

MICRO-HPLC COLUMNS

Micro-HPLC column selection by separation mode	19
Micro-HPLC column selection by manufacturer	19
Introduction to micro-HPLC	20
Keys to defining a well-characterized micro-HPLC column	20
Advantages of using micro-HPLC columns	21
HotSep® micro-HPLC columns: excellent chromatographic performance	22
Types of micro-HPLC columns	23
Micro-HPLC columns: product availability	
Kromasil® C18	27
Kromasil® C8	30
Kromasil® C4	31
Kromasil® Phenyl (PH)	31
Kromasil® Cyano (CN)	32
Kromasil® Amino (NH ₂)	33
Kromasil® Silica	34
Kromasil® Chiral	35
Hypersil ODS (C18)	37
Prontosil C18	38
PLRP-S	40
PL-SAX	44
PL-SCX	44
Nucleosil SAX	45
Nucleosil SCX	45
Other: custom packed columns, batch reservation service, warranty, technical support	46
Care & Use of HotSep® micro-HPLC columns	47
HotSep® micro-guard/trace-enrichment columns	48
HotSep® Tracy	48
HotSep® Protector	50
Packing material availability: complete list	51

Micro-HPLC Column Selection by Separation Mode

Adsorption Chromatography		Normal Phase Chromatography	
Kromasil Silica	34	Kromasil Silica	34
Chiral Chromatography		Kromasil Amino (NH ₂)	33
Kromasil CHI-TMB	35	Reversed-Phase Chromatography	
Kromasil CHI-TBB	35	Kromasil C18	27
Ion-exchange Chromatography		Kromasil C8	30
Nucleosil SAX	45	Kromasil C4	31
Nucleosil SCX	45	Kromasil Phenyl (PH)	31
PL-SAX	44	Kromasil Cyano (CN)	32
PL-SCX	44	Kromasil Amino (NH ₂)	33
		Hypersil ODS	37
		ProntoSIL C18H	38
		PLRP-S	40

Micro-HPLC Column Selection by Manufacturer

This selection is, neither in terms of manufacturers nor in terms of their products, a complete list, and the accuracy of the data is not guaranteed.

Column:	G&T Septech Replacement(s)	
	Silica:	Polymer:
Agilent Technologies		
Zorbax Eclipse-XDB C18 100	Kromasil C18 100	PLRP-S 100
Zorbax Eclipse-XDB C8 100	Kromasil C8 100	
Zorbax Eclipse-XDB C18 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
Zorbax SB C18 100	Kromasil C18 100	PLRP-S 100
Zorbax SB C8 100	Kromasil C8 100	
Zorbax SB Cyano 100	Kromasil Cyano 100	
Grace-Vydac		
Denali 120	Kromasil C18 100	PLRP-S 100
Everest 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
Higgins Analytical		
Clipeus C18	Kromasil C18, ProntoSIL C18	PLRP-S
Clipeus C8	Kromasil C8	
LC Packings		
PepMap C18 100	Kromasil C18 100	PLRP-S 100
PepMap C18 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
Micro-Tech Scientific		
Microsil C18 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
Phenomenex		
Jupiter 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
Luna C18 100	Kromasil C18 100	PLRP-S 100
Luna C8 100	Kromasil C8 100	
Waters		
Symmetry C18 100	Kromasil C18 100	PLRP-S 100
Symmetry C8 100	Kromasil C8 100	
Symmetry C18 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300
X-Terra C18 100	Kromasil C18 100	PLRP-S 100
X-Terra C18 300	Kromasil C18 300, ProntoSIL C18 300	PLRP-S 300

Introduction to micro-HPLC

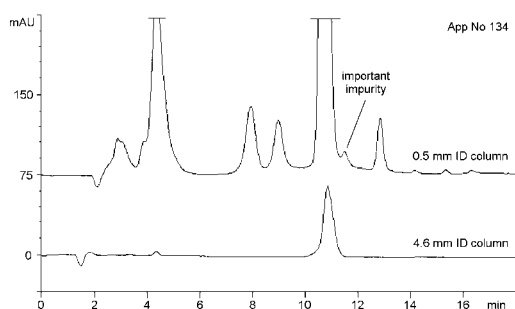
Micro-HPLC plays a crucial role in the modern laboratory by expanding the boundaries of conventional HPLC. Today micro-HPLC is used more or less on routine basis for a wide range of applications ranging from biotechnology to environmental analysis, e.g. peptide mapping by capLC-ESI-MS or analysis of important low-abundant metabolites in plasma.

Keys to defining a “well-characterized” micro-HPLC column by traditional HPLC terms are:

Selectivity

The micro-HPLC column must be able to separate the target compound(s) from other compounds present, e.g. to monitor synthetic products such as peptides or oligonucleotides for identity and purity by separating important impurities from major products. Resolving minor impurities such as deamidation products or oxidized methionine variants place the strongest demands on micro-HPLC column selectivity. In such cases, where high resolution between major product and impurities seldom is obtained, high column efficiency plays a crucial role for achieving sufficient resolution.

Furthermore, in situations where the total sample amount is too small to allow detection of target compounds (e.g., important impurities or metabolites) with conventional HPLC instruments, the use of a micro-HPLC instrument is a necessity. Such an example is shown in Appl. Note 134.

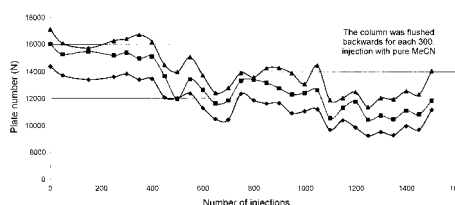


Stability

Although the robustness of micro-HPLC columns cannot match the robustness of their big brothers, the selectivity and efficiency must be constant over numerous injections to ensure robust and reliable assays. Only micro-HPLC columns which are physically and chemically stable and which maintain their chromatographic properties over certain period of time are acceptable for methods used on routine basis. By proper sample preparation and the use of guard columns/column switching, micro-HPLC columns can last for several hundreds injections (Appl. Note 136).

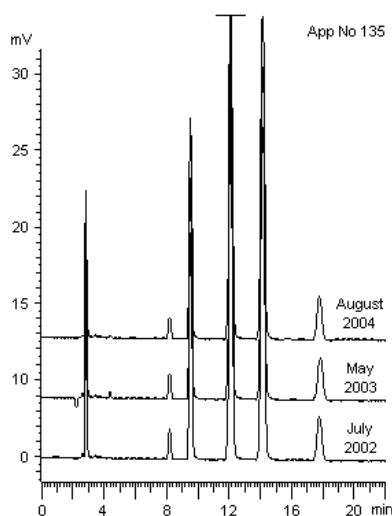
Column Stability

App No 136
Column: 3.5µ Kromasil C18 100A
Dimensions: 0.3 x 150 mm
Mobile Phase: MeCN-water (70:30)
Flow Rate: 3 µL/min
Temp.: 30C
Inj. volume: 50 nL
Detector: UV @ 254 nm
Sample: naphthalene, biphenyl, fluorene



Reproducibility

Micro-HPLC column selectivity and efficiency should remain the same when used micro-columns are replaced with new ones. A big advantage of micro-HPLC columns is that several thousands columns can be made from the same batch of packing material, which eliminate problems with “batch-to-batch”-variations. Appl. Note 135 shows three 3.5µ Kromasil C18 100 Å (0.3 x 150 mm) columns packed and tested over a period of 2 years, which clearly illustrates the high micro-HPLC column reproducibility achievable.



Advantages of using micro-HPLC columns

Improved Limit of Detection!

When only limited sample amounts are available for analysis, HotSep® micro-HPLC columns offer lower limits of detection (LOD) when connected to concentration-sensitive detectors such as ESI-MS or when post-column flow-splitting is applied. For example, a 0.5 mm ID column gives 85x lower LOD than a 4.6 mm ID column. In many applications, the sample amount is simply too small to be detected with conventional HPLC instruments.

Reduced Waste

HotSep® micro-HPLC columns offer a drastic reduction in organic solvents consumption compared to conventional sized HPLC columns. For example, a 1.0 mm ID column will reduce the mobile phase waste by a factor of 21 compared with a standard 4.6 mm ID column.

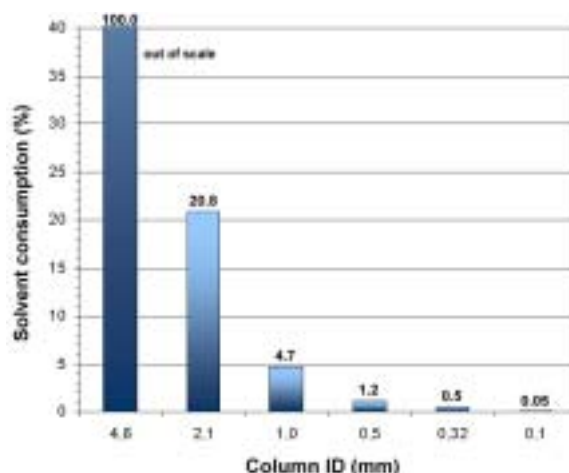
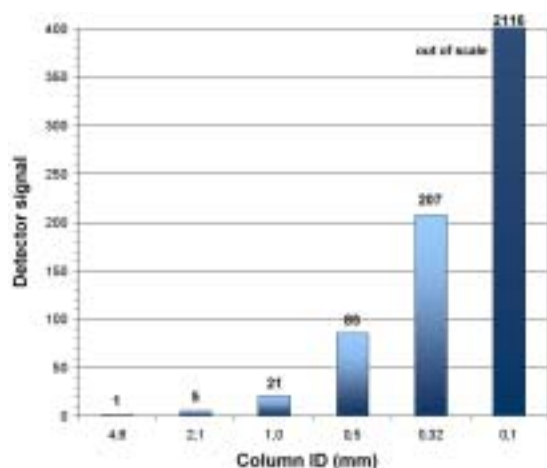
High-Temperature Compatibility

The low thermal mass of micro-HPLC columns is ideal for exploiting temperature to modulate the

chromatography, e.g. preheating of the mobile phase is simplified or often unnecessary. Higher column temperatures increase the mass transfer rate, thereby improving column efficiency and, in general, peaks are sharpened and resolution improved. Higher temperatures usually give reduced retention and thus less organic modifier is required, and the analysis can often be performed at a higher volumetric flow rate with no loss in resolution.

Reduced Column Length – Faster Separations

Many separations can be performed on shorter columns and higher flow-rates without sacrificing the baseline resolution. Furthermore, when using highly selective detectors such as the MS baseline resolution is often not required. However, being able to separate the analyte(s) from the void volume/matrix is preferred, especially when analysing complex matrixes such as biological samples, due to possible suppression effects in the ion source. The HotSep® micro-HPLC columns are therefore offered in 3 cm length for 3µ particles and 5 cm length for 5µ particles. Such short narrow-bore columns offer extremely low solvent consumption per analysis.



Excellent Chromatographic Performance

HotSep® micro-HPLC column facts:

- Fully Validated Columns
- High Reproducibility
- High Robustness
- More than 100,000 plates/m on routine basis

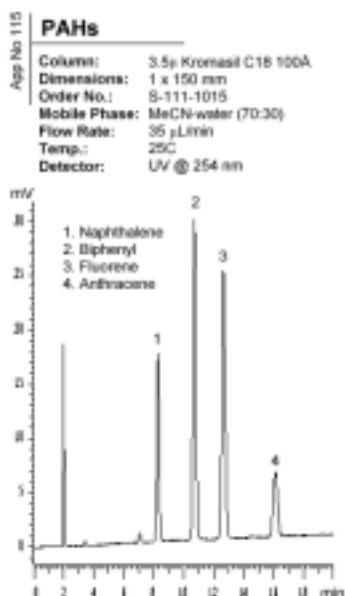
basic and neutral molecules. The HotSep® columns are supplied with stainless steel screens or titanium frits to ensure high biocompatibility.

Our packing procedures enable us to pack the HotSep® micro-HPLC columns with high efficiency. For example, we routinely obtain 80,000 - 90,000 and 100,000 - 130,000 plates/m on columns packed with 5m Kromasil C18 and 3.5m Kromasil C18, respectively.

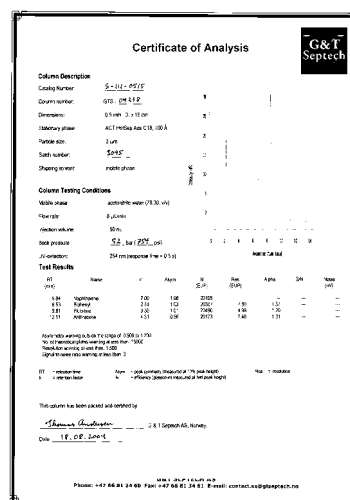
Column Validation

We guarantee that each column is carefully examined and tested before it leaves us. The columns are tested in a commercially available micro-HPLC system to insure that you as a customer can reproduce our results. Each column is delivered with a Certificate of Analysis showing the test results.

MICRO-HPLC COLUMNS



The HotSep® range of micro-HPLC columns are packed with materials from leading reputable manufacturers of stationary phases on the market. A wide range of bonding chemistries combined with ultra-pure silica particles provides excellent chromatographic performance for acidic,



Types of micro-HPLC columns

Column ID (mm)	Column Category	Flow Rate Range (μL/min)	Optimum Flow Rate (μL/min) ^a	Analyte Capacity ^b	Required Amount of Pept./Proteins (fmole)	Bed volume 15 cm column (μL)
1.0	microbore	20-200	35	~10 μg	~1,000	120
0.5	vapillary	5-50	8	~2.5 μg	~250	30
0.3	capillary	2-20	3	~1 μg	~100	10
0.1	nano	0.25-2.5	0.5	~100 ng	~10	2
0.075	nano	0.1-1	0.3	~25 ng	~1	0.7

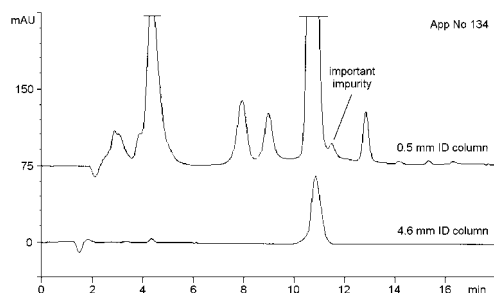
^a For true low dead-volume systems

^b Based on 3.5μ Kromasil C18 100A

HotSep[®] microbore columns (1.0 mm ID)



Microbore HPLC columns offer a convenient compromise between sensitive capillary columns and highly robust conventional-bore columns. A 1.0 mm ID column provides more than a twenty-fold increase in sensitivity over a 4.6 mm ID column when injecting the same amount (mass) of sample (Appl. Note 134). This increase in sensitivity can be critical for accurate quantification of sample limited applications. Moreover, the solvent waste is markedly reduced, offering a greener environment in the laboratory and economical benefits when using expensive solvents.



