Kromasil wettable HPLC phase

The Kromasil new wettable phase is the latest innovation of Nouryon, an HPLC phase that has been specifically designed for polar compounds. This Kromasil 100 Å C18(w) permits loading and run start in 100% aqueous, enabling new opportunities for engineers and scientists working with HPLC.

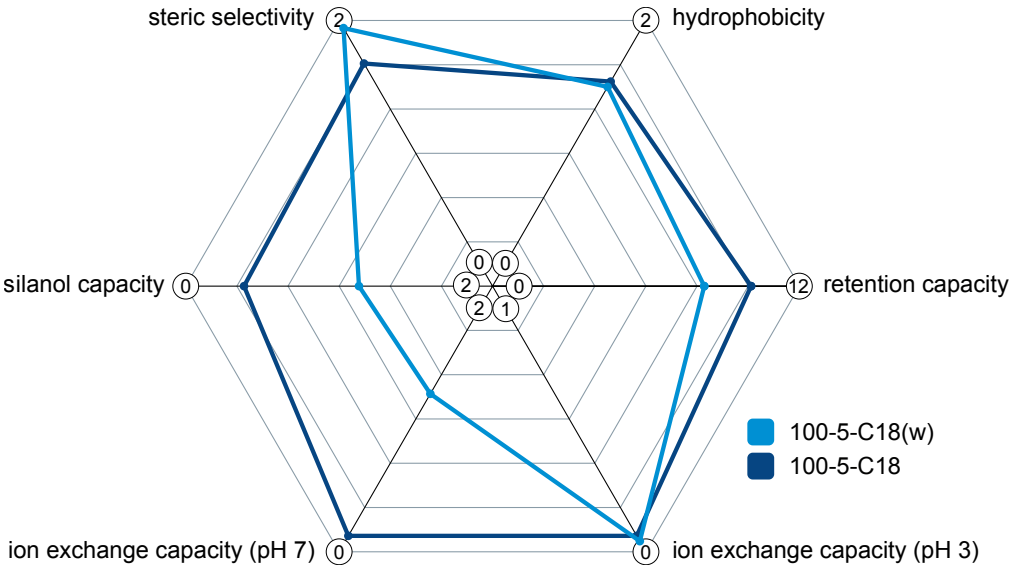
For polar compounds

Whether you are performing separation or purification of APIs, your facility may have to deal with an increasingly number of complex mixtures also containing more polar compounds. Kromasil's expanded portfolio now includes the new wettable C18 phase precisely manufactured for separating and purifying more polar compounds, amino acids and peptides.

Independently, if you work in analysis, development or screening laboratories, pilot facilities or manufacturing, the alternative selectivity offered by Kromasil 100 Å C18 (w) can be of significant benefit for analysis, impurity isolation and API production.

Phase characteristics

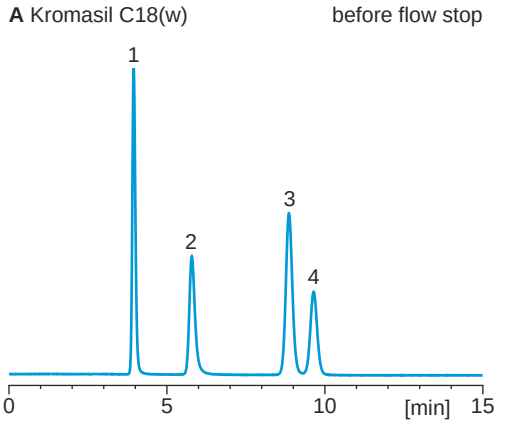
Comparison of phase properties between Kromasil wettable 100-5-C18(w) and the classic 100-5-C18 according to the Tanaka tests set.

