Chromatography

MACHEREY-NAGEL

Columns and supplies

MACHEREY-NAGEL
www.mn-net.com

Contents

Basics

High performance liquid chromatography (HPLC) is part of liquid chromatographic separating processes of substance mixtures and their analysis. At the beginning the technique was also called high pressure liquid chromatography due to the high back pressure of the column. HPLC offers qualitative (identification of substances) and quantitative (concentration determination) analysis by comparison with standard substances. The term HPLC was introduced in the 1970s, for the delineation of the high-performance method to the in the 1930s developed column liquid chromatography (column chromatography). At the beginning of the 21st century the HPLC was complemented by the even more efficient UHPLC (ultra high performance liquid chromatography). Hereby even higher pressures (> 400 bar) result in shorter analysis time and enhanced efficiency enabling a higher sample throughput with smaller sample volumes.

Application

HPLC / UHPLC is used additionally to gas chromatography (GC) for separation and determination of complex substance mixtures composed of low-volatile, polar and ionic, high-molecular or thermal instable substances. Therefore a sufficient solubility of the sample in a solvent or a solvent mixture is required. HPLC / UHPLC is used for purity control of chemicals and industrial products, determination of active agents for drug development, production and testing, environmental analytics, quality and purity control of foods, analysis of ingredients in cosmetics as well as for the isolation of biopolymers.

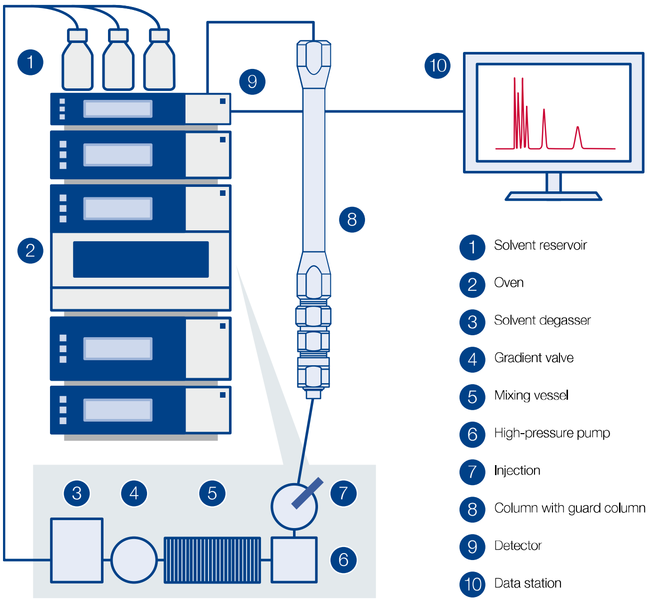
Basic principle

In liquid column chromatography a mobile phase (eluent) flows through a particle filled tube (separation column, stationary phase). In classic column chromatography this tube is a glass column with an inner diameter of several centimeters and a length up to 450 mm or even bigger. The filling material typically consists of coarse-grained particles like silica gel 60. The eluent is transported through the separation column either by hydrostatic pressure or a low-pressure pump with 1.5-2 bar.

In contrast HPLC columns consist of stainless steel with an inner diameter of 2-4.6 mm and a length of 20-300 mm. The column packing, mostly modified porous silica, has generally a particle size of 3, 5, 7 or 10 µm and a pore size of 50, 100, 120 (for low-molecular analytes) or 300-4000 Å (for high-molecular analytes). In UHPLC shorter columns in the range of 20-150 mm length with highly efficient particles of 1.8 µm size (sub-2 µm) are utilized. A guard column of a few millimeters length can be utilized and installed with a specific Column Protection System to increase the column lifetime. HPLC / UHPLC uses a high-pressure pump to transport the eluent from a storage vessel into the system with a column back pressure of up to 600 / 1200 bar.

Instrument

HPLC as well as UHPLC instruments have different building blocks. The storage vessel (eluent reservoir, 1) usually contains a deaerator unit (3) for the solvents. Followed by a gradient valve (4) with mixing chamber (5) in flow direction, which allows the usage of isocratic as well as gradient methods. A high-pressure pump (6) transports the sample into the system. The sample is injected via an injection valve (7). Usually this is operated automatically with a syringe by an autosampler. With the eluent flow the sample is transported to the guard and the separating column (8). For better reproducibility of the separation tempering with a column oven (2) should be performed. The separated substances are determined with a detector (9). In the resulting chromatogram each detector signal of a substance (peak), is related to the retention time of the column. With the data evaluation (10) these peaks can be identified and their concentration can be determined.



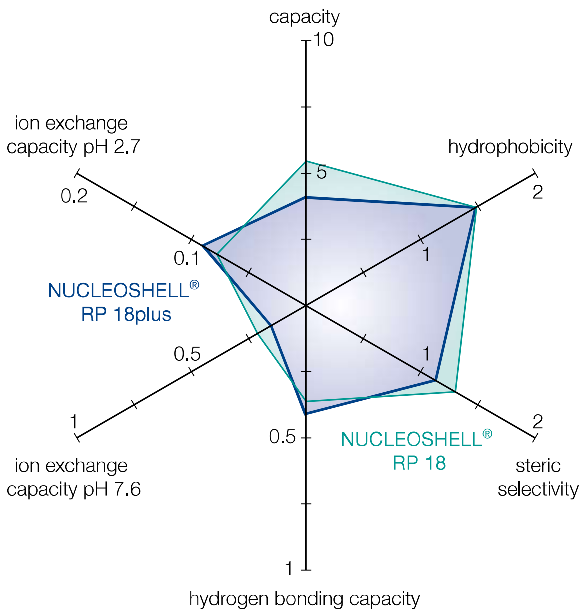
Separation mechanism

While flowing through the column each component of the solved mixture interacts differently with the stationary phase. According to the characteristics of the substance (hydrophobic, polar, ionic, aromatic, sterically hindered etc.) the strength of the interactions vary and thus the compounds are retained by the stationary phase in different ways. Essentially a distinction is drawn between normal phase (NP), reversed phase (RP) and ion exchange chromatography. Depending on the structure of the stationary phase diverse interactions e.g., van der Waals forces or π-π-stacking can occur and different polar mobile phases are required. For polar stationary normal phases (e.g., SiOH, CN, OH, NH_2) non-polar eluents like *n*-heptane, hexane, dichloromethane or 2-propanol are applicable. While for reversed phases (e.g., C_{18}, C_8, C_4, C_2, C_6H_5) typically polar RP eluents (e.g., acetonitrile or methanol with ultrapure water or buffer) and for ion exchange (e.g., SA, SB) aqueous buffers (e.g., phosphate, acetate, citric buffer) come to use.

Selectivity

The characteristic separation behavior of phases under certain conditions is also called selectivity. This is dependent on different parameters like structure and modifications of the base silica gel, nature of the chemical binding or the type of endcapping.