The HotSep[®] microbore columns provide high efficiencies and are available with a variety of packings. The columns are packed in robust 1/8" O.D. glass-lined stainless steel tubing offering smooth internal surface with high biocompatibility and the possibility of operating the column at elevated temperatures. Standard 1/16" end fittings provide simple, universal and robust connections.

Typical flow rates:

1.0 mm ID: 30 - 50 μL/min

Wetted parts:

quartz, stainless steel 316

End fittings:

female 1/16" connections in both ends

HotSep[®] capillary columns (0.5 and 0.3 mm ID)



Capillary HPLC columns offer high sensitivity due to their low dispersion characteristics. For example, a 0.3 mm ID column provides about 200-fold increase in sensitivity over a 4.6 mm ID column when injecting the same amount (mass) of sample. The analytes elute in smaller peak volumes, resulting in higher response for concentration-sensitive detectors (UV, fluorescence, ESI-MS). This increase in sensitivity is often a necessity for accurate quantification of sample limited applications.

The HotSep[®] capillary HPLC columns are ideal for use with detectors requiring very low flow rates, such as ESI-MS. This feature, combined with low dispersion characteristics, has led to a steadily increasing acceptance of such columns in applications where limited sample amounts lead to problems in detection sensitivity. This is relevant in the areas of pharmacokinetics, trace analysis and in particular the expanding fields of bioanalytical and proteomic analysis.

INTRODUCTION TO MICRO-HPLC (cont.)

The HotSep® capillary HPLC columns provide high efficiencies and are available with a variety of packings. The capillary columns are packed in robust glass-lined stainless steel. Standard 1/16" end fittings provide simple, universal and robust connections.

Typical flow rates:

0.3 mm ID: 3 - 5 $\mu\text{L}/\text{min}$

0.5 mm ID: 8 - 10 $\mu\text{L}/\text{min}$

Wetted parts:

quartz, stainless steel 316

End fittings:

female 1/16" connections (1/32" is optional) in both ends

HotSep® nano-HPLC columns (100 and 75 μm)



Nano-HPLC combined with nano-ESI-MS is the cutting-edge technology within miniaturized separation techniques and it is rapidly becoming

the dominating analysis tool within proteomics/peptidomics. The HotSep® nano-HPLC columns provide high efficiencies and reproducibility and they are available with a variety of packings. The columns are packed in PEEK-coated fused silica capillaries (PEEK-SIL), which are flexible and robust for practical use. Standard 1/16" end fittings provide simple, universal and robust connections.

The HotSep® nano-HPLC columns offer ultra-high sensitivity due to their low dispersion characteristics. A 75 μm I.D. column provides a 3760-fold increase in sensitivity over a 4.6 mm I.D. column when injecting the same amount (mass) of sample. The analytes elute in smaller peak volumes, resulting in higher response for concentration-sensitive detectors (UV, fluorescence, ESI-MS). This increase in sensitivity is often a necessity for accurate quantification of sample limited applications.

Typical flow rates:

100 μm ID: 0.3 - 1 $\mu\text{L}/\text{min}$

75 μm ID: 0.2 - 0.5 $\mu\text{L}/\text{min}$

Wetted parts:

fused silica, stainless steel 316

End fittings:

female 1/16" (1/32" is optional) connections in both ends.

Kromasil®

Kromasil® (EKA Chemicals) has superior mechanical and chemical stability, high surface area and narrow pore size distribution. The combination of high pore volume/surface area and high mechanical stability makes this material unique compared to other materials on the market. In addition the surface properties are excellent, making it possible to run even basic compounds without the addition of additives. Extensive control and testing are performed throughout the whole production process, ensuring the highest possible reproducibility.

Introduction to Kromasil packings

Kromasil® silica-based packings are designed to meet the highest demand in HPLC and SFC from nano to process scale. The uniqueness of Kromasil® high-performance spherical silica is the combination of:

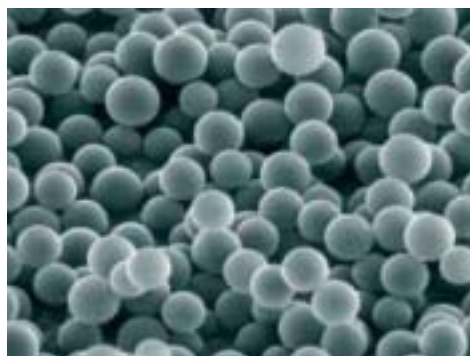
- high surface area
- mechanical strength

Other outstanding properties are:

- chemical purity
- chemical stability
- optimized surface properties
- well-defined pore structure

Kromasil® HPLC silica consists of perfectly spherical, totally porous particles. They are produced in sizes of 3.5–16µ and with a narrow particle size distribution for high efficiency, low pressure drop and best total economy in chromatographic separations.

The pictures to the right show SEM photographs of Kromasil 3.5µ and 10µ.



Kromasil 3.5µ



Kromasil 10µ

Specifications: Kromasil®

PHASE	MODE	END-CAPPED	PARTICLE SIZE (µm)	pH-RANGE	PORE SIZE (Å)	SURFACE AREA (m ² /g)	COVERAGE
C18	RP	yes	3.5, 5, 10	1.5–9.5	100, 300	330, 120	20% C, 8.7% C
C8	RP	yes	3.5, 5, 10	1.5–9.5	100, 300	330, 120	12% C, 4.7% C
C4	RP	yes	3.5, 5, 10	1.5–9.5	100, 300	330, 120	8% C, 2.9% C
Phenyl	RP	yes	5	1.5–9.5	100	330	-
Cyano (CN)	RP/NP	yes	5	1.5–9.5	60	530	12% C, 2.3% N
Amino (NH ₂)	RP/NP	yes	3.5, 5	1.5–9.5	100	330	1.9% N
Silica	NP	no	3.5, 5	1.5–9.5	100	330	-
CHI-DMB	chiral	yes	5	1.5–9.5	100	330	15% C, 0.6% N
CHI-TBB	chiral	yes	5	1.5–9.5	100	330	15.5% C, 20.6% N

Recommended maximum temperature for all Kromasil materials is 50°C.

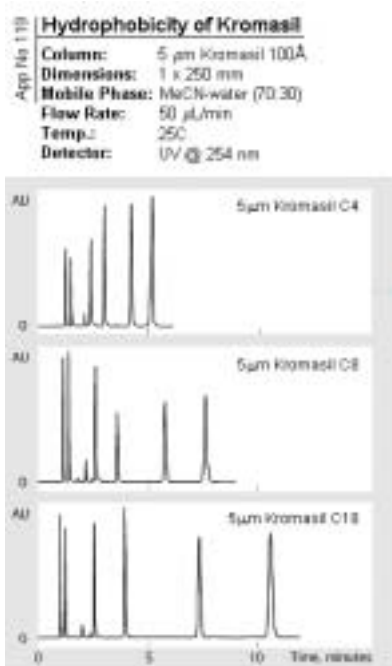
CHI-DMB: O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide
 CHI-TBB: O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide

KROMASIL PACKINGS

CHROMATOGRAPHIC PROPERTIES OF KROMASIL® REVERSED-PHASE COLUMNS

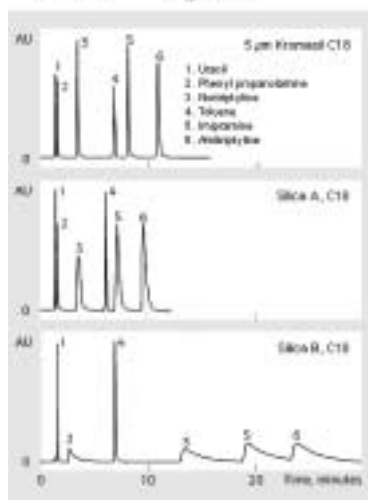
Hydrophobicity

Hydrophobicity determines the separation power of HPLC materials when hydrophobic interactions are dominating. High surface coverage and type of ligand influence this parameter. How retention is influenced by the stationary phase hydrophobicity, is shown in Figure below.



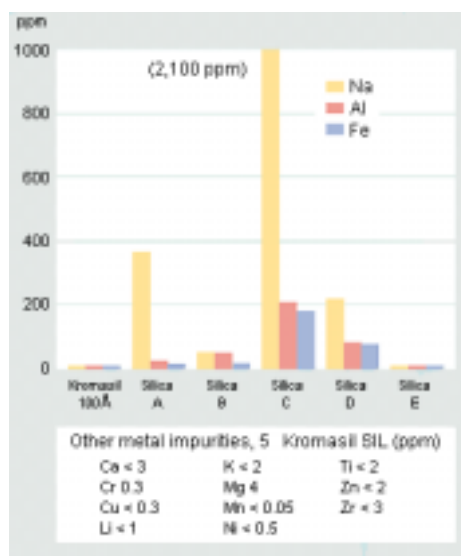
Basic Compounds

App No 110
 Column: 5 µm Kromasil C18 100Å
 Dimensions: 1 x 250 mm
 Order No.: S-121-1025
 Mobile Phase: MeCN: 10 mM sodium phosphate (50:40), pH 7
 Flow Rate: 50 µL/min
 Temp.: 40°C
 Detector: UV @ 215 nm



Metal impurities in the stationary phase

Metal impurities in the silica structure affect the acidity of silanols, creating non-homogeneous structures which strongly complex with chelating compounds. For this reason, metal impurities must be carefully monitored in silica-based materials. In Figure below, the content of metal impurities of Kromasil and other commercial materials is shown.



Ion-Exchange Properties

The interaction of basic samples with acidic silanols is mainly an ion exchange process. These strong interactions may cause undesired tailing when separating basic compounds, such as tricyclic antidepressants, on reversed-phase materials. To illustrate this, the separation of a mixture of tricyclic antidepressants (with toluene as a neutral reference), is shown for Kromasil C18 and analogous commercial materials (see Figure). As shown, Kromasil® materials are ideal for separations of basic compounds.

!!! Development, production and marketing of Kromasil packings are ISO 9001 certified.

Kromasil[®] C18

USP COLUMN CLASSIFICATION: L1

...one of the world's most popular reversed-phase HPLC materials!

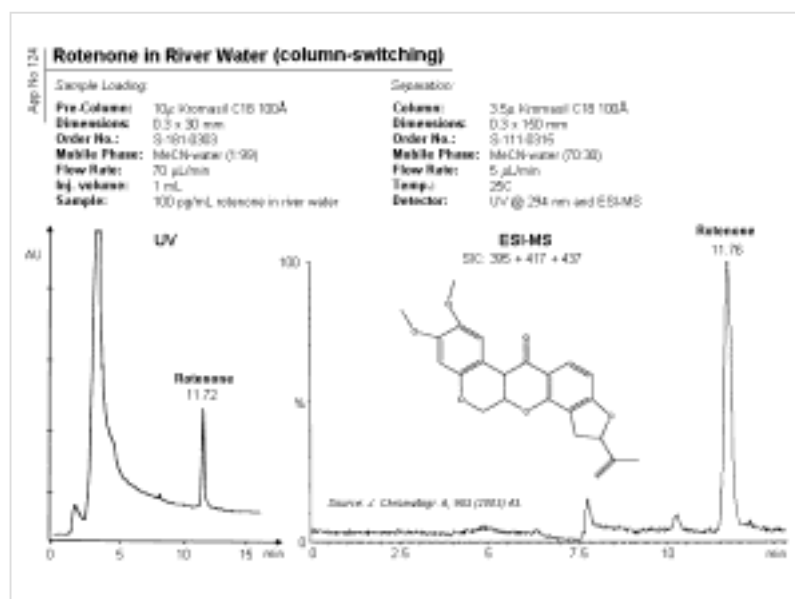
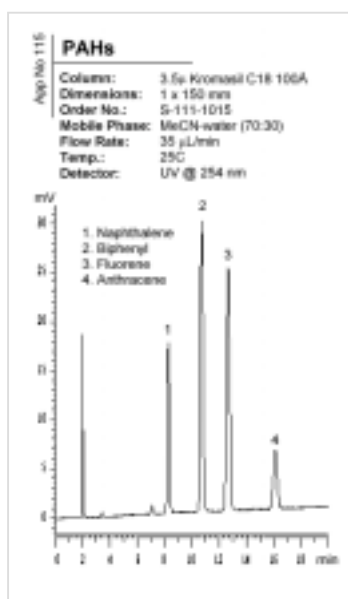
- High efficiency 3.5µ particles available – identical bonding to 5µ
- Very high loading capacity
- Excellent chemical and mechanical stability
- High retentivity
- Separation of basic compounds without additives possible



