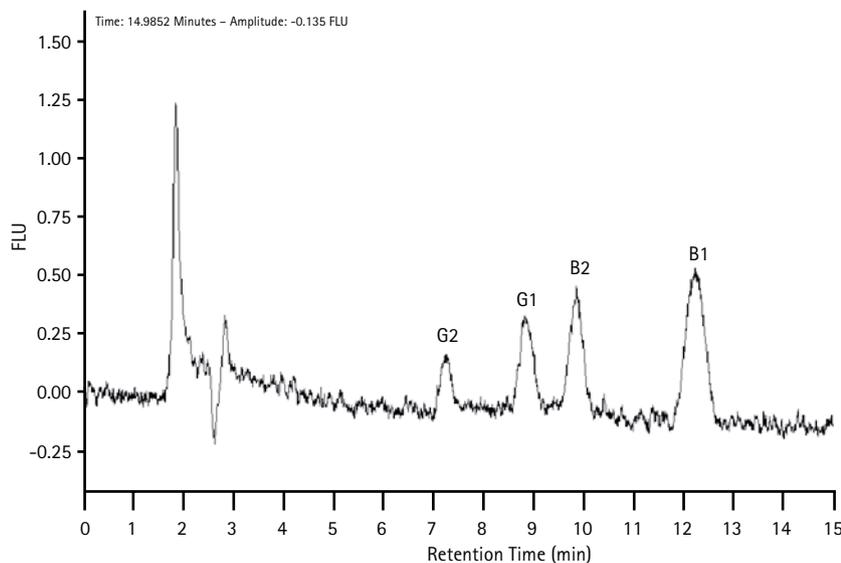


Aflatoxins B1, B2, G1 and G2 are the main toxins produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. They can contaminate food products when storage conditions are favorable to fungal growth. The main aflatoxin contaminations has been reported in maize, peanuts, brazil or pistachio nuts, copra and cottonseeds. Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species. The Internal Agency for Research on Cancer (IARC) has classified all four aflatoxins as group one of carcinogens.

Confirmation of the presence of aflatoxins in a sample by HPLC requires derivatisation of the aflatoxins B1 and G1 in order to enhance their natural fluorescence under UV light and make them more easily detected. Previously, the only options available for derivatising aflatoxins involved the use of either trifluoroacetic acid (TFA) or iodine. Both of these methods are reliable, however they do have some significant limitations which can be overcome by use of a third method with the Coring Cell.

Aflatoxins with iodine derivatisation (EN 12955)

EN 12955: The European standard HPLC method EN 12955 for the determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in cereals, shell-fruits and derived products features highly sensitive fluorescence detection by post column derivatisation with iodine and immunoaffinity column clean up. Iodine derivatisation as a post column technique does have other disadvantages like longer reaction time at higher temperatures (peak broadening), additional HPLC pump necessary and daily preparation of the corrosive iodine solution. This european standard specifies a method for the determination of aflatoxin content of greater than 8 µg/kg.



Chromatographic conditions:

Column:	LiChroCART® 150-4.6 Purospher® STAR RP-18 endcapped, 5 µm
Pre-column:	LiChroCART® 4-4 Purospher® STAR RP-18 endcapped, 5 µm
Mobile phase A:	Water/Acetonitrile/Methanol 65/17.5/17.5 (v/v/v)
Mobile phase B:	Rinse the system after the sequence for 30 minutes with 90/10 Water/Methanol (v/v)
Flow Rate:	1 mL/min
Detection:	Fluorescence EX 365/EM 435
Temperature Oven:	60 °C
Injection volume:	50 µL
Sample:	G2 - 30 pg/mL, G1 - 100 pg/mL, B2 - 30 pg/mL, B1 - 100 pg/mL

Post column derivatisation:

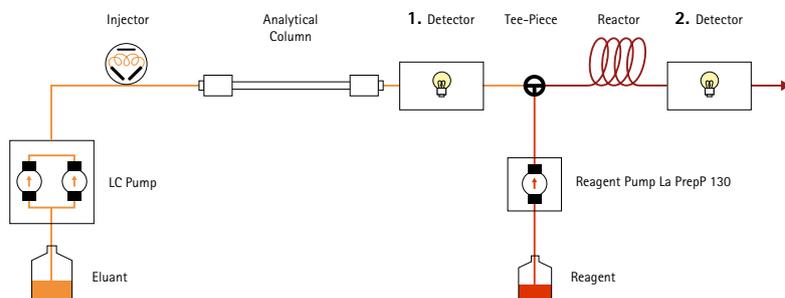
Derivatisation reagent: saturated iodine solution

Reaction pump:

The pump is stopped by timer of the L-2130 pump when the rinsing program is started. Never let this pump run, when the L-2130 pump is stopped. The iodine will damage your column !!!