In recent decades several methods have been developed to compare and distinguish the selectivity of various silica gels and their modifications. In this connection defined substances or substance classes are analyzed and the chromatographic parameters are graphically presented. A frequently applied model in specialist literature is e.g., the TANAKA plot, which allows a quick comparison of different HPLC phases. [4]

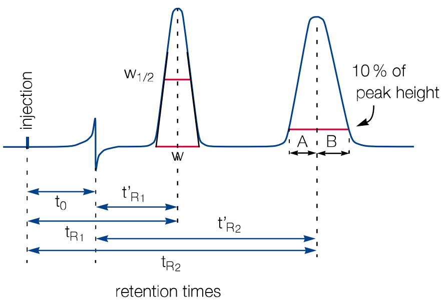


Parameter of the Tanaka diagram:
Capacity = k' (pentylbenzene)
Hydrophobicity = α (pentylbenzene, butylbenzene)
Steric selectivity = α (triphenyl, *o*-terphenyl)
Hydrogen bonding capacity (capacity of silanol) = α (caffeine, phenol)
Ion exchange capacity at pH 2.7 = α (benzylamine, phenol)
Ion exchange capacity at pH 7.6 = α (benzylamine, phenol)

The comparison of NUCLEOSHELL® RP 18 and NUCLEOSHELL RP® 18plus for example shows a lower ion exchange capacity at pH 7.6 for the monomeric NUCLEOSHELL® RP 18plus. The radar chart also reflects a more pronounced steric selectivity of NUCLEOSHELL® RP 18 due to a higher density of modifications with C_{18} chains.

Characteristic parameters

The success of a chromatographic separation depends apart from the stationary and mobile phase also on other characteristics like the quality of the separating column or the linear flow rate. The following schematic chromatogram illustrates the most important parameters which characterize a separation.



Schematic chromatogram

Peak width:	
$w_{1/2}$	peak width at half height
w	peak width of the peak (intersection point of the inflectional tangents with the zero line)
Peak symmetry:	
A	peak front to peak maximum at 10 % of peak height
B	peak maximum to peak end at 10 % of peak height
Retention time::	
t_0	dead time of a column = retention time of a non-retarded substance
t_{R1}, t_{R2}	retention times of components 1 and 2
t'_{R1}, t'_{R2}	net retention times of components 1 and 2

In a chromatographic system the substances differ from each other in their retention time in or on the stationary phase. The time, which is needed by a sample component to migrate from column inlet (sample injection) to the column end (detector) is the retention time t_{R1} or t_{R2}. The dead time t_0 is the time required by an inert compound to migrate from column inlet to column end without any retardation by the stationary phase. Consequently, the dead time is identical with the retention time of the sample component remaining in the stationary phase. The difference of total retention time and dead time yields the net retention time t'_{R1} or t'_{R2}, which is the time a sample component remains in the stationary phase.

$$t'_{R1} = t_{R1} - t_0 \quad \text{bzw.} \quad t'_{R2} = t_{R2} - t_0$$

To compare chromatograms that are recorded with columns of different lengths and internal diameters, as well as different flow rates, the retention time is converted into a dimensionless capacity factor k'.

$$k'_1 = \frac{t_{R1} - t_0}{t_0} \qquad \text{bzw.} \qquad k'_2 = \frac{t_{R2} - t_0}{t_0}$$

The relative retention α, also known as the separation factor, describes the ability of a chromatographic system (stationary and mobile phase) to distinguish between two compounds. This is calculated from the rate of the capacity factors of the substances, where the figure in the denominator is the reference compound.

$$\alpha = \frac{k'_2}{k'_1}$$

The resolution R is a measure for the efficiency of the column to separate two substances. Besides the retention time t_R the peak width at half height $w_{1/2}$ is also included.

$$R = 1.18 \cdot \frac{t_{R2} - t_{R1}}{(w_{1/2})_2 + (w_{1/2})_1}$$

For practical reasons the peak symmetry is calculated at 10 % of peak height. Ideally symmetry should be 1, i.e. A = B. Values > 1 indicate peak tailing, while values < 1 indicate peak fronting.

$$\text{Peak symmetry} = \frac{B}{A}$$

Instead of the mobile phase volumetric flow rate [mL/min], which is controlled at the HPLC instrument, it is advantageous to use the linear velocity u [cm/sec]. The linear velocity is independent of the column cross section and proportional to the pressure drop in the column. The linear velocity can be calculated by means of the dead time, where L is the column length in cm and t_0 the dead time in sec.

$$u = \frac{L}{t_0}$$

The quality of a column packing is determined through the number of theoretical plates N. High N values indicate a high capability to separate complex sample mixtures.

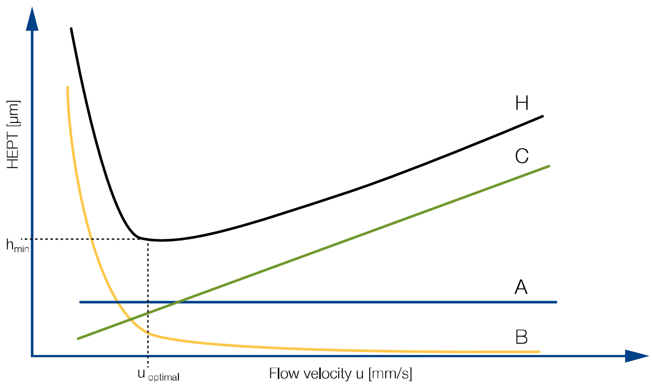
$$N = 5.54 \cdot \left(\frac{t_{R1}}{w_{1/2}}\right)^2$$

The value of the height equivalent to a theoretical plate HEPT is a criterion for the quality of a column. HEPT, is the length, in which the chromatographic equilibrium between mobile and stationary phase has been adjusted once. Its value depends on the particle size, the flow velocity, the mobile phase viscosity and especially on the packing quality. Small HEPT values, meaning a large number of theoretical plates N, facilitate the column to separate complex sample mixtures.

$$H = \frac{L}{N}$$

The Van Deemter equation shows the dependence of the HEPT on the velocity u.

$$H = A + \frac{B}{u} + C \cdot u$$



A term = eddy-diffusion, B term = longitudinal diffusion coefficient, C term = mass transfer coefficient, H = HEPT = height equivalent to a theoretical plate

The A term, also called eddy-diffusion, is a function of the particle size, the B term a function of the diffusion coefficient of the substance in the mobile phase and the C term the retardation of a substance by the interface between stationary and mobile phase. In the point of intersection of h_{min} and u_{opt} the optimal separation efficiency for a column with high peak symmetry for the separated substances is obtained.

Column quality

Each HPLC / UHPLC column of MACHEREY-NAGEL is individually tested according to the most important characteristic parameters in quality control and the results are documented in a certificate of analysis.