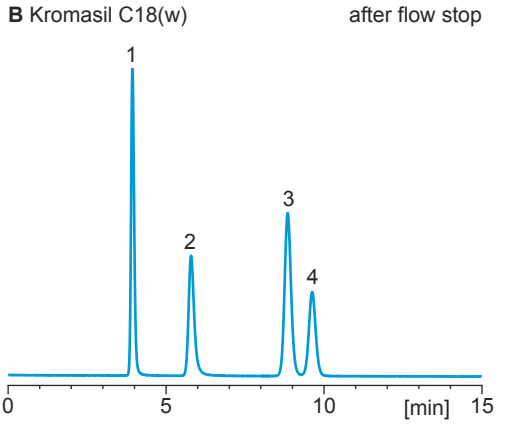


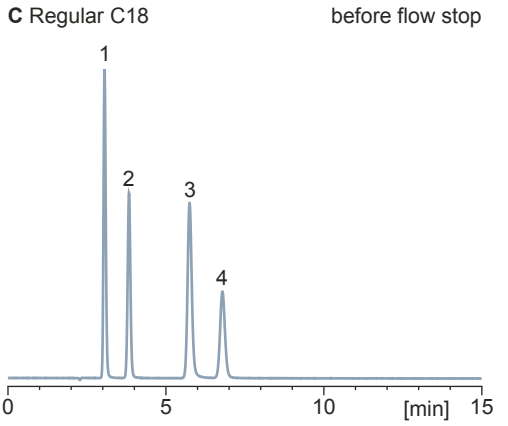
The main differences between wettable C18(w) and classic C18 shows in silanol capacity and ion exchange capacity at pH 7. This is mainly due to the polar embedded end-capping of C18(w).

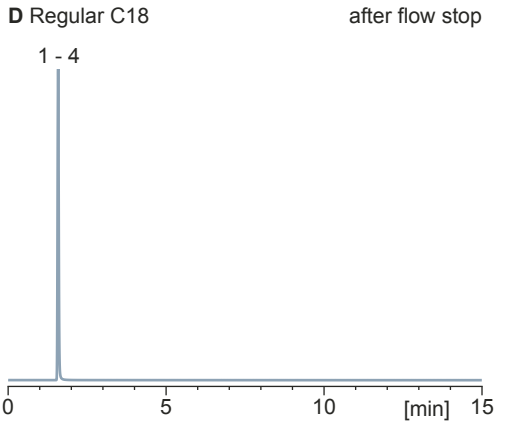
A phase that will withstands dewetting

When comparing a separation of pyrimidine derivatives on Kromasil C18(w) and regular C18 under fully aqueous conditions, different behavior can be observed.









Figures A and C illustrate the difference in selectivity before anything unexpected has happened to the system, such a stop flow situation. The chromatographic result with the wettable phase in figure A shows better retention and selectivity compared to the more hydrophobic C18 in figure C.

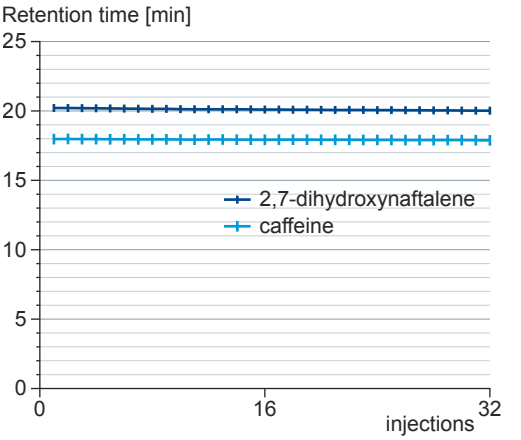
The chromatogram in figure B illustrates that if flow stops and pressure drops the wettable C18(w) will not be affected, continuing to perform just as expected. This is one of the advantages of the wettable phase when dealing with samples that need to be injected under 100% aqueous conditions compared to traditional C18 phases where the regular C18 surface will undergo dewetting, resulting in loss of separation efficiency, as seen in figure D.