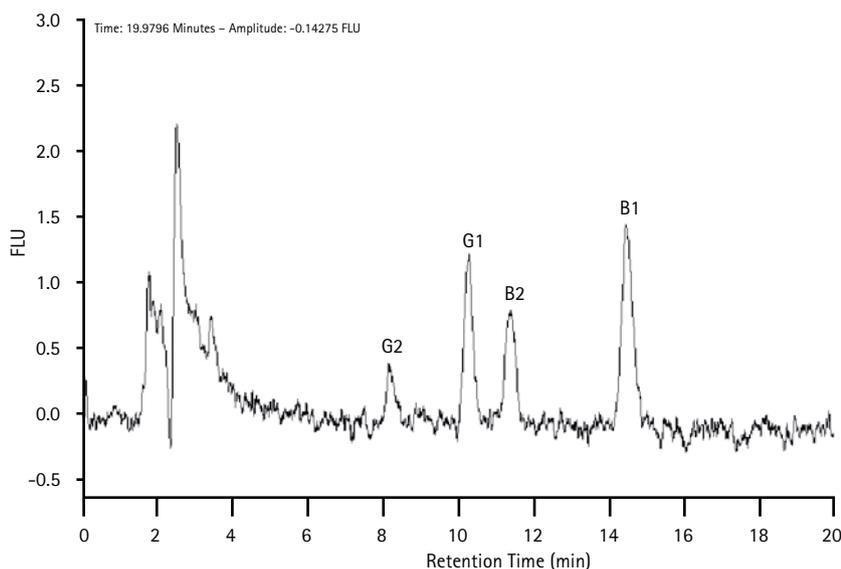
Derivatisation coil: PTFE knitted coil, 5 m x 0.5 mm i.d. or selfmade PEEK coil 3 m x 0.5 mm i.d.

Flow Reaction pump: 0.2 mL/min



Aflatoxins with Coring Cell (§ 35 LMBG)

§35 LMBG: The official German methods for measuring compounds in food products (§35 LMBG methods) features the clean-up of the aflatoxins B1, B2, G1 and G2 by means of an immunoaffinity column and the following HPLC detection with an electrochemical cell (Coring Cell). The Coring Cell is an electrochemical cell which generates the derivatising agent, bromine, from potassium bromide present in the mobile phase. The derivatisation of aflatoxins occurs rapidly (reaction time is approximately 4 seconds) at ambient temperature. A daily preparation of derivatising reagent (iodine) is not necessary and the additional pump for addition of derivatising reagent is not needed.



Chromatographic conditions:

Column: LiChroCART® 150-4.6
Purospher® STAR
RP-18 endcapped, 5 µm

Pre-column: LiChroCART® 4-4
Purospher® STAR
RP-18 endcapped, 5 µm

Mobile phase: Water + 183.1 mg
KBr/L + 154 µL HNO₃ 65 %/L/
Methanol/Acetonitrile
65 % A/17.5 % B/
17.5 % C (v/v/v), Isocratic