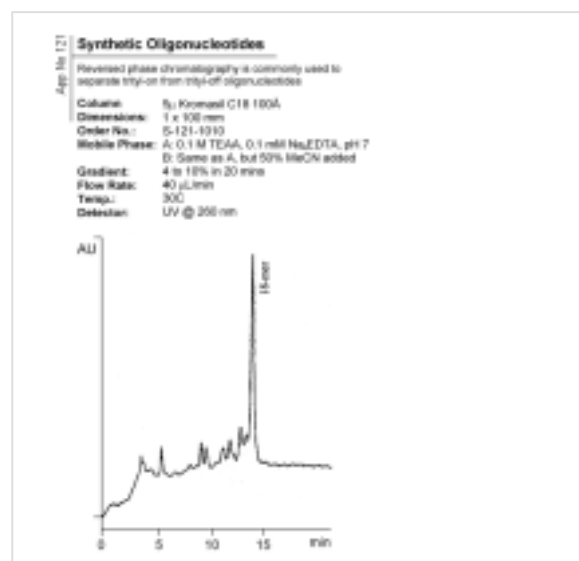
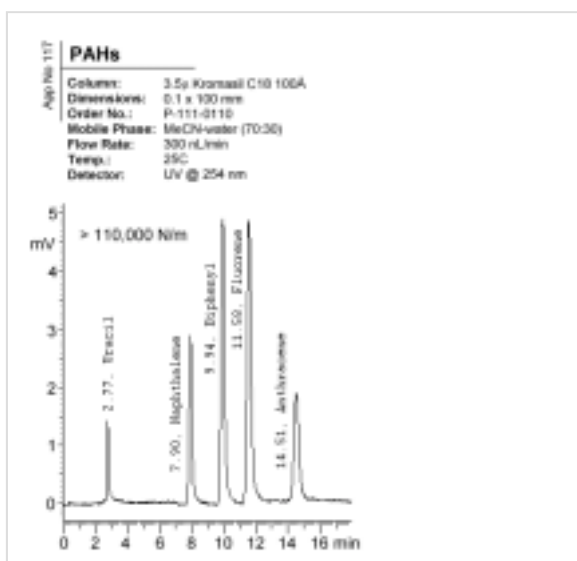
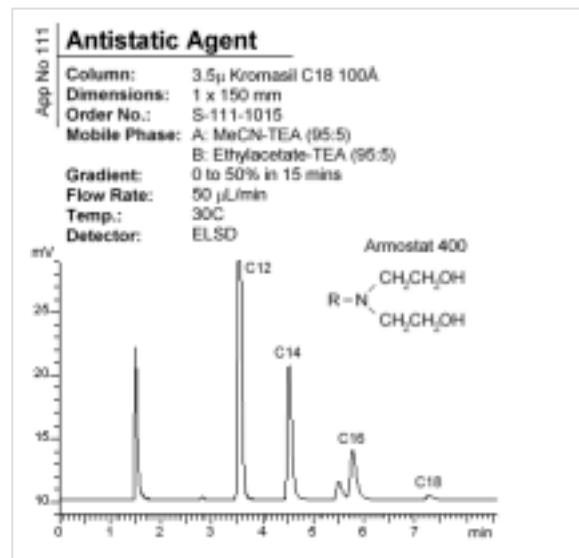
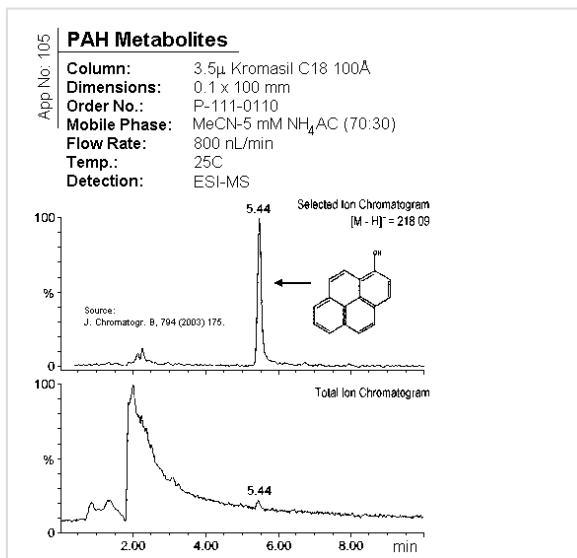
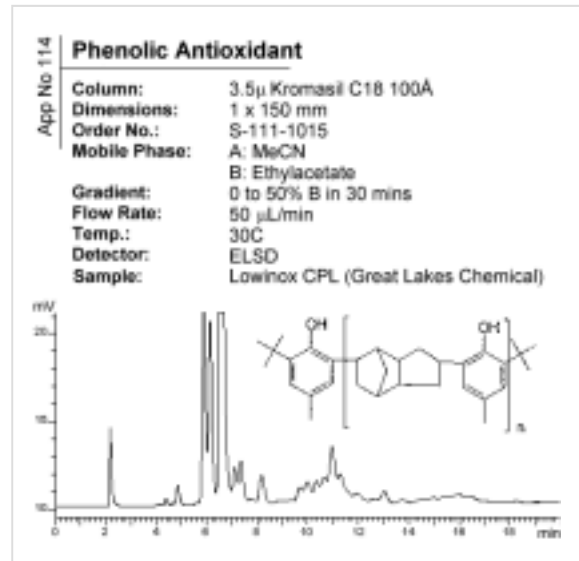
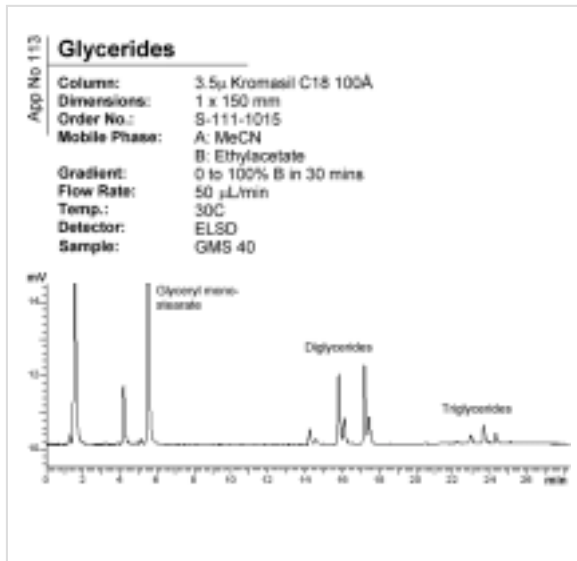
Specifications:

	100Å	300Å
Ligand:	C18	C18
Mode:	RP	RP
Endcapping:	yes	yes
Particle size:	3.5 and 5µ	5µ
pH-stability:	1.5-9.5	1.5-9.5
Actual pore size:	110 ± 25Å	300 ± 25Å
Surface area:	330 m ² /g	120 m ² /g
Pore volume:	0.9 mL/g	-
Mechanical stability:	700 bars	400 bars
Carbon load:	20%, 3.5 µmol/m ²	8%

Recommended max. temperature with aqueous mobile phases is 50°C.



KROMASIL C18 (cont.)



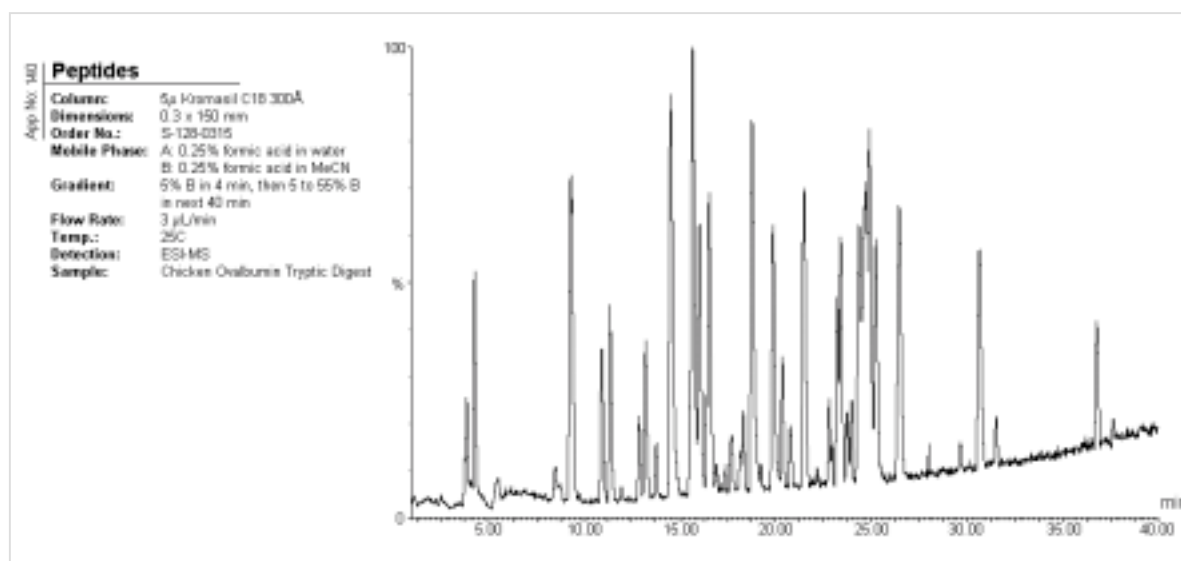
ORDERING INFORMATION – HOTSEP® KROMASIL C18

3.5µ - 100Å COLUMNS			SMALL MOLECULES (MW 100 – 5,000)			
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-111-1003	S-111-1005	S-111-1010	S-111-1015	G-111-10-1	G-111-10-5
0.5 mm	S-111-0503	S-111-0505	S-111-0510	S-111-0515	G-111-05-1	G-111-05-5
0.3 mm	S-111-0303	S-111-0305	S-111-0310	S-111-0315	G-111-03-1	G-111-03-5
0.1 mm	---	---	P-111-0110	P-111-0115	---	---
75 µm	---	---	P-111-00710	P-111-00715	---	---

5µ - 100Å COLUMNS			SMALL MOLECULES (MW 100 – 5,000)			
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-121-1005	S-121-1010	S-121-1015	S-121-1025	G-121-10-1	G-121-10-5
0.5 mm	S-121-0505	S-121-0510	S-121-0515	S-121-0525	G-121-05-1	G-121-05-5
0.3 mm	S-121-0305	S-121-0310	S-121-0315	S-121-0325	G-121-03-1	G-121-03-5
0.1 mm	---	P-121-0110	P-121-0115	---	---	---
75 µm	---	P-121-00710	P-121-00715	---	---	---

5µ - 300Å COLUMNS			MEDIUM MOLECULES (MW 500 – 25,000)			
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-128-1005	S-128-1010	S-128-1015	S-128-1015	G-128-10-1	G-128-10-5
0.5 mm	S-128-0505	S-128-0510	S-128-0515	S-128-0515	G-128-05-1	G-128-05-5
0.3 mm	S-128-0305	S-128-0310	S-128-0315	S-128-0315	G-128-03-1	G-128-03-5
0.1 mm	---	P-128-0110	P-128-0115	---	---	---
75 µm	---	P-128-00715	P-128-00715	---	---	---

Custom dimensions are available upon request



Kromasil[®] C8

USP COLUMN CLASSIFICATION: L7

...excellent alternative to C18 phases when retention is unnecessary high!

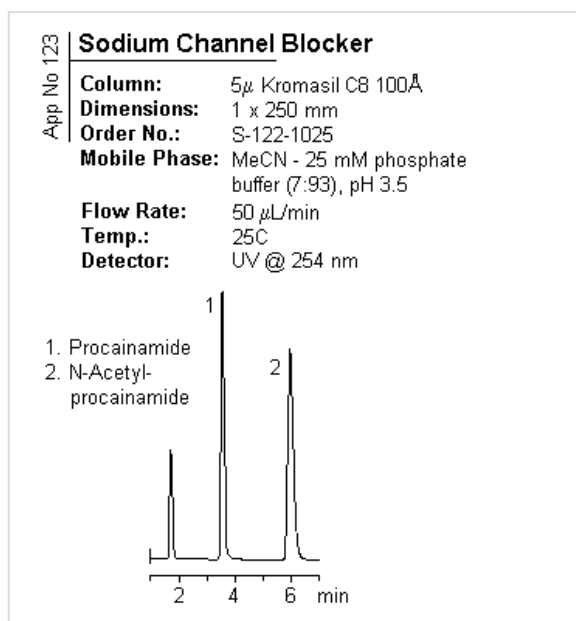
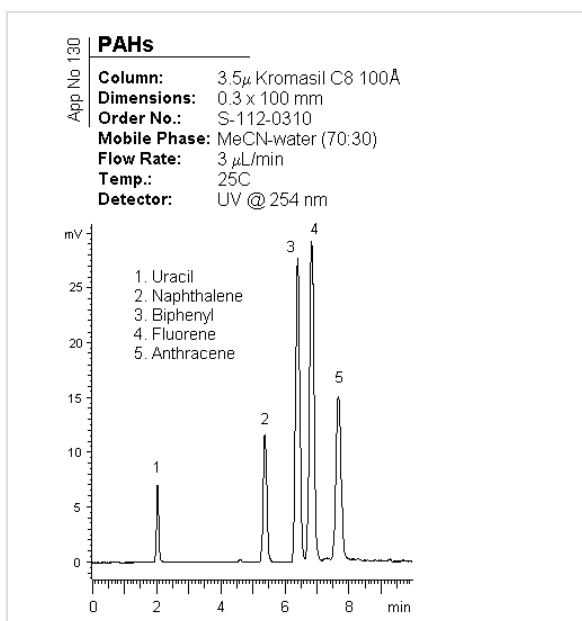
- High efficiency 3.5µ particles available – identical bonding to 5µ
- Excellent chemical and mechanical stability
- Lower retentivity than Kromasil C18 (p. 27), less organic modifier required
- Separation of basic compounds without additives possible



Specifications:

Ligand:	C8
Mode:	RP
Endcapping:	yes
Particle size:	3.5 and 5µ
pH-stability:	1.5-9.5
Actual pore size:	110 ± 25Å
Surface area:	330 m ² /g
Pore volume:	0.6 mL/g
Mechanical stability:	700 bars
Carbon load:	12%, 3.7 µmol/m ²

Recommended max. temperature with aqueous mobile phases is 50°C.



KROMASIL C8 (cont.)