Conditions

Column: Kromasil 100-10-C18(w) 4.6 x 250 mm versus regular C18
Part number: M10WLA25
Substances: 1: cytosine, 2: fluorocytosine, 3: uracil, 4: fluorouracil
Mobile phase: 20 mM potassium phosphate pH 2.5
Temperature: ambient
Flow rate: 1.0 ml/min
Detection: UV @ 254 nm

Chemical stability

The new Kromasil C18(w) will under tough conditions and over 300 column volumes clearly maintain its retentivity with time. This performance assures engineers and researchers of the long term usability of this wettable phase.



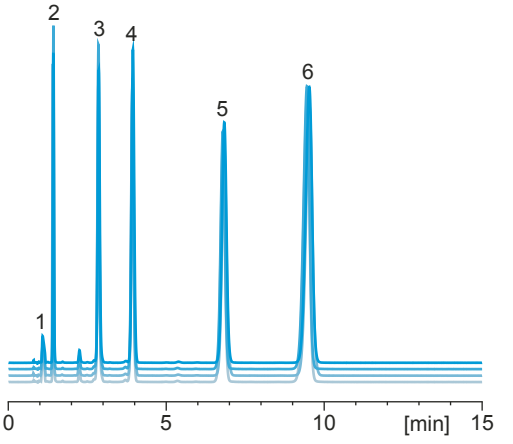
Conditions

Column: Kromasil 100-10-C18(w) 4.6 x 250 mm
Part number: M10WLA25
Substances: caffeine and 2,7-dihydroxynaftalene

step	CV	min	mobile phase
equilibration	3	0 - 12	water / TFA (100/0.1)
loading	1	12 - 16	water / TFA (100/0.1)
gradient	5	16 - 36	from: acetonitrile / water / TFA (0/100/0.1) to: acetonitrile / water / TFA (50/50/0.1)
washing	1.5	36 - 42	acetonitrile / water (70/30)

Reliable quality

Extensive quality control on every raw material together with several in process controls (IPC) ensures Kromasil 100 Å C18(w) to be a high-quality product.



QC test of four batches of Kromasil 100-10-C18(w) showing excellent reproducibility

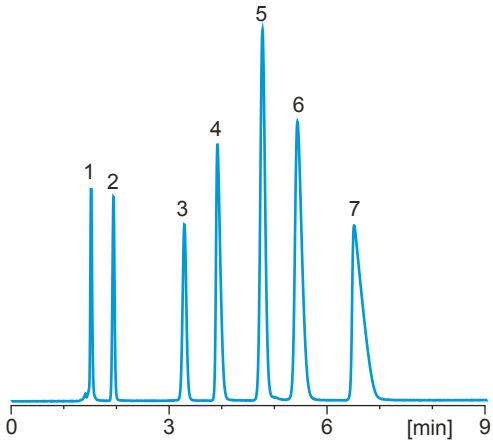
Conditions

Column: Kromasil 100-10-C18(w) 4.6 x 250 mm
Part number: M10WLA25
Substances: 1: sodium nitrite, 2: benzamide, 3: methyl benzoate, 4: toluene, 5: propylbenzene, 6: butylbenzene

Mobile phase: acetonitrile / water (70 / 30)
Temperature: 20 °C
Flow rate: 2.0 ml/min
Detection: UV @ 254 nm

Totally aqueous conditions

When separating organic acids on the Kromasil wettable phase, these small polar compounds show complete resolution even when run under fully aqueous conditions.