Detailed information of the particular properties of the high-purity silica phases NUCLEODUR®, of the established standard silica NUCLEOSIL® and the modern Core-Shell material NUCLEOSHELL® as well as phases for special separations and the equivalent HPLC- and UHPLC-columns can be found on the following pages.

Strict quality specifications for outstanding reliability

- Highest production standard
  our facilities are EN ISO 9001:2008 certified
- Perfect reproducibility from batch to batch and within each lot
- Each column is individually tested and supplied with test chromatogram and test conditions.

Test mixture* for reversed phase columns
in acetonitrile, pack of 1 mL
REF 722394

MACHEREY-NAGEL
MN
EN ISO 9001:2008
CERTIFIED

Furthermore custom-packed columns with different column types, dimensions and particle sizes are available on request.

* This product (REF 722394) contains harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

USP listing

USP specification of MN HPLC phases

Code	Specification	MN HPLC Phases	Page
USP L1	octadecyl silane chemically bonded to porous silica particles 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® C_{18} ec	181
		NUCLEODUR® C_{18} Gravity	158
		NUCLEODUR® C_{18} Gravity-SB	162
		NUCLEODUR® C_{18} HTec	178
		NUCLEODUR® C_{18} Isis	164
		NUCLEODUR® C_{18} PAH	227
		NUCLEODUR® C_{18} Pyramid	166
		NUCLEODUR® PolarTec	168
		NUCLEODUR® Sphinx RP	176
		NUCLEOSHELL® RP 18	200
		NUCLEOSHELL® RP 18plus	202
		NUCLEOSIL® C_{18}	214
		NUCLEOSIL® C_{18} AB	214
		NUCLEOSIL® C_{18} HD	214
		NUCLEOSIL® C_{18} MPN	243
		NUCLEOSIL® C_{18} PAH	229
		NUCLEOSIL® C_{18} PPN	244
USP L3	porous silica particles, 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® SiOH	190
		NUCLEOSIL® SiOH	224
USP L7	octyl silane chemically bonded to totally porous silica particles, 1.8 to 10 µm diameter	NUCLEODUR® C_8 ec	181
		NUCLEODUR® C_8 Gravity	158
		NUCLEOSIL® C_8	217
		NUCLEOSIL® C_8 HD	217
USP L8	an essentially monomolecular layer of aminopropyl silane chemically bonded to totally porous silica gel support, 1.5 to 10 µm diameter	NUCLEODUR® NH_2 / NH_2-RP	188
		NUCLEOSIL® Carbohydrate	246
		NUCLEOSIL® NH_2 / NH_2-RP	221
USP L9	irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm diameter	NUCLEOSIL® SA	223
USP L10	nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® CN / CN-RP	186
		NUCLEOSIL® CN / CN-RP	222

USP specification of MN HPLC phases

Code	Specification	MN HPLC Phases	Page
USP L11	phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® Phenyl-Hexyl	170
		NUCLEODUR® π^2	172
		NUCLEOSHELL® Phenyl-Hexyl	204
		NUCLEODUR® Sphinx RP	176
		NUCLEOSIL® C_6H_5	220
USP L14	silica gel having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm diameter	NUCLEOSIL® SB	223
USP L16	dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm diameter	NUCLEOSIL® C_2	219
USP L17	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the H form, 6 to 12 µm diameter	NUCLEOGEL® ION 300 OA	248
		NUCLEOGEL® SUGAR 810 H	247
USP L19	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the Ca form, 5 to 15 µm particle size	NUCLEOGEL® SUGAR 810 Ca	247
		NUCLEOGEL® SUGAR Ca	248
USP L20	dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm diameter	NUCLEOSIL® OH (Diol)	220
USP L21	a rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm diameter	NUCLEOGEL® RP	245
USP L22	a cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size	NUCLEOGEL® SCX	240
USP L23	an anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, about 10 µm in size	NUCLEOGEL® SAX	240
USP L26	butyl silane chemically bonded to totally porous silica particles, 5 to 10 µm diameter	NUCLEODUR® C_4 ec	241
		NUCLEOSIL® C_4	219
		NUCLEOSIL® C_4 MPN	243
USP L32	a chiral ligand-exchange resin packing · L-proline copper complex covalently bonded to irregular shaped silica particles, 5 to 10 µm diameter	NUCLEOSIL® CHIRAL-1	235
USP L34	strong cation-exchange resin consisting of sulfonated cross-linked PS-DVB copolymer in the Pb form, 5 to 7 µm particle size	NUCLEOGEL® SUGAR Pb	248
USP L36	a 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5 µm aminopropyl silica	NUCLEOSIL® CHIRAL-3	236
USP L40	cellulose tris-(3,5-dimethylphenylcarbamate) coated porous silica particles, 5 to 20 µm diameter	NUCLEOCEL *DELTA*	233
USP L43	pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm diameter	NUCLEODUR® PFP	174
		NUCLEOSHELL® PFP	206
USP L45	beta-cyclodextrin bonded to porous silica particles, R,S-hydroxypropyl ether derivative, 3 to 10 µm diameter	NUCLEODEX β-OH, β-PM	231
USP L58	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the Na form, 6 to 30 µm diameter	NUCLEOGEL® SUGAR Na	248
USP L60	spherical porous silica gel, particle size of 10 µm diameter or smaller, the surface of which has been covalently modified with alkyl amide groups and endcapped	NUCLEODUR® PolarTec	168
		NUCLEOSIL® C_{18} Nautilus	214
USP L75	A chiral-recognition protein, bovine serum albumin (BSA), chemically bonded to silica particles, about 7 µm in diameter, with a pore size of 300 Angstrom	RESOLVOSIL BSA-7	234

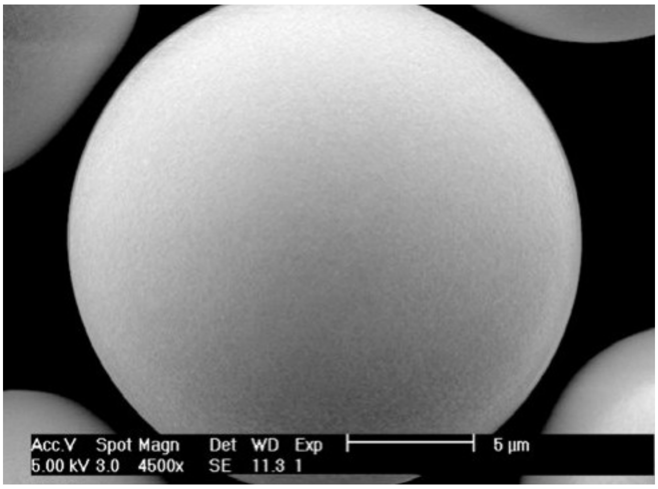
NUCLEODUR® high purity silica for HPLC

NUCLEODUR® is a fully synthetical type B silica (silica of 3rd generation) offering highly advanced physical properties like totally spherical particle shape, outstanding surface microstructure, high pressure stability and low metal content.