ORDERING INFORMATION – HOTSEP® KROMASIL C8

3.5µ - 100Å COLUMNS				SMALL MOLECULES (MW 100 – 5,000)		
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-112-1003	S-112-1005	S-112-1010	S-112-1015	G-112-10-1	G-112-10-5
0.5 mm	S-112-0503	S-112-0505	S-112-0510	S-112-0515	G-112-05-1	G-112-05-5
0.3 mm	S-112-0303	S-112-0305	S-112-0310	S-112-0315	G-112-03-1	G-112-03-5
0.1 mm	---	---	P-112-0110	P-112-0115	---	---
75 µm	---	---	P-112-00710	P-112-00715	---	---

5µ - 100Å COLUMNS				SMALL MOLECULES (MW 100 – 5,000)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-122-1005	S-122-1010	S-122-1015	S-122-1025	G-122-10-1	G-122-10-5
0.5 mm	S-122-0505	S-122-0510	S-122-0515	S-122-0525	G-122-05-1	G-122-05-5
0.3 mm	S-122-0305	S-122-0310	S-122-0315	S-122-0325	G-122-03-1	G-122-03-5
0.1 mm	---	P-122-0110	P-122-0115	---	---	---
75 µm	---	P-122-00710	P-122-00715	---	---	---

5µ - 300Å COLUMNS				MEDIUM MOLECULES (MW 500 – 25,000)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-129-1005	S-129-1010	S-129-1015	S-129-1025	G-129-10-1	G-129-10-5
0.5 mm	S-129-0505	S-129-0510	S-129-0515	S-129-0525	G-129-05-1	G-129-05-5
0.3 mm	S-129-0305	S-129-0310	S-129-0315	S-129-0325	G-129-03-1	G-129-03-5
0.1 mm	---	P-129-0110	P-129-0115	---	---	---
75 µm	---	P-129-00710	P-129-00715	---	---	---

Custom dimensions are available upon request

!!! Kromasil is also available with a C4 phase and a Phenyl phase. Both phases are usually less retentive than Kromasil C8, but the Phenyl phase often has slightly different selectivity and is completely wettable in 100 % aqueous eluents. For ordering information, please refer to p. 51 for correct material codes.

KROMASIL CYANO

Kromasil[®] Cyano (CN)

USP COLUMN CLASSIFICATION: L10

- High efficiency 3.5µ particles available – identical bonding to 5µ
- Excellent chemical and mechanical stability
- Lower retentivity than Kromasil C18 (p. 27), less organic modifier required

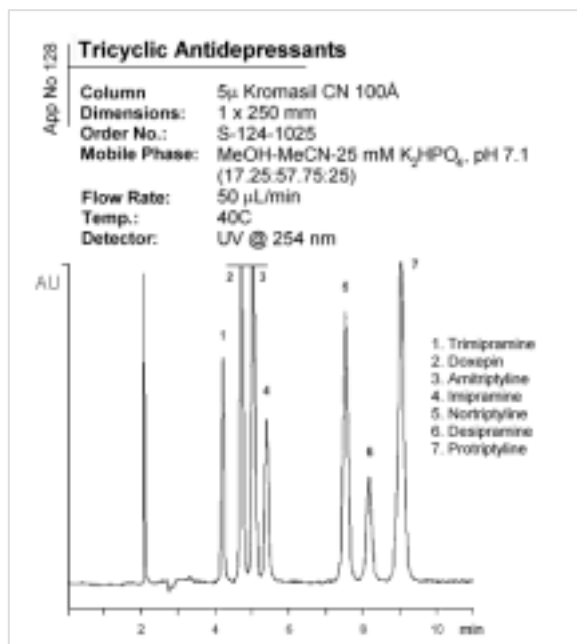
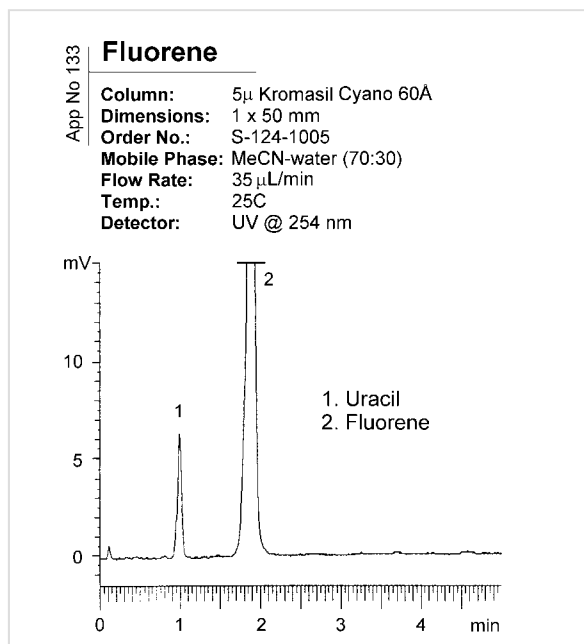
For carboxyl, carbonyl, and amine containing compounds, Kromasil[®] CN offers unique polar selectivity in RP and NP mode.



Specifications:

Ligand:	Cyanopropyl
Mode:	RP/NP
Endcapping:	yes
Particle size:	5µ
pH-stability:	1.5-9.5
Actual pore size:	80 ± 15Å
Surface area:	530 m ² /g
Pore volume:	1.2 mL/g
Mechanical stability:	700 bars
Carbon load:	12% C, 2.3% N

Recommended max. temperature with aqueous mobile phases is 50°C.



ORDERING INFORMATION – HOTSEP[®] KROMASIL CYANO

5µ - 60Å COLUMNS	SMALL MOLECULES (MW 100–5,000)					
	ID	5 cm	10 cm	15 cm	25 cm	Guard
1.0 mm	S-124-1005	S-124-1010	S-124-1015	S-124-1025	G-124-10-1	G-124-10-5
0.5 mm	S-124-0505	S-124-0510	S-124-0515	S-124-0525	G-124-05-1	G-124-05-5
0.3 mm	S-124-0305	S-124-0310	S-124-0315	S-124-0325	G-124-03-1	G-124-03-5

Custom dimensions are available upon request

Kromasil® Amino (NH₂)

USP COLUMN CLASSIFICATION: L8

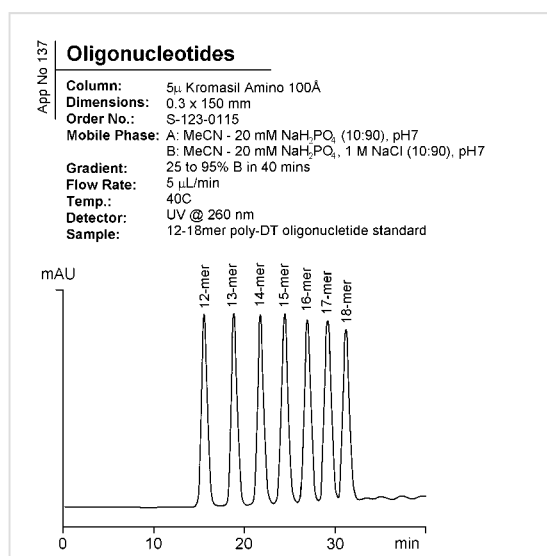
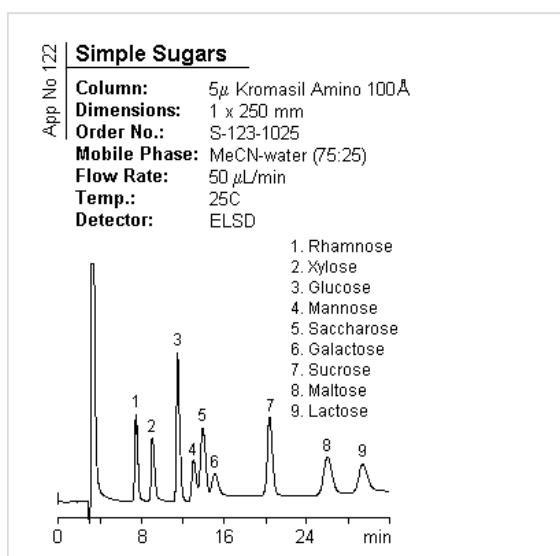
- Ideal for separation of simple and complex sugars, sugar alcohols or other hydrogen-bonding compounds under RP or NP mode
- Stable in 100% aqueous mobile phases
- No anomer resolution in sugar analysis

Specifications:

Ligand:	Aminopropyl
Mode:	RP/NP
Endcapping:	yes
Particle size:	3.5µ and 5µ
pH-stability:	1.5-9.5
Actual pore size:	80 ± 25Å
Surface area:	330 m ² /g
Pore volume:	0.9 mL/g
Mechanical stability:	700 bars
Carbon load:	1.7% N



Recommended max. temperature with aqueous mobile phases is 50°C.



MICRO-HPLC COLUMNS

ORDERING INFORMATION – HOTSEP® KROMASIL AMINO

3.5µ - 100Å COLUMNS		SMALL MOLECULES (MW 100–5,000)				
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-113-1003	S-113-1005	S-113-1010	S-113-1015	G-113-10-1	G-113-10-5
0.5 mm	S-113-0503	S-113-0505	S-113-0510	S-113-0515	G-113-05-1	G-113-05-5
0.3 mm	S-113-0303	S-113-0305	S-113-0310	S-113-0315	G-113-03-1	G-113-03-5

5µ - 100Å COLUMNS		SMALL MOLECULES (MW 100–5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-123-1005	S-123-1010	S-123-1015	S-123-1025	G-123-10-1	G-123-10-5
0.5 mm	S-123-0505	S-123-0510	S-123-0515	S-123-0525	G-123-05-1	G-123-05-5
0.3 mm	S-123-0305	S-123-0310	S-123-0315	S-123-0325	G-123-03-1	G-123-03-5

Custom dimensions are available upon request

KROMASIL SILICA

Kromasil[®] Silica

USP COLUMN CLASSIFICATION: L3

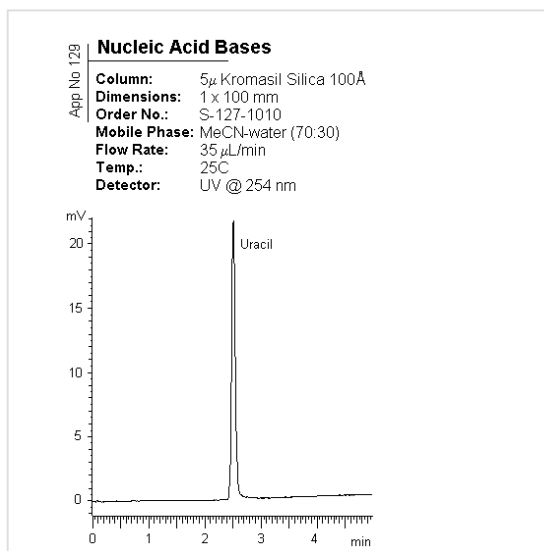
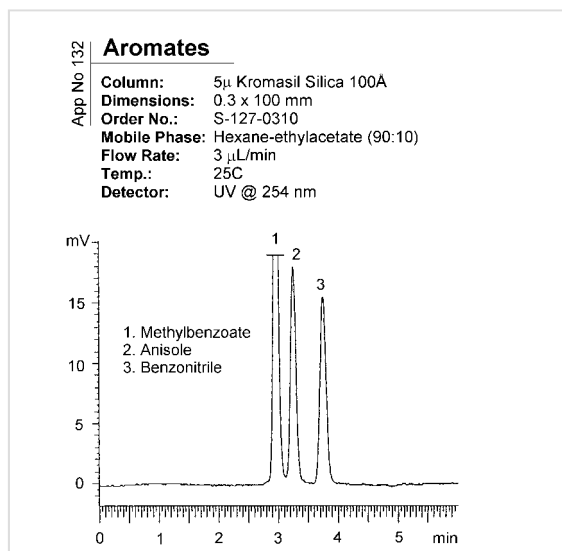
- High surface area and mechanical strength
- Low metal content and well defined pore structure
- Base particle of all other Kromasil phases



Specifications:

Mode:	NP
Particle size:	3.5 μ and 5 μ
pH-stability:	1.5-9.5
Actual pore size:	80 \pm 25Å
Surface area:	330 m ² /g
Pore volume:	0.9 mL/g
Mechanical stability:	700 bars

MICRO-HPLC COLUMNS



ORDERING INFORMATION – HOTSEP[®] KROMASIL SILICA

3.5 μ - 100Å COLUMNS						MW 100 – 5,000
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-117-1003	S-117-1005	S-117-1010	S-117-1015	G-117-10-1	G-117-10-5
0.5 mm	S-117-0503	S-117-0505	S-117-0510	S-117-0515	G-117-05-1	G-117-05-5
0.3 mm	S-117-0303	S-117-0305	S-117-0310	S-117-0315	G-117-03-1	G-117-03-5

5 μ - 100Å COLUMNS						MW 100 – 5,000
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-127-1005	S-127-1010	S-127-1015	S-127-1025	G-127-10-1	G-127-10-5
0.5 mm	S-127-0505	S-127-0510	S-127-0515	S-127-0525	G-127-05-1	G-127-05-5
0.3 mm	S-127-0305	S-127-0310	S-127-0315	S-127-0325	G-127-03-1	G-127-03-5

Custom dimensions are available upon request

Kromasil[®] Chiral

- Chiral network polymers covalently bonded to silica
- Two phases offer complementary enantioselectivity
- Good selectivity for acidic and basic chiral compounds
- Good loadability and good mechanical and chemical stability

Base Silica

Since the surface properties of the silica have a great impact on the enantioselectivity of chiral stationary phases, Kromasil[®] Chiral is made high purity, uniform surface Kromasil premium silica.

Chiral Selectors

Two phases have been developed to compliment each other in selectivity:

Kromasil[®] CHI-TMB

The chiral monomer is O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide.

Kromasil[®] CHI-TBB

The chiral monomer is O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide.

The chiral monomers are reacted with a multi-functional hydrosilane yielding a network polymer incorporating the bifunctional C2-symmetric chiral selector. The chiral polymer is then covalently bonded to functionalized silica.

Performance

The Kromasil[®] Chiral phases separate a broad range of racemates (see Table). The phases are based 5 μ particles to give sharp peaks for best possible resolution. As with most chiral columns, the best selectivity is obtained under normal phase conditions, however these phases are equally usable and stable aqueous conditions.