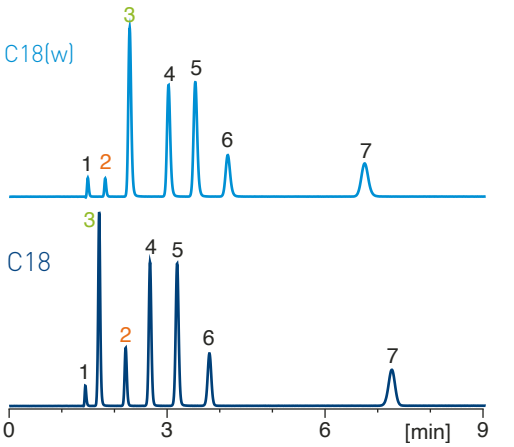


Conditions

Column: Kromasil 100-5-C18(w) 4.6 x 150 mm
Part number: M05WLA15
Substances: 1: oxalic acid, 2: tartaric acid, 3: ascorbic acid, 4: acetic acid, 5: maleic acid, 6: citric acid, 7: fumaric acid
Mobile phase: 25 mM potassium phosphate pH 2.5
Temperature: 25 °C
Flow rate: 1.0 ml/min
Detection: UV @ 210 nm

Alternative selectivity

Since C18(w) has slightly different surface characteristics from a classic C18 as previously illustrated in the Tanaka test, some differences in elution can be expected. Shown here is the separation of a mix of various polar compounds on the wettable C18(w) versus classic C18.



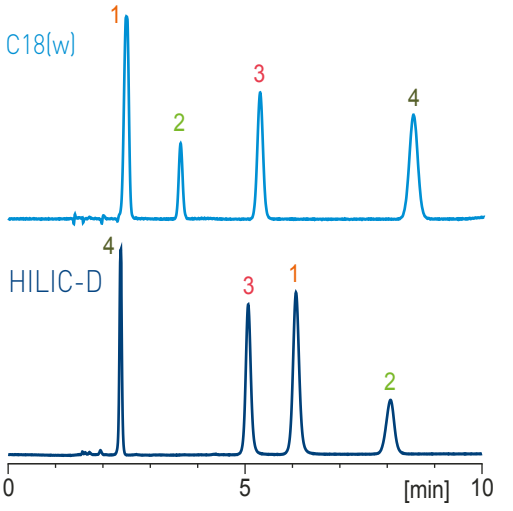
Conditions

Columns: • Kromasil 100-5-C18(w) 4.6 x 150 mm
• Kromasil 100-5-C18 4.6 x 150 mm
Part number: M05WLA15 and M05CLA15, respectively
Mobile phase: methanol / water (70/30)
Flow rate: 1.0 ml/min

Substances: 1: uracil, 2: phenol, 3: caffeine, 4: acetophenone, 5: nitrobenzene, 6: methyl-benzoate, 7: toluene
Temperature: 25 °C
Detection: UV @ 254 nm

Aqueous versus HILIC conditions

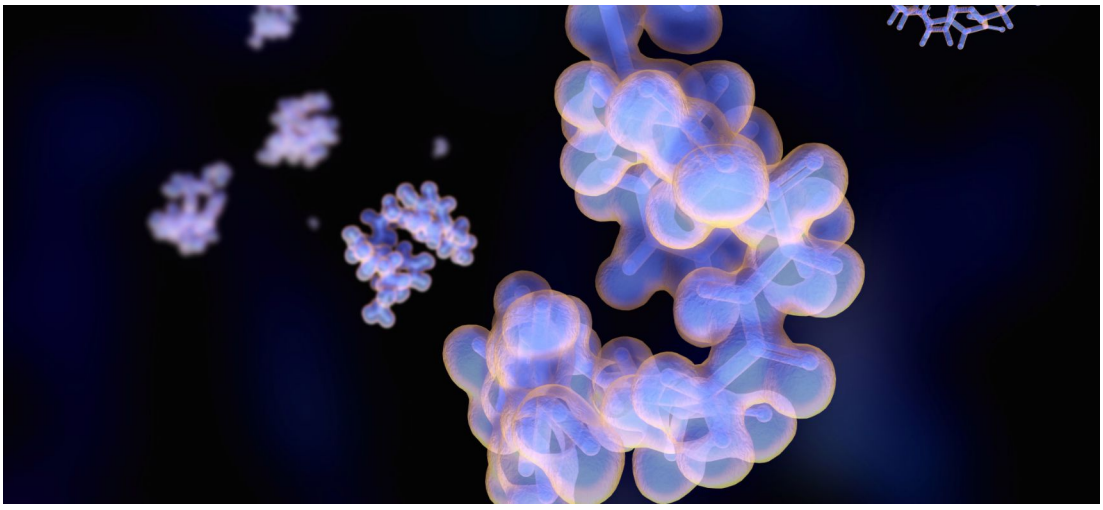
The diversity of available Kromasil phases gives the opportunity for choices best suited for you. Here is a separation example of serotonin and derivatives in highly aqueous versus HILIC conditions. In these conditions, the elution is nearly totally reversed.