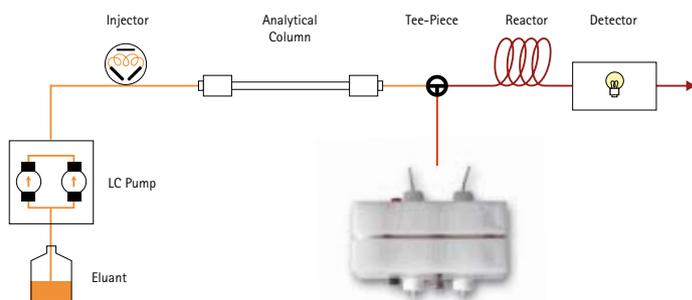
Flow Rate: 1 mL/min

Detection: Fluorescence EX 365/EM 435

Temperature: 40 °C

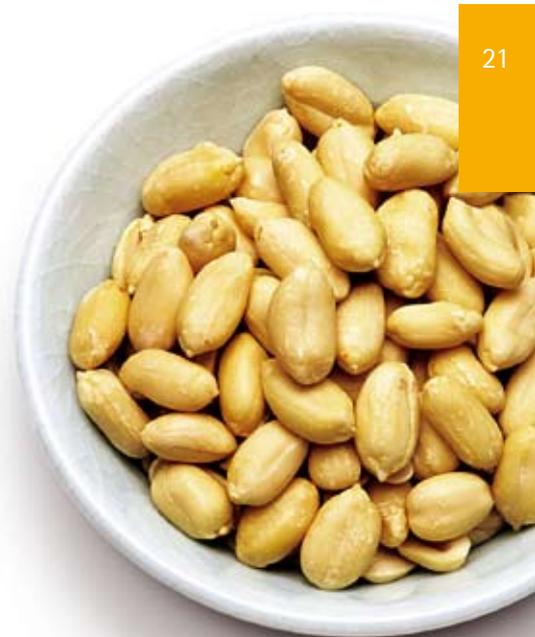
Injection volume: 100 µL

Sample: B1 and G1: 10 pg/mL,
B2 and G2: 2.5 pg/mL



Post column derivatisation:

Derivatisation coil: PEEK coil, 1.38 m x 0.25 mm i.d.



Ordering information

Stainless steel cartridges LiChroCART®

The LiChroCART® columns (75, 125, 150 and 250 mm length and 2, 3, 4 and 4.6 mm i.d.) in the list below require part number 1.51486.0001 manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. LiChroCART® columns 250 - 10 mm require part number 1.51419.0001 manuCART® 10. The short LiChroCART® columns (30 and 55 mm length) can be ordered as a set including the corresponding cartridge holder and one cartridge, or as a pack of 3 cartridges without cartridge holder.

The separate part numbers for the cartridge are as follows:

LiChroCART® cartridge holder for 30 mm cartridge 1.50227.0001
 LiChroCART® cartridge holder for 55 mm cartridge 1.50226.0001

Product	Ord. No.	Particle size	Dimension Length	Dimension i.d.	Contents of one package
Purospher® STAR RP-18 endcapped	1.50237.0001	3 µm	30 mm	2 mm	1 set
Purospher® STAR RP-18 endcapped	1.50238.0001	3 µm	30 mm	2 mm	3 pieces
Purospher® STAR RP-18 endcapped	1.50240.0001	3 µm	55 mm	2 mm	1 set
Purospher® STAR RP-18 endcapped	1.50241.0001	3 µm	55 mm	2 mm	3 pieces
Purospher® STAR RP-18 endcapped	1.50255.0001	5 µm	125 mm	2 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50256.0001	5 µm	250 mm	2 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50253.0001	5 µm	125 mm	3 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50254.0001	5 µm	250 mm	3 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50239.0001	3 µm	30 mm	4 mm	1 set
Purospher® STAR RP-18 endcapped	1.50225.0001	3 µm	30 mm	4 mm	3 pieces
Purospher® STAR RP-18 endcapped	1.50242.0001	3 µm	55 mm	4 mm	1 set
Purospher® STAR RP-18 endcapped	1.50231.0001	3 µm	55 mm	4 mm	3 pieces
Purospher® STAR RP-18 endcapped	1.51460.0001	3 µm	75 mm	4 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50250.0001	5 µm	4 mm	4 mm	10 pieces
Purospher® STAR RP-18 endcapped	1.50251.0001	5 µm	125 mm	4 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50252.0001	5 µm	250 mm	4 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50358.0001	5 µm	150 mm	4.6 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50359.0001	5 µm	250 mm	4.6 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50257.0001	5 µm	250 mm	10 mm	1 piece

Additional dimensions available as customised packings

Stainless steel columns Hibar®

The Hibar® columns are complete with end fittings. When using a guard column with a Hibar® column, we recommend part number 1.51487.0001 guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®

Product	Ord. No.	Particle size	Dimension Length	Dimension i.d.	Contents of one package
Purospher® STAR RP-18 endcapped	1.50036.0001	5 µm	125 mm	4 mm	1 piece
Purospher® STAR RP-18 endcapped	1.50037.0001	5 µm	250 mm	4 mm	1 piece
Purospher® STAR RP-18 endcapped	1.51455.0001	5 µm	150 mm	4.6 mm	1 piece
Purospher® STAR RP-18 endcapped	1.51456.0001	5 µm	250 mm	4.6 mm	1 piece