Mechanical and Chemical Stability

The Kromasil[®] Chiral phases are one of the most mechanically stable of HPLC silicas. The high stability is given by the nature of the network polymer covalently bonded to the silica. Both phases can be used with most solvents and buffers in the mobile phase without degradation. TFA buffers can, under certain conditions, cause some hydrolysis of the

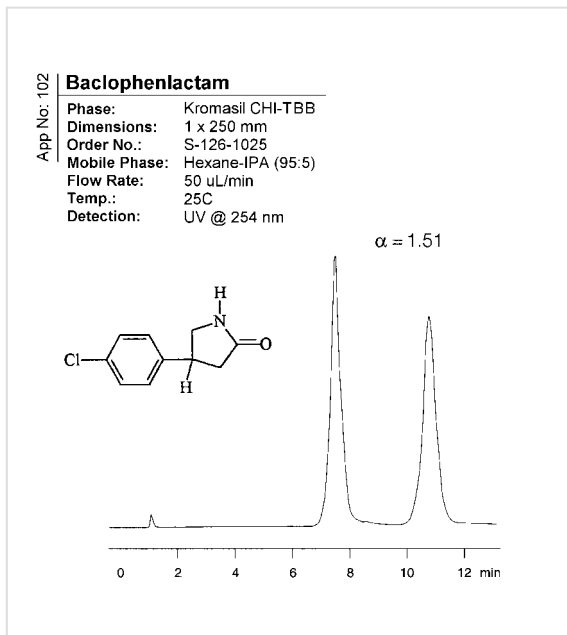
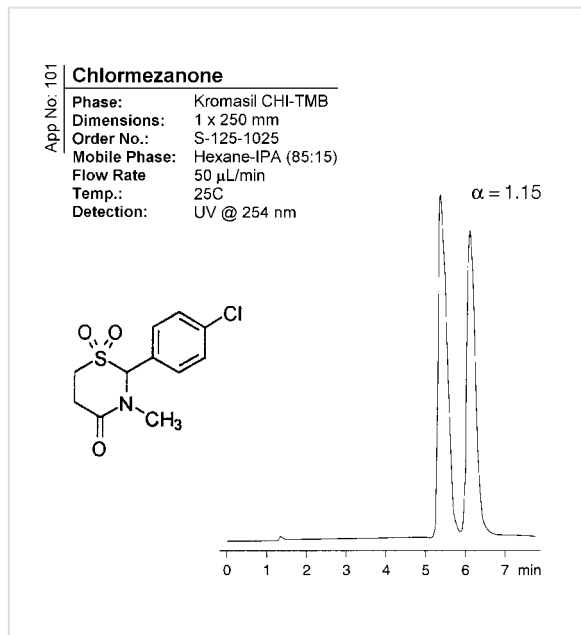
Selected Applications on Kromasil CHI-TMB and CHI-TBB

	Separation Factor (α)	
	CHI-TMB	CHI-TBB
Benzodiazepinones		
Oxazepam	1.16	1.35
Lorazepam	1.48	1.60
Lopirazepam	1.64	1.57
Ketazolam	1.13	---
Camazepam	1.06	1.25
Temazepam	1.10	---
Profens		
Carprofen	---	1.63
Ketoprofen	1.10	1.25
Naproxen	---	2.04
Ibuprofen	1.12	1.76
Flurbiprofen	1.58	1.73
Benoxaprofen	---	1.72
Priprofen	---	1.73
Benzothiadiazines and Related compounds		
Bendroflumethiazide	1.32	1.18
Parafthiazide	---	1.34
Epithiazide	1.21	---
Penfluthiazide	1.25	---
Trichloromethiazide	1.30	---
Metolazone	1.29	---
Quineyhazone	1.17	---
Amino Alcohols		
Metoprolol	1.14	---
Propranolol	1.06	---
Clenbuterol	1.12	1.23
Barbiturates		
Benzonal	1.18	---
Hexobarbital	1.19	---
Phenylphenobarbital	1.06	---
Hydantoins		
Mephentoin	1.43	---
Miscellaneous		
Morpholep	1.23	---
Glutethimide	1.08	---
Omeprazole	1.14	1.40
Warfarin	1.06	---
Chlorthalidone	1.69	---
Chlormezanone	1.15	---
Mefloquine	1.71	---
Chloroquine	1.26	---
Desethylchloroquine	1.14	---
Baclophenlactam	1.37	1.51
1,1'-Bi-(2-naphthol)	2.75	---
2-(Octylsulfinyl)-benz. acid	1.57	---
Bupivacaine	---	1.60
Carticaine	---	1.24
p-Chlorophenprocoumon	---	1.24
Etodolac	---	1.24
Indapamide	1.25	---
Metolazone	1.24	---
Oxamniquine	1.26	---
Phenprocoumon	---	1.15
Promethazine	---	1.32

Loadability

Kromasil[®] Chiral phases have a high loading capacity compared to other chiral phases. This is due to the high surface area of the silica and the high chiral ligand density.

KROMASIL CHIRAL (cont.)



!!! Check previous page for a list of applications on Kromasil CHI-TMB and Kromasil CHI-TBB

ORDERING INFORMATION – HOTSEP® KROMASIL CHI-TMB

5 μ - 100Å COLUMNS					MW 100–5,000	
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-125-1005	S-125-1010	S-125-1015	S-125-1025	G-125-10-1	G-125-10-5
0.5 mm	S-125-0505	S-125-0510	S-125-0515	S-125-0525	G-125-05-1	G-125-05-5
0.3 mm	S-125-0305	S-125-0310	S-125-0315	S-125-0325	G-125-03-1	G-125-03-5

ORDERING INFORMATION – HOTSEP® KROMASIL CHI-TBB

5 μ - 100Å COLUMNS					MW 100–5,000	
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-126-1005	S-126-1010	S-126-1015	S-126-1025	G-126-10-1	G-126-10-5
0.5 mm	S-126-0505	S-126-0510	S-126-0515	S-126-0525	G-126-05-1	G-126-05-5
0.3 mm	S-126-0305	S-126-0310	S-126-0315	S-126-0325	G-126-03-1	G-126-03-5

Custom dimensions are available upon request

Hypersil™ ODS (C18)

USP COLUMN CLASSIFICATION: L1

- Slightly different selectivity than modern C18 phases when separating amines
- Not recommended for highly basic compounds

Hypersil ODS (Thermo Hypersil-Keystone) is a classical and well-known reversed-phase material for experienced users of HPLC. This material is end-capped, but not well deactivated and may give interactions between the analytes and the silanols on the stationary phase. Hence, it may provide a slightly different selectivity than Kromasil C18. Not recommended for highly basic compounds.

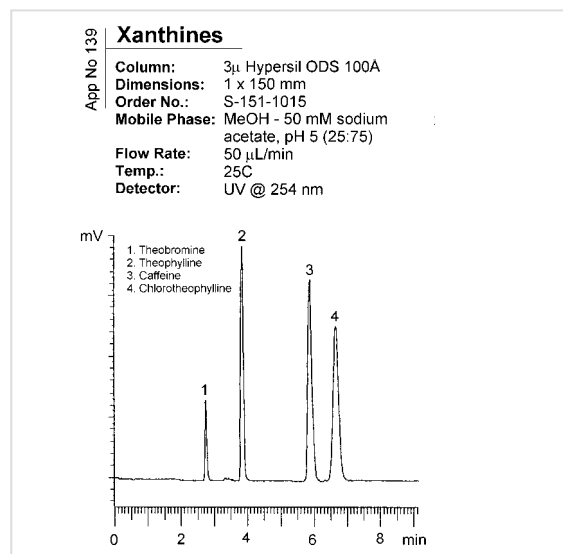
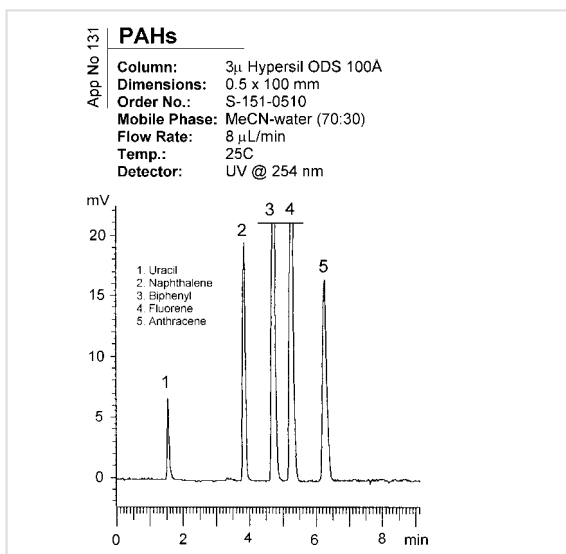


Recommended max. temperature with aqueous mobile phases is 50°C.

Specifications:

Ligand:	C18
Mode:	reversed
Endcapping:	yes
Particle size:	3µ
pH-stability:	2-8
Actual pore size:	120Å
Surface area:	170 m ² /g
Carbon Load:	10% C

MICRO-HPLC COLUMNS



ORDERING INFORMATION – HOTSEP® HYPERSIL ODS

ID	3µ - 100Å COLUMNS			SMALL MOLECULES (MW 100–5,000)		
	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-151-1003	S-151-1005	S-151-1010	S-151-1015	G-151-10-1	G-151-10-5
0.5 mm	S-151-0503	S-151-0505	S-151-0510	S-151-0515	G-151-05-1	G-151-05-5
0.3 mm	S-151-0303	S-151-0305	S-151-0310	S-151-0315	G-151-03-1	G-151-03-5
0.1 mm	---	---	P-151-0110	P-151-0115	---	---
75 µm	---	---	P-151-00710	P-151-00715	---	---

Custom dimensions are available upon request

ProntoSIL C18

USP COLUMN CLASSIFICATION: L1

...excellent high-purity silica-based reversed-phase material for separation of biomolecules



Specifications:

	100Å	300Å
Ligand:	C18	C18
Mode:	RP	RP
Endcapping:	yes	yes
Particle size:	3µ	3µ
pH-stability:	1.5-9.5	1.5-9.5
Act. pore size:	120	300
Surface area:	300 m ² /g	100 m ² /g
Pore volume:	1.0 mL/g	0.9 mL/g
Mech. stability:	700 bars	450 bars
Carbon load:	17%	7%

Recommended max. temperature with aqueous mobile phases is 50°C.

ProntoSIL C18H (Bischoff Chromatography) is an ultra-pure, spherical, completely porous silica gel. It is manufactured under the most stringently controlled conditions, guaranteeing constant particle and pore distribution, as well as constant size and volume of the pores. This manufacturing process permits no performance-reducing micropores, but yields constant specific surface area. ProntoSIL packing materials have extraordinary purity and are free from metallic contaminants that could hinder optimum peak shape.

Many years of experience in the development of ProntoSIL C18 phases have led to more than the usual physical parameters such as specific surface area, pore size, pore volume and percentage carbon content. Additional parameters provided include silica gel composition, metallic impurities, hydrophobic strength, peak symmetry for basic analytes, silanol capacity and ion exchange sites. These parameters are determined by procedures such as NMR and ICP in addition to chromatographic techniques. This multiplicity of characteristics yields extremely reproducible carrier materials providing a basis for reliable, constant, batch-independent, high-performance separation columns.

Ultra-Pure Silica Gel

The silica gel utilized in the manufacture of

ProntoSIL is 99.999% pure silica gel. A particle size distribution analyzer is used to test each batch for constant particle size. Surface area, pore size and pore volume are determined by a BET analyzer. AAS and ICP are utilized to test for metallic impurities. The extremely low level of metallic impurities guarantees that no sample will be adsorbed and no complexes will be formed during chromatographic analysis.

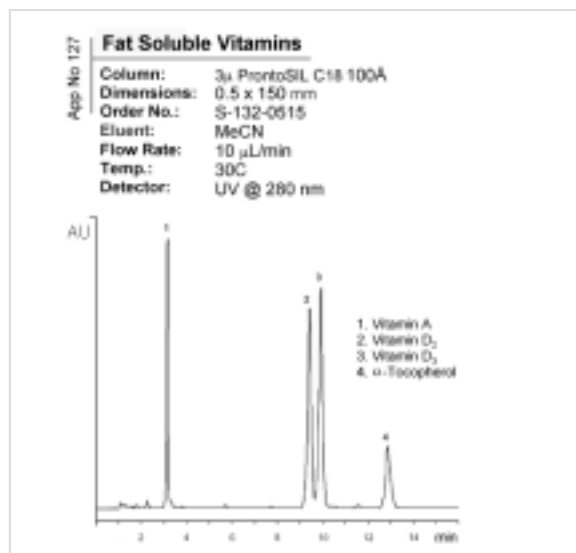
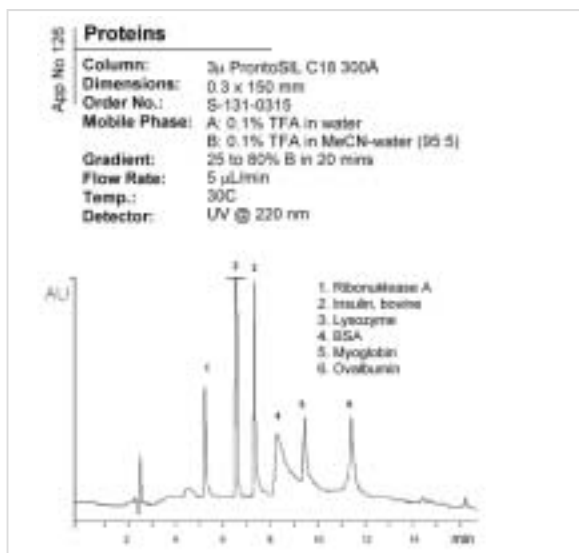
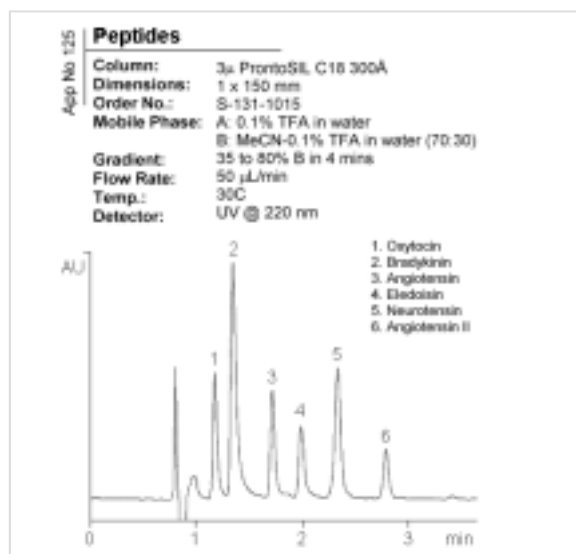
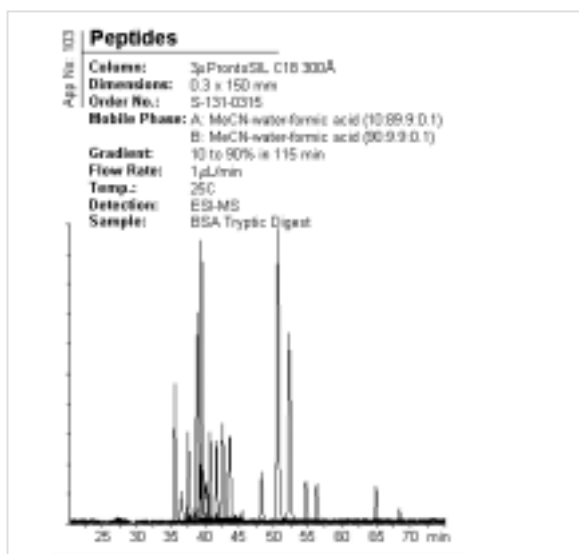
Bonding and End-Capping

The silica gel is chemically modified using the most convenient procedures, in part developed by Bischoff. Efficient bonding and the elimination of harmful silanol group residues is monitored by NMR. The absence of silanol group residues is shown by the excellent chromatographic performance with respect to basic and acid analytes. Both yield equally good chromatograms.