Conditions

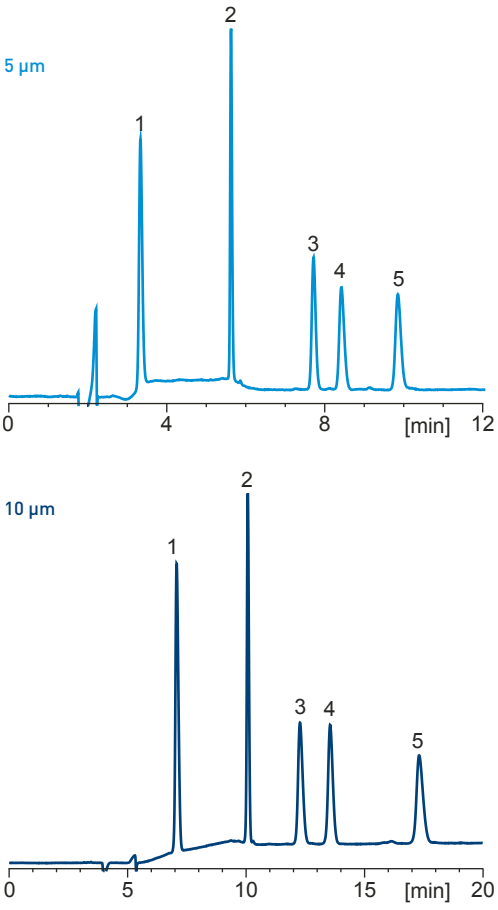
Columns: • Kromasil 100-5-C18(w) 4.6 x 150 mm
• Kromasil 60-5-HILIC-D 4.6 x 150 mm
Part number: M05WLA15 and S05HIA15, respectively
Mobile phases:
Aqueous: acetonitrile / ammonium acetate, 50 mM, pH 4 (10/90)
HILIC: acetonitrile / ammonium acetate, 5 mM, pH 4 (80/20)

Substances: **1**: 5-hydroxy-L-tryptophan, **2**: serotonin, **3**: L-tryptophan, **4**: 5-hydroxyindoleacetic acid
Temperature: ambient
Flow rate: 1.0 ml/min
Detection: UV @ 280 nm



Scalability

Good selectivity and reproducibility enable easy scale-up of analytical methods to preparative methods. This example shows the separation of a peptide mix on Kromasil C18(w) columns with 5 and 10 µm particles. Conditions are adapted to respective particle size optimum and column length.