NUCLEODUR® as a state-of-the-art silica is the ideal base material for modern HPLC phases. It is the result of MACHEREY-NAGEL's pioneering research in chromatography for more than 40 years.

In RP liquid chromatography the efficiency of the packing is strongly affected by the quality of the base silica itself. Shortcomings in the surface geometry of the particles or metal contaminants are the main reasons for inadequate coverage with the covalently bonded alkylsilanes in the subsequent derivatization steps. It is well known, that poor surface coverage and, in consequence, high activity of residual free silanols often results in peak tailing or adsorption, particularly with basic compounds.

Particle shape and surface symmetry



NUCLEODUR® silicas are synthesized in a unique and carefully controlled manufacturing process which provides silica particles, which are totally spherical. The picture shows the outstanding smoothness of the NUCLEODUR® surface.

Purity

As already mentioned above, a highly pure silica is required for achieving symmetric peak shapes and maximum resolution. Inclusions of, e.g., iron or alkaline earth metal ions on the silica surface are largely responsible for the unwanted interactions with ionizable analytes, e.g., amines or phenolic compounds.

NUCLEODUR® is virtually free of metal impurities and low acidic surface silanols. Elemental analysis data of NUCLEODUR® 5 µm measured by AAS are listed below.

Elementary analysis (metal ions) of NUCLEODUR® 100-5		
Aluminum	< 5	ppm
Iron	< 5	ppm
Sodium	< 5	ppm
Calcium	< 10	ppm
Titanium	< 1	ppm
Zirconium	< 1	ppm
Arsenic	< 0.5	ppm
Mercury	< 0.05	ppm

Pressure stability

The totally spherical and 100 % synthetic silica gel exhibits an outstanding mechanical stability, even at high pressures and elevated eluent flow rates. In addition, after several cycles of repeated packing, no significant drop in pressure can be observed. The latter is of prime importance for preparative and process-scale applications.

NUCLEODUR® silica is available with two pore sizes – 110 Å pore size as standard material and as 300 Å widepore material for the separation of biomolecules, like peptides and proteins.

Physical data of NUCLEODUR®		
	Standard	Widepore
Pore size	110	300 Å
Surface area (BET)	340 m^2/g	100 m^2/g
Pore volume	0.9 mL/g	0.9 mL/g
Density	0.47 g/mL	0.47 g/mL

NUCLEODUR® modifications

Several different surface modifications based on NUCLEODUR® silica have been developed over the last years providing a full range of specified HPLC phases and an ideal tool for every separation.

For a summary of important properties of our NUCLEODUR® phases please see page 152.

1.8 µm particles for increased separation efficiency

Key feature

- Decrease of analysis time (ultra fast HPLC)
- Shorter columns with high separation efficiency and significant improvement of resolution and detection sensitivity
- Suitable for LC/MS due to low bleeding characteristics

Fractionation

- NUCLEODUR® 1.8 µm particles are fractionated to limit the increase in back pressure.

Availability

- The following NUCLEODUR® phases are available in 1.8 µm:
  C_{18} Gravity, C_8 Gravity, C_{18} Gravity-SB, C_{18} Isis, C_{18} Pyramid, PolarTec, Phenyl-Hexyl, PFP, Sphinx RP, C_{18} HTec and HILIC

Advantages of 1.8 µm particle size

Miniaturization started in the early stage of HPLC with the reduction of particle size from 10 µm via 7 µm to standard 5 µm – still the most used particle diameter in analytical HPLC – to 3 µm spherical particles. With the introduction of 1.8 µm NUCLEODUR® particles researchers have turned over a new leaf in HPLC column technology, featuring extraordinary improvements in terms of plate numbers, column efficiency and resolution compared with 3 µm particles.

Increased separation efficiency by higher number of theoretical plates (N):

- 50 x 4.6 mm NUCLEODUR® C_{18} Gravity
- 3 µm: N ≥ 100 000 plates/m (h-values≤ 10)
- 1.8 µm: N ≥ 166 667 plates/m (h-values≤ 6)

Increase of the plate number by ~ 67 % offers the possibility of using shorter columns with equal plate number resulting in a decrease of analysis time.

Significant improvement in resolution

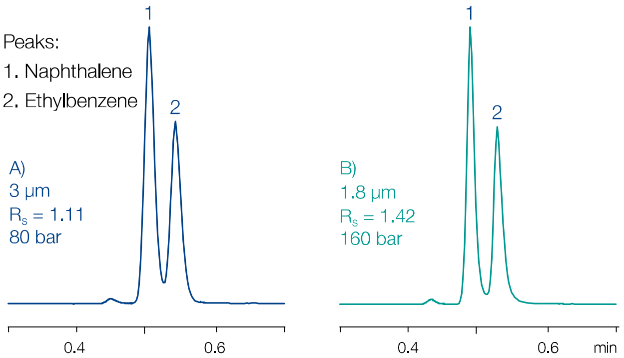
$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_i}{k'_i + 1}\right)$$

R_s = resolution, α = selectivity (separation factor), k_i' = retention
N = plate number with N ∝ $1/d_P$, d_P = particle diameter

Resolution as a function of particle size

Column: 50 x 4 mm NUCLEODUR® C_{18} Gravity
A) 3 µm, B) 1.8 µm
Eluent: acetonitrile – water (80:20, v/v)
Flow rate: 2 mL/min
Detection: UV, 254 nm

Peaks:
1. Naphthalene
2. Ethylbenzene



Use of 1.8 µm instead of 3 µm particles leads to an increase of resolution by a factor of 1.29 (29 %) since the resolution is inversely proportional to the square root of the particle size.

Column back pressure

Due to the smaller particles the back pressure will increase according to

$$\Delta_P = \frac{\Phi \cdot L_C \cdot \eta \cdot u}{d_P^2}$$

Δ_P = pressure drop, Φ = flow resistance (nondimensional), LC = column length, η = viscosity, u = linear velocity, d_P = particle diameter

The high sphericity of the NUCLEODUR® particles and the very narrow particle size distribution allow to keep the back pressure on a moderate level.

Comparison of back pressures

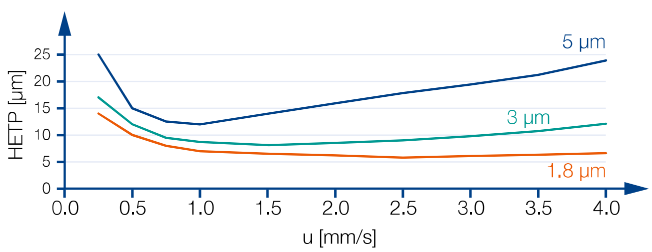
Eluent 100 % methanol, flow rate 1.5 mL/min
temperature 22 °C, column dimensions 50 x 4.6 mm

	NUCLEODUR® C_{18} Gravity	Competitor
3 µm	70 bar	–
1.8 µm	130 bar	170 bar

Higher flow rates and shorter run times

The optimal flow rate for 1.8 µm particles is higher than for 3 and 5 µm particles (see figure – the flow rate should be at the van Deemter minimum).