Packed High-Performance Separation Columns

The spherical, mechanically stable particles of silica gel with extremely narrow size distribution ensure constant column packing at lowest operating pressures in micro-HPLC columns. We offer **ProntoSIL C18H**, which is a standard reversed-phase material for separating hydrophobic analytes. A particular benefit is the maximum surface area and complete end-capping

PRONTOSIL C18 (cont.)



MICRO-HPLC COLUMNS

ORDERING INFORMATION – HOTSEP® PRONTOSIL C18

3 μ - 100Å COLUMNS				SMALL MOLECULES (MW 100 – 5,000)		
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-132-1003	S-132-1005	S-132-1010	S-132-1015	G-132-10-1	G-132-10-5
0.5 mm	S-132-0503	S-132-0505	S-132-0510	S-132-0515	G-132-05-1	G-132-05-5
0.3 mm	S-132-0303	S-132-0305	S-132-0310	S-132-0315	G-132-03-1	G-132-03-5
0.1 mm	---	---	P-132-0110	P-132-0115	---	---
75 μ m	---	---	P-132-00710	P-132-00715	---	---

3 μ - 300Å COLUMNS				MEDIUM MOLECULES (MW 500 – 25,000)		
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-131-1003	S-131-1005	S-131-1010	S-131-1015	G-131-10-1	G-131-10-5
0.5 mm	S-131-0503	S-131-0505	S-131-0510	S-131-0515	G-131-05-1	G-131-05-5
0.3 mm	S-131-0303	S-131-0305	S-131-0310	S-131-0315	G-131-03-1	G-131-03-5
0.1 mm	---	---	P-131-0110	P-131-0115	---	---
75 μ m	---	---	P-131-00710	P-131-00715	---	---

Custom dimensions are available upon request

PLRP-S

USP COLUMN CLASSIFICATION: L21

...the world's most popular polymeric reversed-phase HPLC material!

PLRP-S (Polymer Laboratories, UK) is a rigid macroporous styrene/divinylbenzene (PS/DVB) HPLC phase that has outstanding chemical and physical stability. PLRP-S HPLC media is inherently hydrophobic and reproducible, and does not require a bonded alkyl chain, e.g. C8 or C18 to confer hydrophobicity.

Advantages of PLRP-S

- Outstanding chemical stability
- High pressure capability (>8,000 psi for 100Å)
- Highly reproducible
- Easily, regenerated, sanitized and sterilized
- Temperature stability (min. 150°C in water)
- Gradients from 1 to 100% organic modifier
- Unlimited buffer concentration

PLRP-S Quality

PLRP-S is manufactured by Polymer Laboratories using state-of-the-art manufacturing techniques. The process is tightly controlled and monitored throughout, ensuring product quality and reproducibility at the manufacturing stage.

The media/columns undergo rigorous QC for:

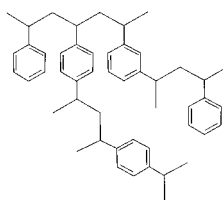
- Particle size and distribution
- Pore size
- Swell and physical stability
- Leachables
- Efficiency
- Asymmetry
- Retention factors
- Resolution
- Permeability/pressure

Pore Sizes and Particle Sizes

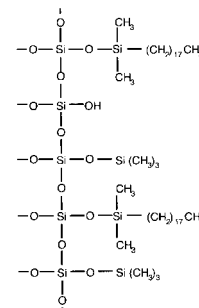
The PLRP-S family consists of a wide range of pore and particle sizes, all with the identical fundamental adsorptive characteristics and chemistry:

- The PLRP-S 100Å has the optimum pore size for the analysis of small molecules, small peptides and oligonucleotides.

Chemical Structure of PLRP-S



Chemical Structure of C18 Reversed Phase Silica



- The PLRP-S 300Å is best suited to the analysis of large peptides and globular proteins.
- The PLRP-S 1000Å is required for large biomolecules, e.g. fibrous proteins and DNA fragments.
- The PLRP-S 4000Å gigaporous material is designed for the analysis of very large biomolecules or for high speed/high resolution separations.

Long lifetimes

PLRP-S media, being a macroporous PS/DVB polymer with no bonded phase, has no residual reactive sites which often arise in silica-based products and is free from silanols and heavy metal ions. The polymeric nature of PLRP-S prevents dissolution of the stationary phase. Columns therefore last significantly longer as voids are unlikely to form. This feature even allows the use of high temperature (min. 150°C) superheated water as an eluent without fear of damage to the stationary phase.

Column Regeneration

A wide range of clean-up procedures can be used to regenerate the original characteristics and prolong the use of the column. Theoretically, and in practice, there can be no leakage or ageing due to removal of bonded ligands.

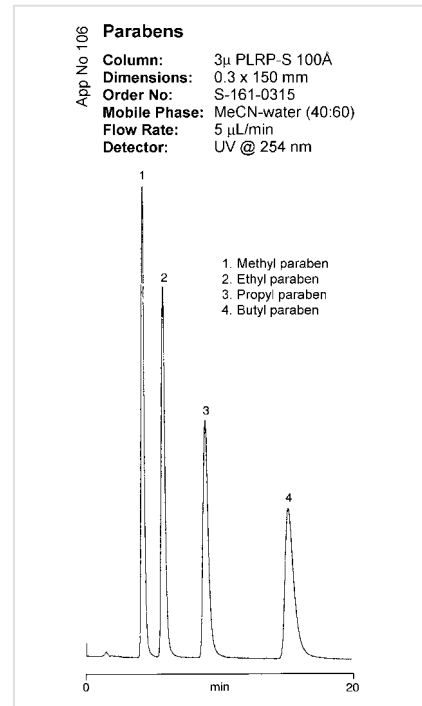
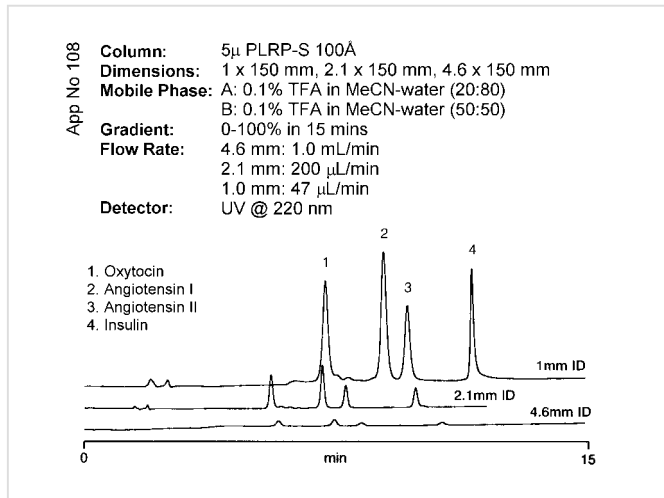
Easily Sterilized

PLRP-S columns can easily be sterilized using sodium hydroxide or other reagents.

Specifications:

pH range:	1-14
Buffer Content:	unlimited
Organic Modifier:	1-100%
Temperature:	min. 150°C in water
Pressure (max.):	4,000 psi/300 bar (3μ)

Recommended literature: L.L. Lloyd, J. Chromatogr. A, 544 (1991) 201.



PLRP-S is ideal for biomolecule analysis

Reversed phase HPLC has become the method of choice for the analysis and purification of peptides and proteins. The PLRP-S materials, with a range of optimized pore sizes, are ideally suited to this application area. Accessibility and high permeability of the molecules to the internal surface of the porous particle give excellent stability and capacity, and the high chemical and physical stability of PLRP-S columns enable reproducible resolution with greatly extended column lifetimes. The PLRP-S packing is completely insoluble, and will not contaminate isolated fractions with leachable bonded phase. The ability to operate over the entire pH range and virtually all mobile phase compositions enables greater selectivity and unrestricted clean-up procedures to be used.

PLRP-S Advantages

PLRP-S does not suffer from the same problems and silica-based particles with bonded phases.

No residual acidic silanol groups:

- No peak tailing/loss of resolution due to interaction with basic amino acid residues – lysine, arginine.
- No ion pairing agent or amine needed to mask silanol interactions.
- No loss of basic peptides/proteins due to non-specific interactions.

No stripping of hydrophobic bonded phase:

- No change in retention/selectivity as a function of column age.
- No contamination of valuable peptide/protein with hydrophobic ligand.

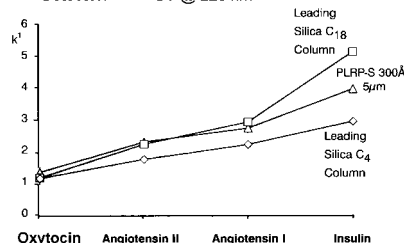
No dissolution of base particle:

- No loss of column performance – voiding
- No contamination of valuable peptide/protein with silica

The underlying hydrophobic retention characteristics of the PLRP-S media are comparable to RP-silica based packings. However, subtle differences due to the potential for additional pi-pi bond interactions can be utilized to further improve the resolution.

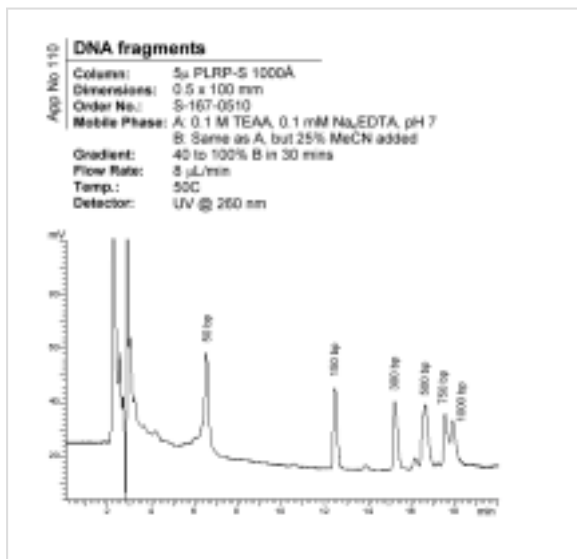
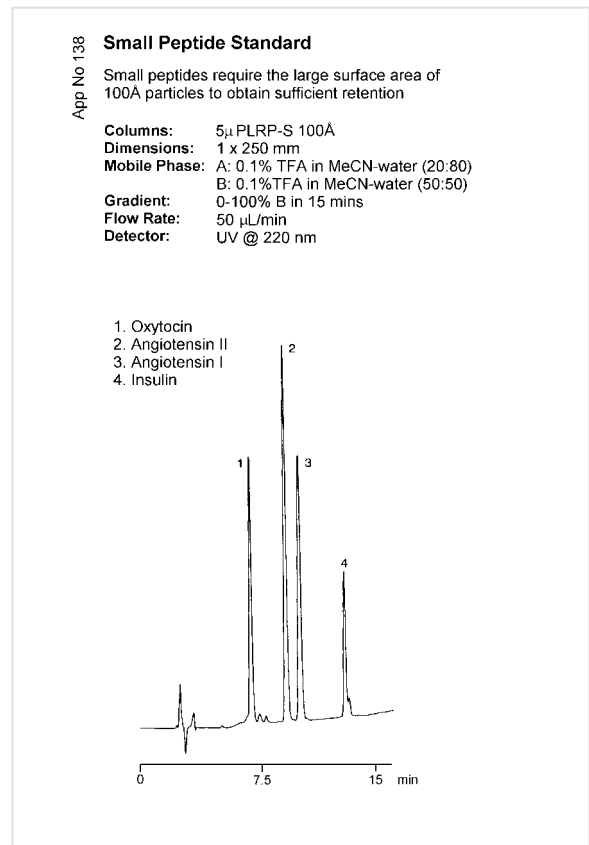
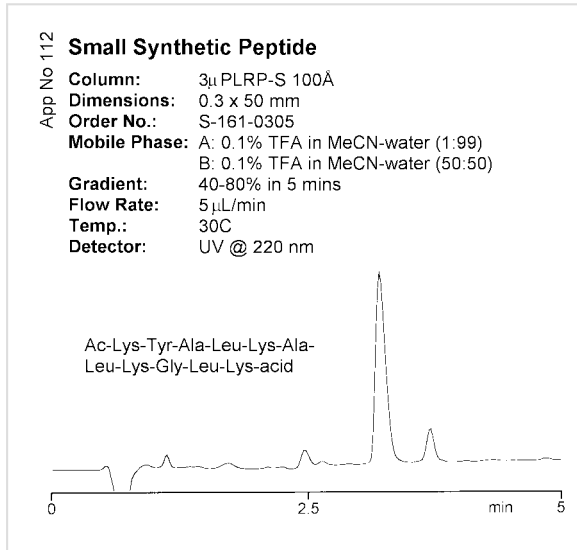
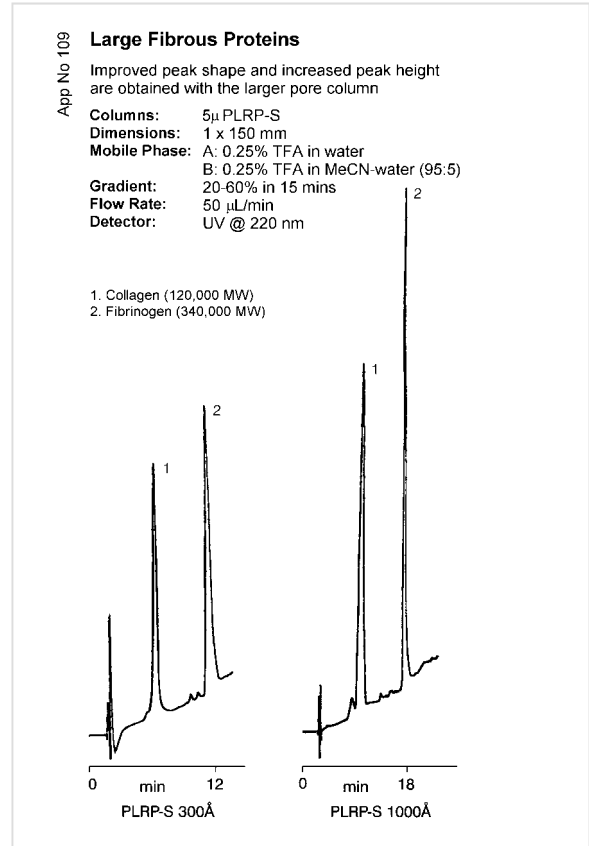
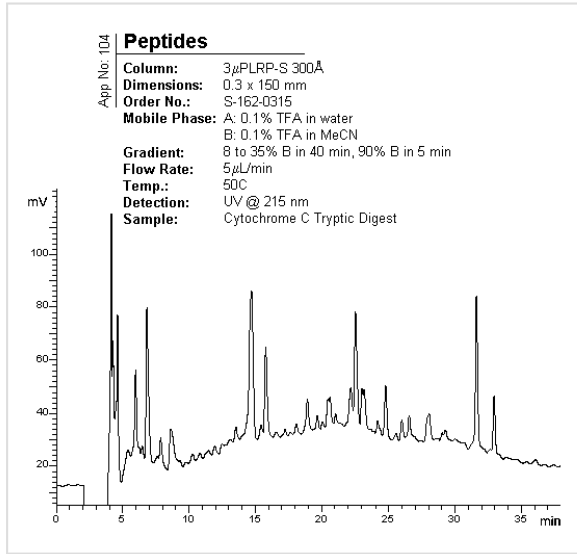
App No 107 Gradient elution of 4 peptide hormones