Additional dimensions available as customised packings

Ordering information of customized packings

For ordering please combine the ordering number of the column hardware (A) and the sorbent number (B):

Example	Customized packing catalog number of LiChroCART® 125-2	1.50195.
	Sorbent number of Purospher® STAR RP-18 endcapped, 5 µm	7185
	Ordering number of LiChroCART® 125-2 Purospher® STAR RP-18 endcapped, 5 µm	1.50195.7185

Stainless steel cartridges LiChroCART®

A	Product	Cat. No.	Dimension Length	Dimension i.d.	Contents of one package
	LiChroCART® 10-2	1.50201.*	10 mm	2 mm	*as specified (sorbent numbers)
	LiChroCART® 30-2	1.50229.*	30 mm	2 mm	*as specified (sorbent numbers)
	LiChroCART® 55-2	1.50234.*	55 mm	2 mm	*as specified (sorbent numbers)
	LiChroCART® 125-2	1.50195.*	125 mm	2 mm	*as specified (sorbent numbers)
	LiChroCART® 250-2	1.50190.*	250 mm	2 mm	*as specified (sorbent numbers)
	LiChroCART® 30-3	1.50233.*	30 mm	3 mm	*as specified (sorbent numbers)
	LiChroCART® 55-3	1.50236.*	55 mm	3 mm	*as specified (sorbent numbers)
	LiChroCART® 125-3	1.50175.*	125 mm	3 mm	*as specified (sorbent numbers)
	LiChroCART® 250-3	1.50177.*	250 mm	3 mm	*as specified (sorbent numbers)
	LiChroCART® 4-4	1.50173.*	4 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 25-4	1.50172.*	25 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 30-4	1.50302.*	30 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 55-4	1.50228.*	55 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 75-4	1.50171.*	75 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 125-4	1.50170.*	125 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 250-4	1.50174.*	250 mm	4 mm	*as specified (sorbent numbers)
	LiChroCART® 100-4.6	1.51448.*	100 mm	4.6 mm	*as specified (sorbent numbers)
	LiChroCART® 125-4.6	1.51442.*	125 mm	4.6 mm	*as specified (sorbent numbers)
	LiChroCART® 150-4.6	1.51431.*	150 mm	4.6 mm	*as specified (sorbent numbers)
	LiChroCART® 250-4.6	1.51432.*	250 mm	4.6 mm	*as specified (sorbent numbers)
	LiChroCART® 10-10	1.50178.*	10 mm	10 mm	*as specified (sorbent numbers)
	LiChroCART® 75-10	1.51449.*	75 mm	10 mm	*as specified (sorbent numbers)
	LiChroCART® 100-10	1.51445.*	100 mm	10 mm	*as specified (sorbent numbers)
	LiChroCART® 125-10	1.51443.*	125 mm	10 mm	*as specified (sorbent numbers)
	LiChroCART® 150-10	1.51444.*	150 mm	10 mm	*as specified (sorbent numbers)
	LiChroCART® 250-10	1.50179.*	250 mm	10 mm	*as specified (sorbent numbers)

Stainless steel columns Hibar® RT

A	Product	Cat. No.	Dimension Length	Dimension i.d.	Contents of one package
	Hibar® RT 250-3	1.00423.*	250 mm	3 mm	*as specified (sorbent numbers)
	Hibar® RT 30-4	1.51196.*	30 mm	4 mm	*as specified (sorbent numbers)
	Hibar® RT 125-4	1.50181.*	125 mm	4 mm	*as specified (sorbent numbers)
	Hibar® RT 250-4	1.50182.*	250 mm	4 mm	*as specified (sorbent numbers)
	Hibar® RT 100-4.6	1.50013.*	100 mm	4.6 mm	*as specified (sorbent numbers)
	Hibar® RT 125-4.6	1.50012.*	125 mm	4.6 mm	*as specified (sorbent numbers)
	Hibar® RT 150-4.6	1.50009.*	150 mm	4.6 mm	*as specified (sorbent numbers)
	Hibar® RT 250-4.6	1.00424.*	250 mm	4.6 mm	*as specified (sorbent numbers)
	Hibar® RT 250-10	1.50183.*	250 mm	10 mm	*as specified (sorbent numbers)

Purospher® STAR RP-18 endcapped sorbent numbers

B	Product	Sorbent No.	Particle size
	Purospher® STAR RP-18 endcapped	*.7184	3 µm
	Purospher® STAR RP-18 endcapped	*.7185	5 µm



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