Van Deemter curves



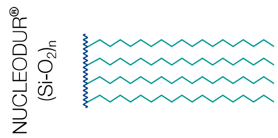
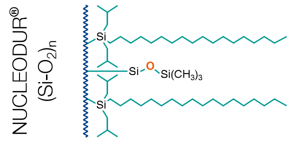
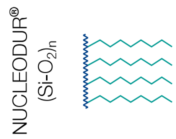
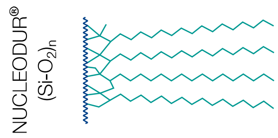
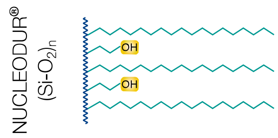
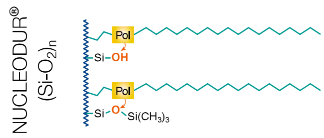
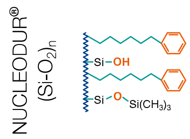
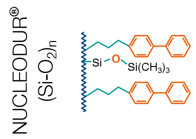
Column 50 x 4.6 mm, acetonitrile – water (50:50, v/v), analyte toluene

Technical requirements

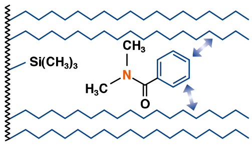
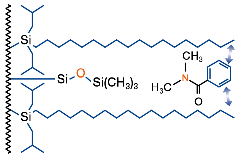
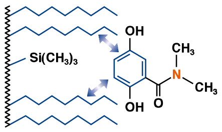
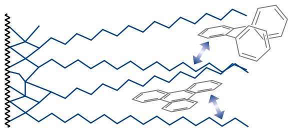
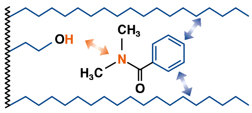
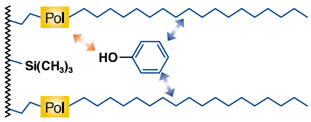
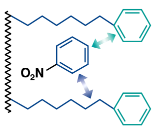
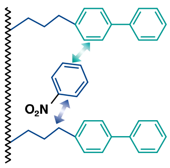
To gain best results with 1.8 µm particles certain technical demands must be met including pumps for flow rates of 2–3 mL with pressures of 250–1000 bar, minimized dead volume, and fast data recording.

NUCLEODUR® phase overview

Overview of NUCLEODUR® HPLC phases

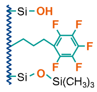
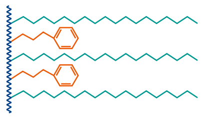
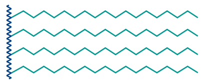
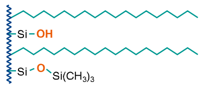
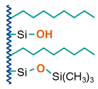
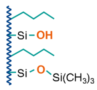
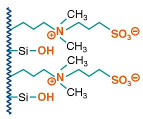
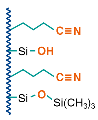
Phase	Specification	Page	Characteristic*	Stability	Structure
C_{18} Gravity	octadecyl, high density coating, multi-endcapping 18 % C · USP L1	158	A ●●●●● B ◖ C ●●◖	pH 1–11, suitable for LC/MS	
C_{18} Gravity-SB	octadecyl (monomeric), extensive endcapping 13 % C · USP L1	162	A ●●●● B ●●◖ C -	pH 1–9, suitable for LC/MS	
C_8 Gravity	octyl, high density coating, multi-endcapping 11 % C · USP L7	158	A ●●◖ B ● C ●◖	pH 1–11, suitable for LC/MS	
C_{18} Isis	octadecyl phase with specially crosslinked surface modification endcapping 20 % C · USP L1	164	A ●●●●◖ B ●● C ●●●●●	pH 1–10, suitable for LC/MS	
C_{18} Pyramid	octadecyl with polar endcapping 14 % C · USP L1	166	A ●●●● B ●●◖ C ●●	stable in 100 % aqueous eluent, pH 1–9, suitable for LC/MS	
PolarTec	octadecyl with embedded polar group 17 % C · USP L1 and L60	168	A ●●●● B ●●◖ C ●●●●	stable in 100 % aqueous eluent, pH 1–9, suitable for LC/MS	
Phenyl-Hexyl	phenylhexyl, multi-endcapping 10 % C · USP L11	170	A ●● B ●●● C ●	pH 1–10, suitable for LC/MS	
π^2	biphenylpropyl, multi-endcapping 17 % C · USP L11	172	A ●● B ●●●● C ●●●	pH 1.5–10	

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity

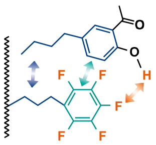
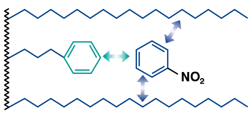
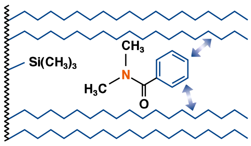
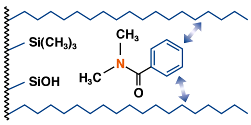
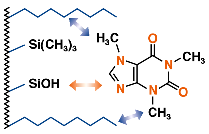
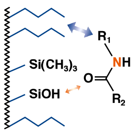
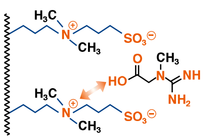
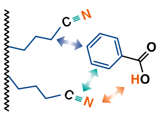
Application	Similar phases**	Interactions · retention mechanism	
in general compounds with ionizable functional groups such as basic pharmaceuticals and pesticides	NUCLEOSIL® C_{18} HD Xterra® RP18 / MS C18; Luna® C18(2), Gemini®, Synergi® Max RP; Zorbax® Extend-C18; Inertsil® ODS III; Purospher® STAR RP-18; Hypersil™ BDS	hydrophobic (van der Waals interactions)	
overall sophisticated analytical separations, especially for polar compounds, e.g., antibiotics, water-soluble vitamins, organic acids	–	hydrophobic (van der Waals interactions) with additional polar interactions	
like C_{18} Gravity, however, generally shorter retention times for nonpolar compounds	NUCLEOSIL® C_8 HD Xterra® RP8 / MS C8; Luna® C8; Zorbax® Eclipse XDB-C8	hydrophobic (van der Waals interactions)	
high steric selectivity, thus suited for separation of positional and structural isomers, planar / nonplanar molecules	NUCLEOSIL® C_{18} AB Inertsil® ODS-P; Pro C18 RS	steric and hydrophobic	
basic pharmaceuticals, very polar compounds, organic acids	Aqua, Synergi® Hydro-RP; AQ; Atlantis® dC18; Polaris® C18-A	hydrophobic and polar (H bonds)	
basic pharmaceuticals, organic acids, pesticides, amino acids, water-soluble vitamins	NUCLEOSIL® C_{18} Nautilus ProntoSIL® C18 AQ, Zorbax® Bonus-RP, Polaris® Amide-C18; Ascentis® RP Amide, SymmetryShield™ RP18; SUPELCOSIL™ LC-ABZ+; HyPURITY™ ADVANCE; ACCLAIM Polar AD.II	hydrophobic and polar (H bonds)	
aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics	Luna® Phenyl-Hexyl; Zorbax® Eclipse Plus Phenyl-Hexyl; Kromasil® Phenyl-Hexyl	π-π and hydrophobic	
aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics	Pinnacle® DB Biphenyl; Ultra Biphenyl	π-π and hydrophobic	