Dimensions: 1 x 250 mm
Mobile Phase: A: 0.1% TFA in MeCN-water (20:80)
 B: 0.1% TFA in MeCN-water (50:50)
Gradient: 0-100% B in 15 mins
Flow Rate: 50 μ L/min
Detector: UV @ 220 nm



The observed absolute retention for four peptides on leading C18 and C4 silica columns and PLRP-S indicates the similarity of the interaction. The retention characteristics of the PLRP-S column offer excellent performance over a wide range of peptides and proteins.

PLRP-S (cont.)



PLRP-S (cont.)

ORDERING INFORMATION – HOTSEP® PLRP-S

3μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-161-1003	S-161-1005	S-161-1010	S-161-1015	G-161-10-1	G-161-10-5
0.5 mm	S-161-0503	S-161-0505	S-161-0510	S-161-0515	G-161-05-1	G-161-05-5
0.3 mm	S-161-0303	S-161-0305	S-161-0310	S-161-0315	G-161-03-1	G-161-03-5
0.1 mm	---	---	P-161-0110	P-161-0115	---	---
75 μm	---	---	P-161-00710	P-161-00715	---	---

3μ - 300Å COLUMNS		MEDIUM MOLECULES (MW 500 – 25,000)				
ID	3 cm	5 cm	10 cm	15 cm	Guard	Guard/5pk
1.0 mm	S-162-1003	S-162-1005	S-162-1010	S-162-1015	G-162-10-1	G-162-10-5
0.5 mm	S-162-0503	S-162-0505	S-162-0510	S-162-0515	G-162-05-1	G-162-05-5
0.3 mm	S-162-0303	S-162-0305	S-162-0310	S-162-0315	G-162-03-1	G-162-03-5
0.1 mm	---	---	P-162-0110	P-162-0115	---	---
75 μm	---	---	P-162-00710	P-162-00715	---	---

5μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-165-1005	S-165-1010	S-165-1015	S-165-1025	G-165-10-1	G-165-10-5
0.5 mm	S-165-0505	S-165-0510	S-165-0515	S-165-0525	G-165-05-1	G-165-05-5
0.3 mm	S-165-0305	S-165-0310	S-165-0315	S-165-0325	G-165-03-1	G-165-03-5
0.1 mm	---	P-165-0110	P-165-0115	---	---	---
75 μm	---	P-165-00710	P-165-00715	---	---	---

5μ - 300Å COLUMNS		MEDIUM MOLECULES (MW 500 – 25,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-166-1005	S-166-1010	S-166-1015	S-166-1025	G-166-10-1	G-166-10-5
0.5 mm	S-166-0505	S-166-0510	S-166-0515	S-166-0525	G-166-05-1	G-166-05-5
0.3 mm	S-166-0305	S-166-0310	S-166-0315	S-166-0325	G-166-03-1	G-166-03-5
0.1 mm	---	P-166-0110	P-166-0115	---	---	---
75 μm	---	P-166-00710	P-166-00715	---	---	---

5μ - 1000Å COLUMNS		LARGE MOLECULES (MW 5,000 – 500,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-167-1005	S-167-1010	S-167-1015	S-167-1025	G-167-10-1	G-167-10-5
0.5 mm	S-167-0505	S-167-0510	S-167-0515	S-167-0525	G-167-05-1	G-167-05-5
0.3 mm	S-167-0305	S-167-0310	S-167-0315	S-167-0325	G-167-03-1	G-167-03-5
0.1 mm	---	P-167-0110	P-167-0115	---	---	---
75 μm	---	P-167-00710	P-167-00715	---	---	---

5μ - 4000Å COLUMNS		VERY LARGE (MW 100,000+)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-168-1005	S-168-1010	S-168-1015	S-168-1025	G-168-10-1	G-168-10-5
0.5 mm	S-168-0505	S-168-0510	S-168-0515	S-168-0525	G-168-05-1	G-168-05-5
0.3 mm	S-168-0305	S-168-0310	S-168-0315	S-168-0325	G-168-03-1	G-168-03-5
0.1 mm	---	P-168-0110	P-168-0115	---	---	---
75 μm	---	P-168-00710	P-168-00715	---	---	---

Custom dimensions are available upon request

PL-SAX/PL-SCX

PL-SAX | $\sim \text{N}(\text{CH}_3)_3^+$

Protein Binding Capacity of BSA:

PL-SAX 1000Å: 80 mg/mL
PL-SAX 4000Å: 35 mg/mL

- Macroporous polymer-based strong anion-exchange material
- Ideal for separation of nucleic acids and proteins

PL-SAX (Polymer Laboratories, UK) is a hydrophilic, strong anion exchange chromatographic packing material. The combination of the rigid macroporous PS/DVB polymer matrix and chemically stable quaternized PEI coating allows the analysis of biomolecules over a wide range of mobile phase conditions and pH. The media's physical stability allows it to be used with high eluent flow rates and high speed gradients for very rapid separations. This excellent stability ensures both rapid equilibration between separations and the ability to use aggressive clean-up procedures employing high salt, NaOH, mineral and organic acids, and a wide range of organic solvents.

ORDERING INFORMATION – HOTSEP® PL-SAX

5µ - 1000Å COLUMNS				LARGE MOLECULES (MW 5,000 – 500,000)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-171-1005	S-171-1010	S-171-1015	S-171-1025	G-171-10-1	G-171-10-5
0.5 mm	S-171-0505	S-171-0510	S-171-0515	S-171-0525	G-171-05-1	G-171-05-5
0.3 mm	S-171-0305	S-171-0310	S-171-0315	S-171-0325	G-171-03-1	G-171-03-5

5µ - 4000Å COLUMNS				VERY LARGE (MW 100,000+)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-172-1005	S-172-1010	S-172-1015	S-172-1025	G-172-10-1	G-172-10-5
0.5 mm	S-172-0505	S-172-0510	S-172-0515	S-172-0525	G-172-05-1	G-172-05-5
0.3 mm	S-172-0305	S-172-0310	S-172-0315	S-172-0325	G-172-03-1	G-172-03-5

PL-SCX | $\sim \text{SO}_3^-$

Protein Binding Capacity of Lysozyme:

PL-SAX 1000Å: 60 mg/mL
PL-SAX 4000Å: 30 mg/mL

- Macroporous polymer-based strong cation-exchange material
- Ideal for separation of nucleic acids and proteins

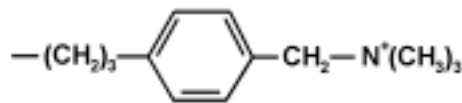
PL-SCX (Polymer Laboratories, UK) is a macroporous PS/DVB matrix, functionalized with a very hydrophilic strong cation exchange coating. This process is controlled to provide the optimum density of strong cation exchange moieties for the analysis, separation and purification of a wide range of biomolecules, from small peptides to large proteins.

ORDERING INFORMATION – HOTSEP® PL-SCX

5µ - 1000Å COLUMNS				LARGE MOLECULES (MW 5,000 – 500,000)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-173-1005	S-173-1010	S-173-1015	S-173-1025	G-173-10-1	G-173-10-5
0.5 mm	S-173-0505	S-173-0510	S-173-0515	S-173-0525	G-173-05-1	G-173-05-5
0.3 mm	S-173-0305	S-173-0310	S-173-0315	S-173-0325	G-173-03-1	G-173-03-5

5µ - 4000Å COLUMNS				VERY LARGE (MW 100,000+)		
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-174-1005	S-174-1010	S-174-1015	S-174-1025	G-174-10-1	G-174-10-5
0.5 mm	S-174-0505	S-174-0510	S-174-0515	S-174-0525	G-174-05-1	G-174-05-5
0.3 mm	S-174-0305	S-174-0310	S-174-0315	S-174-0325	G-174-03-1	G-174-03-5

Nucleosil[®] SAX



- Spherical silica-based strong anion-exchange material
- Capacity of about 1 meq/g

Nucleosil SAX is a strongly basic anion exchanger produced by quaternary ammonium modification of spherical silica. The carbon load is 10%, pore size is 100Å and the pH stability at 20°C is 2-8. The capacity is about 1 meq/g.

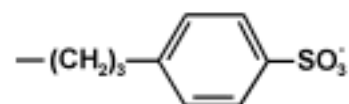
ORDERING INFORMATION – HOTSEP[®] NUCLEOSIL SAX

5μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-191-1005	S-191-1010	S-191-1015	S-191-1025	G-191-10-1	G-191-10-5
0.5 mm	S-191-0505	S-191-0510	S-191-0515	S-191-0525	G-191-05-1	G-191-05-5
0.3 mm	S-191-0305	S-191-0310	S-191-0315	S-191-0325	G-191-03-1	G-191-03-5

10μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-192-1005	S-192-1010	S-192-1015	S-192-1025	G-192-10-1	G-192-10-5
0.5 mm	S-192-0505	S-192-0510	S-192-0515	S-192-0525	G-192-05-1	G-192-05-5
0.3 mm	S-192-0305	S-192-0310	S-192-0315	S-192-0325	G-192-03-1	G-192-03-5

Nucleosil[®] SCX

USP COLUMN CLASSIFICATION: L9



- Spherical silica-based strong cation-exchange material
- Capacity of about 1 meq/g

Nucleosil SCX is a strongly acidic cation exchanger produced by sulphonic acid modification of spherical silica. The carbon load is 6.5%, pore size is 100Å and the pH stability at 20°C is 2-8. The capacity is about 1 meq/g.

ORDERING INFORMATION – HOTSEP[®] NUCLEOSIL SCX

5μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-195-1005	S-195-1010	S-195-1015	S-195-1025	G-195-10-1	G-195-10-5
0.5 mm	S-195-0505	S-195-0510	S-195-0515	S-195-0525	G-195-05-1	G-195-05-5
0.3 mm	S-195-0305	S-195-0310	S-195-0315	S-195-0325	G-195-03-1	G-195-03-5

10μ - 100Å COLUMNS		SMALL MOLECULES (MW 100 – 5,000)				
ID	5 cm	10 cm	15 cm	25 cm	Guard	Guard/5pk
1.0 mm	S-196-1005	S-196-1010	S-196-1015	S-196-1025	G-196-10-1	G-196-10-5
0.5 mm	S-196-0505	S-196-0510	S-196-0515	S-196-0525	G-196-05-1	G-196-05-5
0.3 mm	S-196-0305	S-196-0310	S-196-0315	S-196-0325	G-196-03-1	G-196-03-5

G&T Septech offers...

Custom Packed Columns

In addition to the range of micro-HPLC column dimensions previously listed, we also manufacture tailor-made columns that might be required for your particular application. For 0.1-0.5 mm ID columns, other column hardware materials such as PEEKsil are also available on request. Please contact your local distributor or us for further details about our custom packing service.

Batch Reservation Service

For challenging applications/validated methods we also offer a batch reservation service, which completely eliminates batch related reproducibility concerns. Based on your projected column usage, we will reserve the quantity of packing material you need and use it each time you order a new column.