Conditions

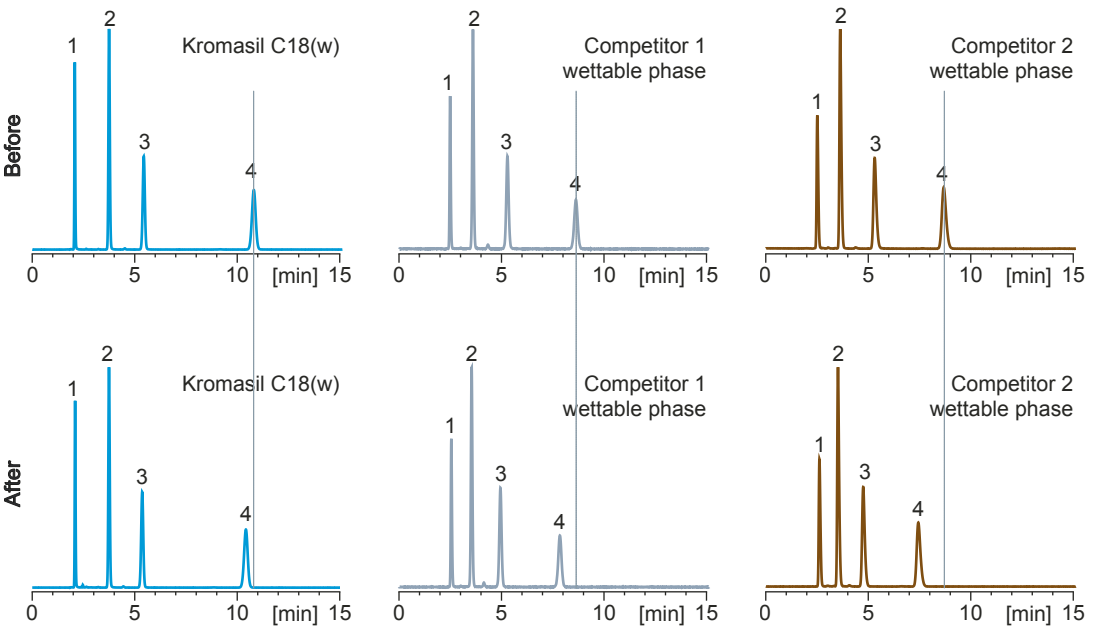
Columns: • Kromasil 100-5-C18(w) 4.6 x 150 mm
• Kromasil 100-10-C18(w) 4.6 x 250 mm

Part numbers: M05WLA15 and M10WLA25, respectively
Mobile phase: acetonitrile / water / 0.1% TFA
Gradient (5 µm): 0 min: 12%, 3 min: 26%, 13 min: 28% acetonitrile
Gradient (10 µm): 0 min: 12%, 4.3 min: 26%, 18.6 min: 28% acetonitrile

Substances: 1: gly-tyr, 2: val-tyr-val, 3: met-enkephalin, 4: angiotensin II, 5: leu-enkephalin
Temperature: 25 °C
Flow rate (5 µm): 1.0 ml/min
Flow rate (10 µm): 0.7 ml/min
Detection: UV @ 220 nm

Best in prep-class

We benchmarked the Kromasil C18(w) phase against other wettable preparative phases on the market.



Chromatograms before and after accelerated acidic hydrolysis test showing better maintained selectivity on Kromasil compared to other wettable phases.

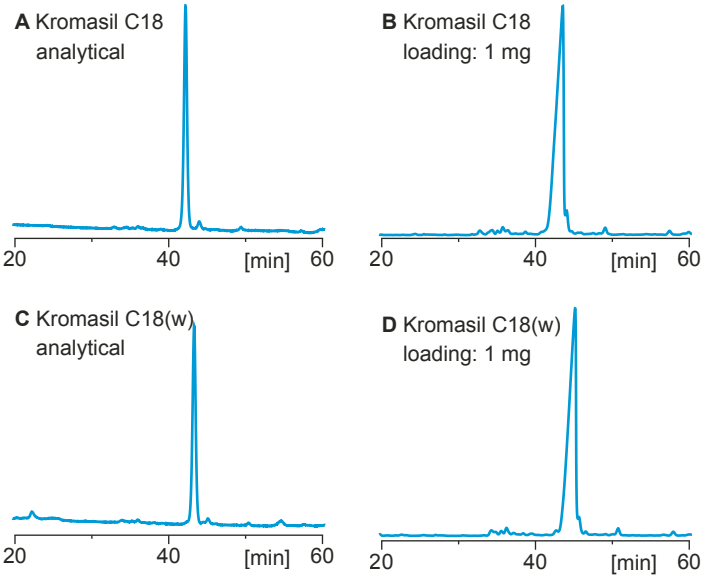
Conditions

Columns: Kromasil 100-10-C18(w) 4.6 x 250 mm and other wettable C18.
Substances: 1: uracil, 2: 2-phenylethanol, 3: butyl-4-hydroxybenzoate, 4: naphtalene
Temperature: 80 °C during hydrolysis, 25 °C during analysis
Mobile phase hydrolysis: methanol / water / TFA (5/95/0.1) 120 column volumes
Mobile phase analysis: acetonitrile / water (70/30)
Flow rate: 1.0 ml/min
Detection: UV @ 254 nm

