

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

#### 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.

5 1 0 5 10 [min] 15

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,

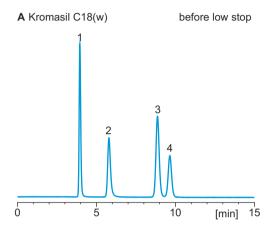
4: toluene, 5: propylbenzene, 6: butylbenzene

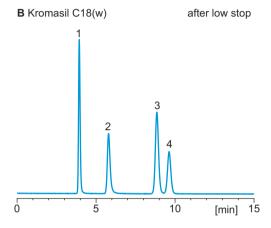
Mobile phase: acetonitrile / water (70 / 30)

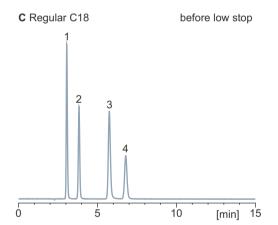
Temperature: 20 °C Flow rate: 2.0 ml/min Detection: UV @ 254 nm

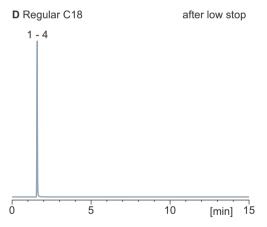
## A phase that will not collapse

In this example, we compare a separation of pyrimidine derivatives on regular C18 with Kromasil C18(w) under fully aqueous conditions.









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 fig. A shows better retention and selectivity compared to the more hydrophobic C18 in fig. 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 the surface will collapse resulting 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

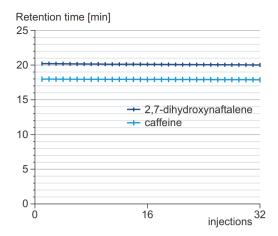
Substances: 1: cytosine, 2: fluorocytosine, 3: uracil, 4: fluorouracil

Mobile phase: 20 mM potassium phosphate pH 2.5

Temperature: ambient Flow rate: 1.0 ml/min Detection: UV @ 254 nm

## Chemical stability

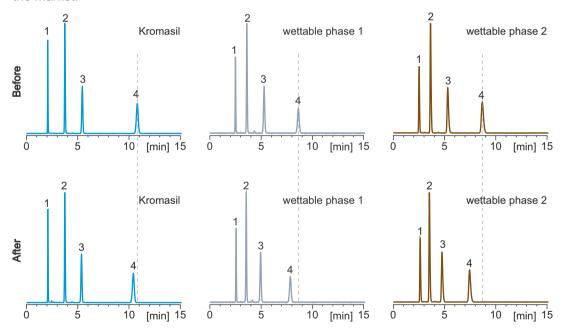
The new Kromasil C18(w) will under tough conditions and over 300 column volumes clearly maintain its retentivity with time. This performance assures engineers and researchers of the long term usability of this wettable phase.



Conditions Column: Kromasil 100-10-C18(w) 4.6 x 250 mm step min mobile phase Part number: M10WLA25 0 - 12 water / TFA (100/0.1) equilibration Substances: caffeine and 2,7-dihydroxynaftalene 12 - 16 water / TFA (100/0.1) loading gradient 16 - 36 from: acetonitrile / water / TFA (0/100/0.1) acetonitrile / water / TFA (50/50/0.1) washing 36 - 42 acetonitrile / water (70/30)

#### Best in 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

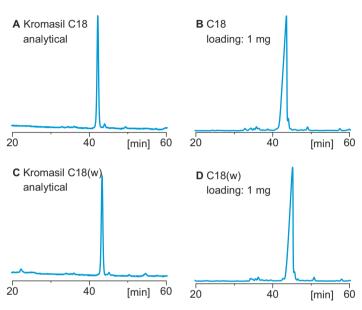
Mobile phase analysis: acetonitrile / water (70/30)

Flow rate: 1.0 ml/min Detection: UV @ 254 nm

## Fully aqueous conditions when you need it

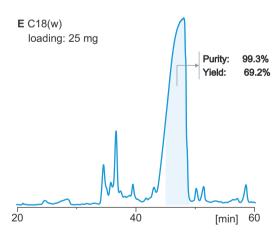
With Kromasil C18(w), you can load your preparative samples under fully aqueous conditions, increasingly important benefit for researchers today as more polar structures are being considered, reduces organic solvent consumption, cuts costs and address sustainability goals.