** phases which provide a similar selectivity based on chemical and physical properties

Overview of NUCLEODUR® HPLC phases

Phase	Specification	Page	Characteristic*	Stability	Structure
PFP	pentafluorophenylpropyl, multi-endcapping 8 % C · USP L43	174	A ●● B ●●●● C ●●●●	pH 1–9, suitable for LC/MS	NUCLEODUR® $(Si\text{-}O_2)_n$ 
Sphinx RP	bifunctional, balanced ratio of propylphenyl and octadecyl, endcapping 15 % C · USP L1 and L11	176	A ●●● B ●●◖ C ●	pH 1–10, suitable for LC/MS	NUCLEODUR® $(Si\text{-}O_2)_n$ 
C_{18} HTec	octadecyl, high density coating, high capacity, multi-endcapping 18 % C · USP L1	178	A ●●●●● B ◖ C ●●◖	pH 1–11, suitable for LC/MS	NUCLEODUR® $(Si\text{-}O_2)_n$ 
C_{18} ec	octadecyl, medium density, endcapping available in 110 Å and 300 Å pore size 17.5 % / 4 % C · USP L1	181	A ●●●● B ● C ●●●◖	pH 1–9	NUCLEODUR® $(Si\text{-}O_2)_n$ 
C_8 ec	octyl, medium density, endcapping 10.5 % C · USP L7	181	A ●● B ●◖ C ●●◖	pH 1–9	NUCLEODUR® $(Si\text{-}O_2)_n$ 
C_4 ec	butyl, medium density, endcapping, 300 Å pore size 2.5 % C · USP L26	181	A ● B ●● C ●◖	pH 1–9	NUCLEODUR® $(Si\text{-}O_2)_n$ 
HILIC	zwitterionic ammonium – sulfonic acid phase 7 % C	184	A ● B ●●●●● C -	pH 2–8.5	NUCLEODUR® $(Si\text{-}O_2)_n$ 
CN / CN-RP	cyano (nitrile) for NP and RP separations 7 % C · USP L10	186	A ● B ●●●● C -	pH 1–8, stable towards highly aqueous mobile phases	NUCLEODUR® $(Si\text{-}O_2)_n$ 

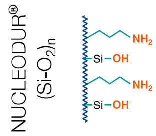
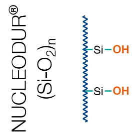
* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity

Application	Similar phases**	Interactions · retention mechanism	
aromatic and unsaturated compounds, halogen compounds, phenols, isomers, polar pharmaceuticals, antibiotics	ACQUITY® CSH Fluoro-Phenyl; Hypersil™ GOLD PFP; Luna® PFP(2); Discovery® HS F5; Allure® PFP Propyl; Ultra II PFP Propyl	polar (H bond), dipole-dipole, π-π and hydrophobic	
compounds with aromatic and multiple bond systems	no similar phases	π-π and hydrophobic	
robust and well base deactivated C_{18} phase; all separation tasks with preparative potential	Xterra® RP18 / MS C18 / SunFire™ C18; Luna® C18(2), Gemini®, Synergi® Max RP; Zorbax® Extend-C18; Inertsil® ODS III; Purospher® STAR RP-18; Hypersil® BDS	hydrophobic (van der Waals interactions)	
robust C_{18} phase for routine analyses	NUCLEOSIL® C_{18} Spherisorb® ODS II; Symmetry® C18; Hypersil® ODS; Inertsil® ODS II; Kromasil® C18; LiChrospher® RP-18	hydrophobic (van der Waals interactions) some residual silanol interactions	
robust C_8 phase for routine analyses	NUCLEOSIL® C_8 ec / C_8 Spherisorb® C8; Symmetry® C8; Hypersil® MOS; Kromasil® C8; LiChrospher® RP-8	hydrophobic (van der Waals interactions) some residual silanol interactions	
biological macromolecules like proteins or peptides	Jupiter® C4; ACE® C4	hydrophobic (van der Waals interactions) some residual silanol interactions	
hydrophilic compounds such as polar organic acids and bases, polar natural compounds	Sequant™ ZIC®-HILIC; Obelisc™	ionic / hydrophilic and electrostatic	
polar organic compounds (basic drugs), molecules containing π-electron systems	NUCLEOSIL® CN / CN-RP	π-π and polar (H bond), hydrophobic	

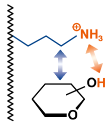
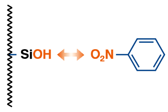
** phases which provide a similar selectivity based on chemical and physical properties

NUCLEODUR® phase overview

Overview of NUCLEODUR® HPLC phases

Phase	Specification	Page	Characteristic*	Stability	Structure
 NH_2 / NH_2-RP	aminopropyl for NP and RP separations 2.5 % C · USP L8	188	A ● B ●●●● C -	pH 2–8, stable towards highly aqueous mobile phases	
 SiOH	unmodified high purity silica · USP L3	190	A - B - C -	pH 2–8	

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity

Application	Similar phases**	Interactions · retention mechanism	
sugars, sugar alcohols and other hydroxy compounds, DNA bases, polar compounds in general	NUCLEOSIL® NH_2 / NH_2-RP	polar / ionic and hydrophobic	
polar compounds in general	NUCLEOSIL® SiOH	polar / ionic	

** phases which provide a similar selectivity based on chemical and physical properties

NUCLEODUR® C_{18} Gravity · C_8 Gravity nonpolar high density phase · USP L1 (C_{18}) · USP L7 (C_8)

Key feature
- Suitable for LC/MS and HPLC at pH extremes (pH 1–11)
- Superior base deactivation
- Ideal for method development

Technical data
- Available as octadecyl (C_{18}) and octyl (C_8), multi-endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm for C_{18}, 1.8 and 5 µm for C_8; 7, 10, 12 and 16 µm particles for preparative purposes on request
- Carbon content 18 % for C_{18}, 11 % for C_8

Recommended application
- Overall sophisticated analytical separations
- Compound classes separated include pharmaceuticals, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants

Base deactivation

NUCLEODUR® C_{18} Gravity and NUCLEODUR® C_8 Gravity are based on the ultrapure NUCLEODUR® silica. Derivatization generates a homogeneous surface with a high density of bonded silanes (~18 % C for C_{18}, ~11 % C for C_8). Thorough endcapping suppresses any unwanted polar interactions between the silica surface and the sample, which makes "Gravity" particularly suitable for the separation of basic and other ionizable analytes. Even strongly basic pharmaceuticals like amitriptyline are eluted without tailing under isocratic conditions. For a discussion of the different retention behavior of C_{18} phases compared to C_8 phases see page 182.