Fully aqueous conditions when you need it

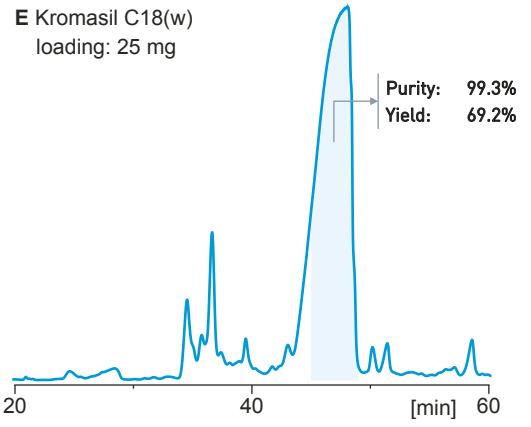
With Kromasil C18(w), you can load your preparative samples under fully aqueous conditions, increasingly important benefit for researchers today as more polar structures are being considered, reduces organic solvent consumption, cuts costs and address sustainability goals.

Kromasil C18 (w) implementation can also be of benefit for facilities that have not fully implemented explosion proof requirements to meet industry standards.



Loading of peptide sample on Kromasil C18 and Kromasil C18(w).

When comparing analytical results between Kromasil Classic C18 (figure A) and the new Kromasil C18(w) (figure C), retention times are noticeably similar for both stationary phases. Also, when proceeding to an overloaded step, retention pattern for the main peak and the impurities are comparable as seen, figures B and D.



The scale-up result of the purification on Kromasil C18(w), for this sample, is shown in figure E, where the fractions pooled provide very high purity and the given yield. If the purity requirements were lower, then more fractions could be pooled and yield increased accordingly.

Conditions

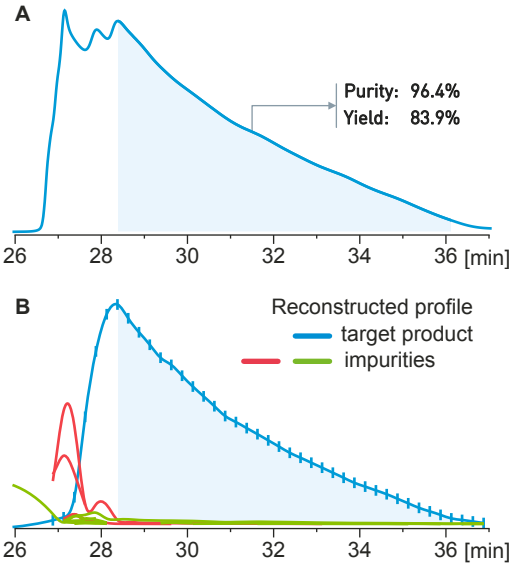
Columns: Kromasil 100-10-C18(w) 4.6 x 250 mm
Part number: M10WLA25.
Substance: crude of bivalirudin in feed solution
Temperature: 25 °C
Flow rate: 0.7 ml/min
Detection: UV @ 280 nm

Equilibrium and feed solutions:
C18: acetonitrile / ammonium acetate, 0.2 M (5/95)
C18(w): ammonium acetate, 0.2 M
Mobile phase: acetonitrile / ammonium acetate, 0.2 M
Gradient: 0 min: 10%, 60 min: 30% acetonitrile

Follow the standards

In this preparative example, we purify a peptide following Chinese Pharmacopeia ChP 2015 standard for wetted stationary phases. The results achieved is shown in figure A, with high purity of 96.4% and yield of 83.9%.