Warranty

HotSep® micro-HPLC columns are warranted to be free from defects in materials or workmanship. G&T Septech AS will promptly replace any defective column unless such defects are attributed to customer abuse, misuse or neglect. Please contact your local distributor or us for further information.

Technical Support

For technical support, please contact your local distributor or G&T Septech AS.

Care and Use of HotSep[®] micro-HPLC columns

Installation and testing

Remove the column from its container and retain the container for storing the column when not in use. The flow direction during the column packing process is indicated on the column tag. Operate the column with the mobile phase flowing in this direction. Before connecting the column outlet to the detector, flush the column with mobile phase, this will prevent small particles, settled on the column frits during shipping and handling, from being washed into the detector. With high performance columns, significant efficiency will be lost if long lengths of large I.D. tubing are employed. For optimum performance, we recommend the following connecting tubing dimensions:

Column ID (mm)	Flow rate (μL/min)	Tubing ID (μm)
0.075 / 0.1 *	0.2 / 0.3	25
0.3	3	< 75
0.5	10	< 125
1.0	40	< 200

* nano-LC columns require a dedicated nano-injector even when using column-switching for sample loading (max. 0.1 mm bore).

Filters and guard columns can further reduce column performance if not properly selected and maintained. After connecting to the HPLC system, begin to pump an appropriate mobile phase to equilibrate the column. Enclosed along with your new HotSep[®] micro-HPLC column you will find a performance test chromatogram (Certificate of Analysis) generated on your column. The mobile phase used for the separation of the test mixture is the shipping solvent.

Care and maintenance

The following guidelines will be helpful for most columns prepared with rigid silica-based packings:

Pressure: The column backpressure depends on the packing material and the mobile phase used. With extended use a gradual increase in pressure is usually seen, however, a sudden increase in pressure signals a plugging problem that should be corrected (see column cleaning). However, do not exceed a maximum pressure of 500 bars on your column. Usually, this is not a relevant problem for most users, as most HPLC pumps have a maximum pressure below this limit. Exceptions are packings with pore size of 300Å and larger.

Temperature / column oven

The hardware of HotSep[®] micro-HPLC columns tolerates temperatures up to 150°C. However, at high temperatures bonded phases can be lost over time and a decrease in efficiency and peak symmetry might be observed due to dissolution of the silica particles. Column temperatures above 60°C are not recommended for silica-based particles. We recommend using a column oven to assure reproducible retention times.

Filters and guard columns

Column life is improved with in-line filters or guard columns. Contact our technical personnel for help in choosing guard protection.

Mobile phase solvents

All common HPLC grade organic solvents can be used with your HotSep[®] micro-HPLC column. Buffers made from acetate, formate, citrate and phosphate salts can be used up to 0.2 M without adverse effects. As long as the appropriate pH range is not exceeded, organic modifiers and ion pair reagents can be used. However, some ion-pair reagents could be difficult to flush from the column, and columns used with these reagents should be dedicated to the particular analysis involved. Limit the use of strong bases, and avoid strong acids. Do not mix solutions that might precipitate or gel in the column or in the system. The pH range for your column should usually not exceed 1.5-9.5.

Column lifetime

The lifetime of your HotSep[®] micro-HPLC column is highly dependent on the sample and the employed conditions and cannot be generalized. Maximize column lifetime by making sure that samples and mobile phases are clean and particle free, and by using a guard column and/or filters.

Column storage

When storing your HotSep[®] micro-HPLC column, flush it with acetonitrile/methanol after cleaning and seal it. Do not store columns containing buffers, salt solutions, acidic mobile phases or tetrahydrofuran.

HotSep[®] Tracy

- trace-enrichment columns for column-switching applications in micro-HPLC

- Trace-enrichment
- Sample clean-up
- Desalting

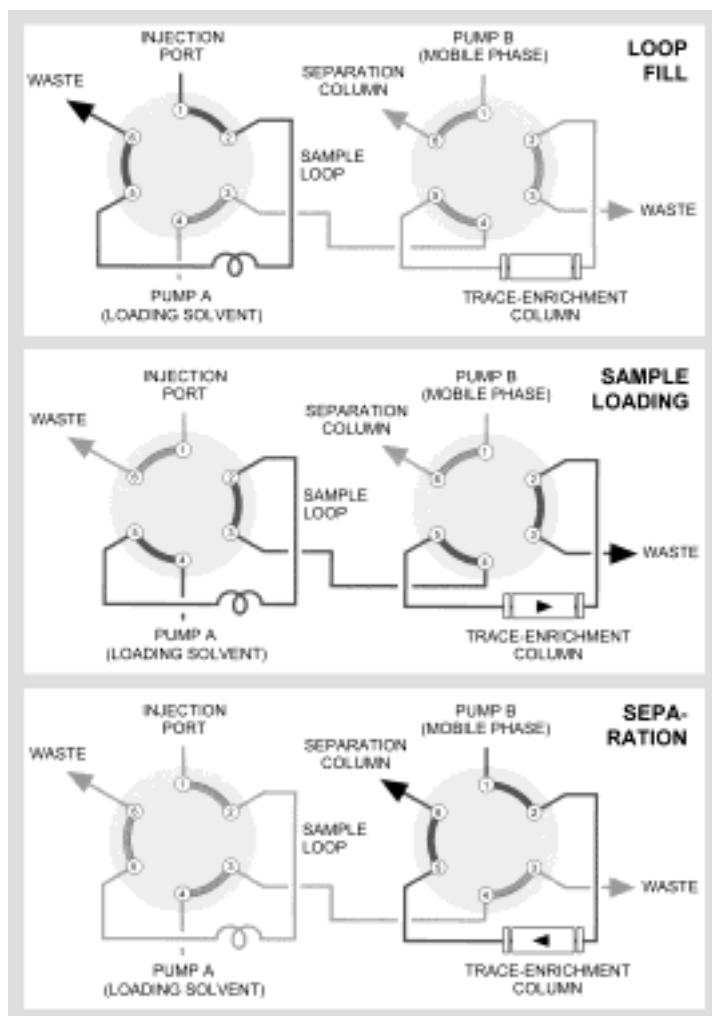


The HotSep[®] Tracy columns are highly suited for column-switching applications, particularly for concentration of low-abundant analyte(s) or clean-up/desalting of biological samples. The short

column length allows higher flow rate compared with the micro-HPLC column to reduce turnover times between injections.

Column-switching example I:

Typical set-up for performing large volume injection and sample clean-up/desalting in micro-HPLC



Application example:

Quantification of drug metabolites in blood plasma

Trace-enrichment column:
HotSep[®] Tracy
5 μ Kromasil C8 100Å
(0.3 x 5 mm)

Separation column:
5 μ HotSep[®] Kromasil C18 100Å
(0.3 x 150 mm)

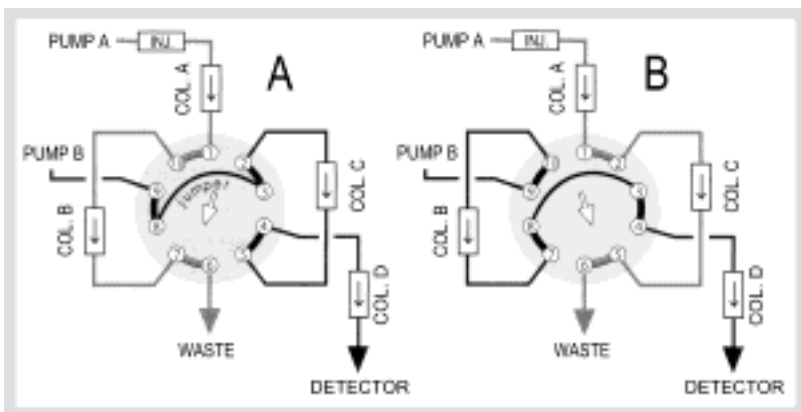
With autosampler:

When using automated injection, the left valve is replaced by the autosampler.

MICRO-HPLC TRACE-ENRICHMENT COLUMNS

Column-switching example II:

2D set-up with two trap columns for collection of next fraction (from 1st dim), while the previous fraction is separated in the 2nd dimension.



Typical application:

2D micro-HPLC set-up for separation of complex peptide mixtures

Col. A:
strong cation-exchanger, e.g.
5µ HotSep® PL-SCX 300Å
(1 x 150 mm)

Col. B = Col. C:
reversed-phase trap column, e.g.
HotSep® Tracy
5µ Kromasil C8 300Å
(1 x 5 mm)

Col. D:
reversed-phase column, e.g.
3µ HotSep ProntoSIL C18 300Å
(0.3 x 150 mm)

ORDERING INFORMATION – HOTSEP® TRACY

When ordering a trace-enrichment column, please replace X with the appropriate material code:

COLUMN ID	Part No.	
	1pk	5pk
1.0 mm	T-X-10-1	T-X-10-5
0.5 mm	T-X-05-1	T-X-05-5
0.3 mm	T-X-03-1	T-X-03-5

Example: 5pk of 3.5µ Kromasil C18 100Å (0.5 mm ID)

⇒ Part No: **T-111-05-5**

Popular Packing Materials:

3.5µ Kromasil C18 100Å	X = 111	3µ PLRP-S 100Å	X = 161
3.5µ Kromasil C8 100Å	X = 112	3µ PLRP-S 300Å	X = 162
5µ Kromasil C18 100Å	X = 121	5µ PLRP-S 100Å	X = 165
5µ Kromasil C8 100Å	X = 122	5µ PLRP-S 300Å	X = 166
5µ Kromasil C18 300Å	X = 128	5µ PLRP-S 1000Å	X = 167
		5µ PLRP-S 4000Å	X = 168
10µ Kromasil C18 100Å	X = 181		
		5µ Nucleosil SAX 100Å	X = 191
3µ ProntoSIL C18 100Å	X = 132	10µ Nucleosil SAX 100Å	X = 192
3µ ProntoSIL C18 300Å	X = 131	5µ Nucleosil SCX 100Å	X = 195
		10µ Nucleosil SCX 100Å	X = 196
3µ Hypersil ODS 100Å	X = 151		

See p. 51 or www.gtseptech.no for updated packing material list

HotSep[®] Protector

- Cost-effective protection of any micro-HPLC column
- < 10 % loss in column efficiency guaranteed
- Very low dead volume



The HotSep[®] Protector micro-HPLC guard columns prolong the lifetime of any micro-HPLC column. When placed between the injector and the column, the 5 mm long micro-guard columns will trap particles and adsorb impurities present in the mobile phase or sample, while the practically

dead volume-free design guarantees minimal dispersion and maintains high column efficiency. Periodical replacement of inexpensive guard columns provides optimal and cost-effective protection of your micro-HPLC columns.

ORDERING INFORMATION – HOTSEP[®] PROTECTOR

GUARD COLUMN ID	Part No.	
	1pk	5pk
1.0 mm	G-X-10-1	G-X-10-5
0.5 mm	G-X-05-1	G-X-05-5
0.3 mm	G-X-03-1	G-X-03-5

Example: 5pk of 0.5 mm ID 3.5 μ m Kromasil C18 100Å guard columns

⇒ Part No: **G-111-05-5**

Column Coupler

This short and elegant fingertight column coupler made of PEEK is ideal for connecting a HotSep[®] Protector guard column in front of a micro-HPLC column. Choose between three different dimensions, each optimized to introduce a minimum of dead volume to the system. The flexible end fittings (1/16") make it easy to obtain dead-volume free connections.



ORDERING INFORMATION – COLUMN COUPLER

GUARD COLUMN ID	ID (μ m)	Dead Volume (μ L)	Part No.
1.0 mm	175	0.93	G-101-10
0.5 mm	100	0.31	G-101-05
0.3 mm	65	0.13	G-101-03

Packing Material Availability

COMPLETE LIST

Eka Chemicals		Thermo Hypersil-Keystone	
3.5µ Kromasil C18 100Å	X = 111	3µ Hypersil ODS 100Å	X = 151
3.5µ Kromasil C8 100Å	X = 112		
3.5µ Kromasil C4 100Å	X = 114	MacHerey-Nagel	
3.5µ Kromasil Amino 100Å	X = 113	5µ Nucleosil SAX 100Å	X = 191
3.5µ Kromasil Silica 100Å	X = 117	10µ Nucleosil SAX 100Å	X = 192
		5µ Nucleosil SCX 100Å	X = 195
5µ Kromasil C18 100Å	X = 121	10µ Nucleosil SCX 100Å	X = 196
5µ Kromasil C18 300Å	X = 128		
5µ Kromasil C8 100Å	X = 122	Polymer Laboratories	
5µ Kromasil C8 300Å	X = 129	3µ PLRP-S 100Å	X = 161
5µ Kromasil C4 100Å	X = 120	3µ PLRP-S 300Å	X = 162
5µ Kromasil C4 300Å	X = 130	5µ PLRP-S 100Å	X = 165
5µ Kromasil Phenyl 100Å	X = 182	5µ PLRP-S 300Å	X = 166
5µ Kromasil Amino 100Å	X = 123	5µ PLRP-S 1000Å	X = 167
5µ Kromasil Cyano 60Å	X = 124	5µ PLRP-S 4000Å	X = 168
5µ Kromasil CHI-TMB 100Å	X = 125		
5µ Kromasil CHI-TBB 100Å	X = 126	5µ PL-SAX 1000Å	X = 171
5µ Kromasil Silica 100Å	X = 127	5µ PL-SAX 4000Å	X = 172
		5µ PL-SCX 1000Å	X = 173
10µ Kromasil C18 100Å	X = 181	5µ PL-SCX 4000Å	X = 174
10µ Kromasil C8 100Å	X = 182		
10µ Kromasil C4 100Å	X = 183	ZirCrom Separations	
Bischoff Chromatography		3µ ZirChrom-PBD 300Å	X = 141
3µ ProntoSIL C18 100Å	X = 132	3µ ZirChrom-DB 300Å	X = 142
3µ ProntoSIL C18 300Å	X = 131	3µ ZirChrom-CARB 300Å	X = 143
		3µ ZirChrom-MS 300Å	X = 144

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