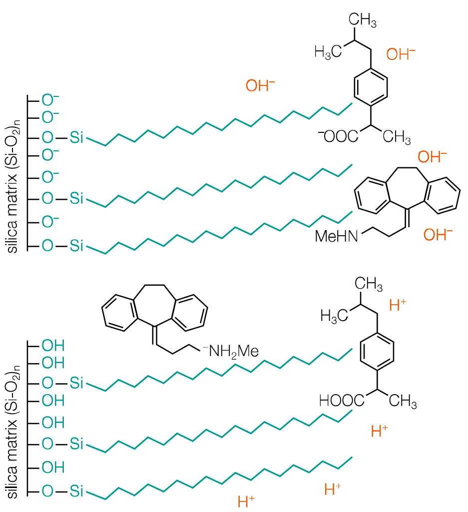
Enhanced pH stability

One major disadvantage of silica stationary phases is limited stability at strongly acidic or basic pH. Cleavage of the siloxane bonding by hydrolysis, or dissolution of the silica will rapidly lead to a considerable loss in column performance. Conventional RP phases are usually not recommended to be run with mobile phases at pH > 8 or pH < 2 for extended periods of time. The special surface bonding technology and the low concentration of trace elements of NUCLEODUR® C_{18} and C_8 Gravity allow for use at an expanded pH range from pH 1 to 11.

Benefits of enhanced pH stability

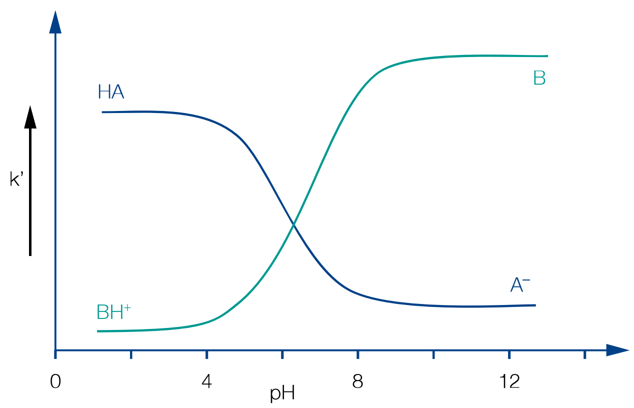
An expanded pH range is often required in method development. Many nitrogen containing compounds like basic drugs are protonated at acidic or neutral pH and exhibit poor retention on a standard C_{18} phase. The retention behavior can be improved by working at a higher pH, where the analyte is no longer protonated, but formally neutrally charged, as a rule between pH 9–10. For acidic analytes it is exactly in inverse proportion, maximum retention can be attained at low pH.

Surface silanols at different pH values



The figure above shows the extent of protonation of surface silanols and of two exemplary analytes at acidic and alkaline pH. The following graph explains the general correlation between retention and pH.

Correlation between retention and pH for basic and acidic compounds



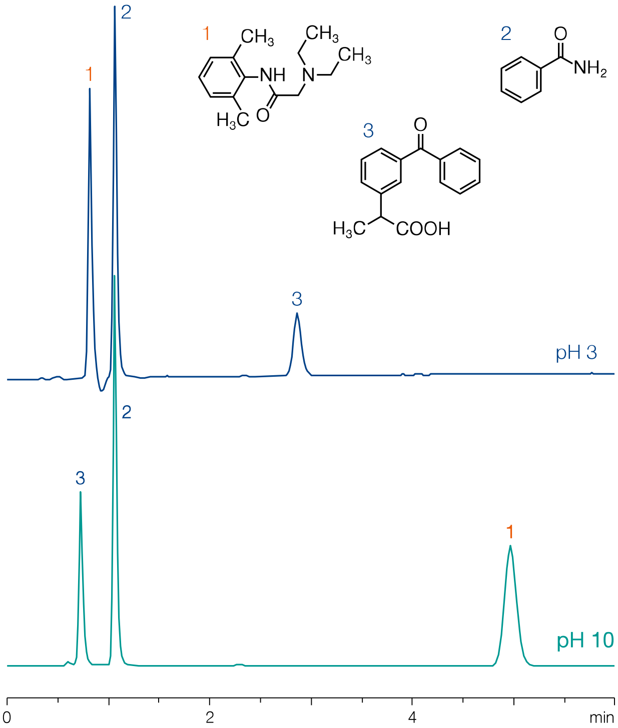
An example how selectivity can be controlled by pH is the separation of the acid ketoprofen, the base lidocaine and benzamide. Under acidic conditions the protonated lidocaine is eluted very fast due to lack of sufficiently strong hydrophobic interactions between analyte and C_{18} chains, while the formally neutral ketoprofen is eluted after about 3 min. However, at pH 10 a reversal of the elution order, with a visibly longer retention time for the basic lidocaine, is observed.

Influence of the pH value on selectivity

MN Appl. No. 120860

Column:	125 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	A) acetonitrile – 10 mmol/L ammonium formate, pH 3.0 (50:50, v/v); B) acetonitrile – 10 mmol/L ammonium bicarbonate, pH 10.0 (50:50, v/v)
Flow rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV, 230 nm
Injection:	2 µL

Peaks:
1. Lidocaine
2. Benzamide
3. Ketoprofen



As mentioned above, pH stability of the stationary phase can be helpful for improving selectivity in method development. The following figure shows the separation of 4 basic drugs under acidic and basic conditions.

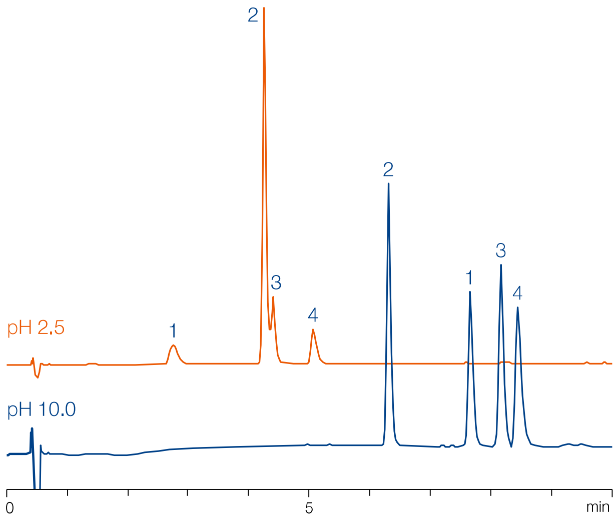
At pH 2.5 the protonated analytes exhibit poor retention (early elution) and in addition an inadequate resolution for papaverine and noscapine, whilst the formally non ionized molecules can be baseline separated due to the better retention pattern at alkaline pH.