By using the ChP 2015, the wanted displacement effect is noticeable, see red and green lines in figure B, where Kromasil C18(w) first presses out impurities from the main peak at a 20 mg sample load.

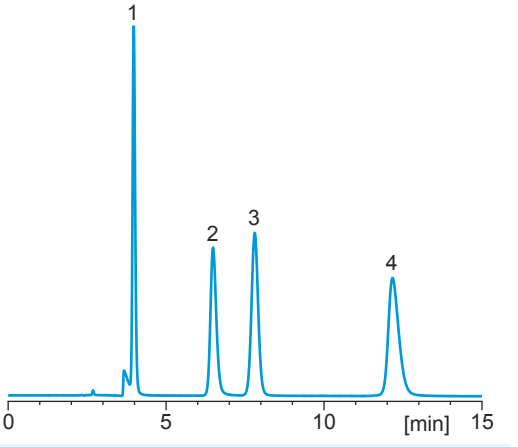


Conditions

Columns: Kromasil 100-10-C18(w) 4.6 x 250 mm
Part number: M10WLA25.
Sample: thymalfasin (crude) in 20 mM potassium phosphate
Sample load: 20 mg
Mobile phase: acetonitrile / water / 0.1 % TFA
Gradient: 0 min: 0%, 5.1 min: 16.3%, 40.1 min: 24.4%, 40.2 min: 80%, 50.2 min: 80% acetonitrile
Flow rate: 0.7 ml/min
Temperature: 25 °C
Detection: UV @ 235 nm

Catecholamines

Small phenolic compounds, catecholamines, can also be easily separated under aqueous conditions on Kromasil C18(w).



Conditions

Column: Kromasil 100-10-C18(w) 4.6 x 250 mm
Part number: M10WLA25
Substances: 1: norepinephrine, 2: epinephrine, 3: L-DOPA, 4: dopamine
Mobile phase: 20 mM potassium phosphate pH 4.6
Temperature: ambient
Flow rate: 1.0 ml/min
Detection: UV @ 220 nm

Characteristics

Particle size distribution:
(Coulter Multisizer)
dv_{90}/dv_{10}: 10 µm <1.70
5 µm < 1.55

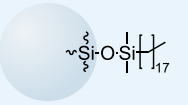
Chemical purity:
Typical values (AAS or ICP)
Na <10 ppm,
Al < 5 ppm,
Fe < 5 ppm

Specific surface area:
(multi-point BET)
320 m²/g

Pore volume:
(N_2-adsorption)
0.9 ml/g

Pore size:
(N_2-adsorption)
110 Å

Pore size distribution:
(N_2-adsorption)
80% ± 25 Å
(97% of the surface is accessible for toluene, which indicates low amounts of inaccessible micro pores.)



C18(w)

Octadecyl
USP: L1
Coverage: 2.5 µmol/m²
Element content: 15% C
Packed density: 0.60 g/ml

Polar embedded end-capping.

Availability

Kromasil C18(w) wettable phase is available in 5 and 10 µm particle size as slurry-packed HPLC columns in sizes as listed in the table below. 10 µm particles are also available as bulk material for DAC packing.

Material	Size	Part number
Kromasil 100-5-C18(w)	4.6 × 150 mm	M05WLA15
Kromasil 100-5-C18(w)	4.6 × 250 mm	M05WLA25
Kromasil 100-5-C18(w)	4.0 × 125 mm	M05WLB1F
Kromasil 100-10-C18(w)	bulk	M10WLblk
Kromasil 100-10-C18(w)	4.6 × 250 mm	M10WLA25
Kromasil 100-10-C18(w)	10 × 250 mm	M10WLP25
Kromasil 100-10-C18(w)	21.2 × 250 mm	M10WLQ25

Other sizes available upon request