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 the new Kromasil C18(w) (Figure C) and Kromasil Classic C18 (Figure A), it is noticed similar retention times 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 fig 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 bivalirudine 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)

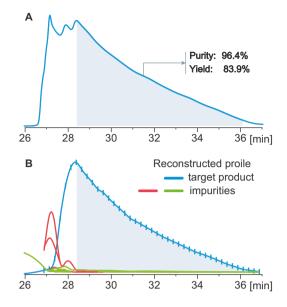
C18(w):ammonium acetate, 0.2 M

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

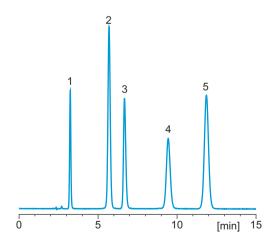
Mobile phase: acetonitrile / water / 0.1 % TFA

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

## Not only for peptides

Here is an example of a separation of organic acids: small molecules, but also polar compounds that show complete resolution when run under aqueous conditions.



#### Conditions

Column: Kromasil 100-10-C18(w) 4.6 x 250 mm

Part number: M10WLA25

Substances: 1: tartaric acid, 2: ascorbic acid,

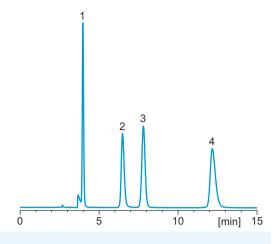
3: acetic acid, 4: citric acid, 5: fumaric acid

Mobile phase: 20 mM potassium phosphate pH 2.5

Temperature: ambient Flow rate: 1.0 ml/min Detection: UV @ 210 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) dv9ødv₁: ≪1.70

#### Chemical purity:

Typical values (AAS or ICP)

Na <10 ppm, Al <5 ppm, Fe < 5 ppm

#### Speciic surface area:

(multi-point BET) 320 m²/g

#### Pore volume:

 $\begin{array}{c} \text{(N}_{\mathcal{Z}} \text{adsorption)} \\ \text{0.9 ml/g} \end{array}$ 

## Pore size:

(N<sub>2</sub>-adsorption) 110 Å

#### Pore size distribution:

(N<sub>2</sub>-adsorption)

80% ± 25 Å

(97% of the surface is accessible for toluene, which indicates low amounts ofi naccessible micro pores.)

amounts of naccessible 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 10 µm particle size as bulk and slurry-packed HPLC columns in sizes as listed in the table.



Material	Size	Part number	Rremașii
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	M10WLP25	



## info@vdsoptilab.de

The moment you adopt our Kromasil High Performance Concept, you join thousands of chromatographers who share a common goal: to achieve better separations when analyzing or isolating pharmaceuticals or other substances.

Not only will you benefit from our patented silica technology, but you gain a strong partner with a reliable track record in the eld of silica products. For the past 70 years, we have pioneered new types of silica. Our long experience in the eld of silica chemistry is the secret behind the development of Kromasil, and the success of our Separation Products group. Kromasil is available in bulk and in high-pressure slurry-packed columns.

The development, production and marketing of Kromasil are ISO 9001 certified.

Kromasil is a brand of Nouryon, a global specialty chemicals leader. Industries worldwide rely on our essential chemistry in the manufacture of everyday products. Building on our nearly 400-year history and operations in over 80 countries, the dedication of our 10 000 employees, and our shared commitment to safety, sustainability, and innovation, we have established a world-class business and built strong partnerships with our customers.



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