Separation of basic alkaloids

MN Appl. No. 118010

Column:	125 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	A) acetonitrile B) 20 mmol/L $(NH_4)_2HPO_4$, pH 2.5 / 10.0 10 % A (1 min) → 75 % A in 10 min
Flow rate:	1.0 mL/min; Temperature 25 °C
Detection:	UV, 254 nm; Injection 2 µL

Peaks:
1. Lidocaine
2. Papaverine
3. Noscapine
4. Diphenhydramine



The following chromatogram demonstrates the stability of NUCLEODUR® C_{18} Gravity under alkaline conditions. The ultrapure Gravity with its unique high density surface bonding technology withstands strong alkaline mobile phase conditions.

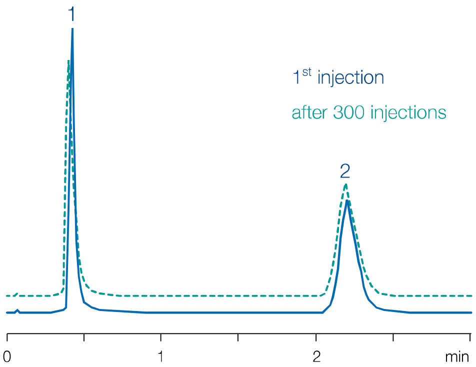
NUCLEODUR® columns

Stability of NUCLEODUR® C_{18} Gravity at pH 11

MN Appl. No. 120850

Column:	50 x 4.6 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	methanol – water – ammonia (20:80:0.5, v/v/v), pH 11
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	UV, 254 nm
Injection:	2.0 µL

Peaks:
1. Theophylline
2. Caffeine



Even after 300 injections no loss of column efficiency – identified, e.g., by peak broadening or decrease in retention times – could be observed.

Under alkaline conditions dissolution of the silica support is possible, resulting in dead volume and thus peak broadening. It is worth mentioning, that this phenomenon also depends on type and concentration of buffers, as well as on the temperature. It is well known that the use of phosphate buffers, particularly at elevated temperatures, can reduce column lifetime even at moderate pH. If possible, phosphate buffers should be replaced by less harmful alternatives.

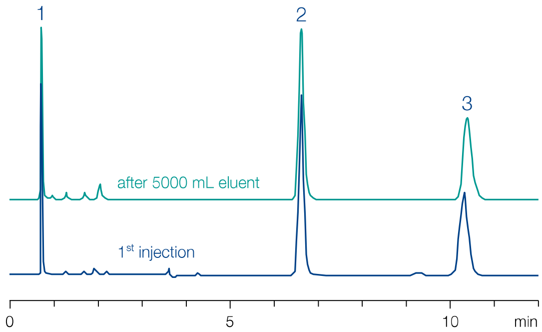
The following chromatograms show the excellent column stability of NUCLEODUR® C_{18} Gravity in acidic conditions. Retention times of all three compounds in the column performance test remain consistent and virtually unchanged, even after the column is run with 5000 mL eluent. Due to the extremely stable surface modification, no cleavage of the Si-O-Si bonding occurs, column deterioration is therefore successfully prevented.

Stability of NUCLEODUR® C_{18} Gravity at pH 1.5

MN Appl. No. 120840

Column:	125 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	acetonitrile – 1 % TFA in water (50:50, v/v), pH 1.5
Flow rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV, 230 nm
Injection:	5 µL

Peaks: 1. Pyridine, 2. Toluene, 3. Ethylbenzene



Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Gravity, 1.8 µm octadecyl phase, particle size 1.8 µm, 18 % C · UHPLC							
Analytical EC columns							
2 mm	760078.20	760079.20	760071.20	760076.20		760075.20	
3 mm	760078.30	760079.30		760076.30			
4 mm	760078.40	760079.40		760076.40			
4.6 mm	760078.46	760079.46		760076.46			
EC guard columns*		4 x 2 mm: 761901.20		4 x 3 mm: 761901.30			
NUCLEODUR® C_{18} Gravity, 3 µm octadecyl phase, particle size 3 µm, 18 % C							
Analytical EC columns							
2 mm		760080.20		760084.20	760081.20	760083.20	760082.20
3 mm		760080.30		760084.30	760081.30	760083.30	760082.30
4 mm		760080.40		760084.40	760081.40	760083.40	760082.40
4.6 mm		760080.46	760086.46	760084.46	760081.46	760083.46	760082.46
EC guard columns*		4 x 2 mm: 761902.20		4 x 3 mm: 761902.30			

Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Gravity, 5 µm octadecyl phase, particle size 5 µm, 18 % C							
Analytical EC columns							
2 mm		760102.20		760104.20	760100.20	760103.20	760101.20
3 mm		760102.30		760104.30	760100.30	760103.30	760101.30
4 mm		760102.40		760104.40	760100.40	760103.40	760101.40
4.6 mm		760102.46	760106.46	760104.46	760100.46	760103.46	760101.46
EC guard columns*		4 x 2 mm: 761903.20		4 x 3 mm: 761903.30			
Preparative VarioPrep columns							
10 mm		762103.100			762109.100		762113.100
21 mm		762103.210			762109.210		762113.210
32 mm							762113.320
40 mm						762100.400	762113.400
VP guard columns ***		10 x 8 mm: 762160.80		10 x 16 mm: 762160.160		15 x 32 mm: 762163.320	
NUCLEODUR® C_{18} Gravity, 10 µm octadecyl phase, particle size 10 µm, 18 % C							
Preparative VarioPrep columns							
21 mm							762250.210
40 mm							762250.400
VP guard columns **				10 x 16 mm: 762160.160		15 x 32 mm: 762163.320	

Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_8 Gravity, 1.8 µm octyl phase, particle size 1.8 µm, 11 % C · UHPLC							
Analytical EC columns							
2 mm	760756.20	760755.20	760760.20	760757.20		760759.20	
3 mm	760756.30	760755.30		760757.30			
4 mm	760756.40	760755.40		760757.40			
4.6 mm	760756.46	760755.46		760757.46			
EC guard columns*		4 x 2 mm: 761905.20		4 x 3 mm: 761905.30			
NUCLEODUR® C_8 Gravity, 5 µm octyl phase, particle size 5 µm, 11 % C							
Analytical EC columns							
2 mm		760750.20		760754.20	760751.20	760752.20	760753.20
3 mm		760750.30		760754.30	760751.30	760752.30	760753.30
4 mm		760750.40		760754.40	760751.40	760752.40	760753.40
4.6 mm		760750.46	760749.46	760754.46	760751.46	760752.46	760753.46
EC guard columns*		4 x 2 mm: 761907.20		4 x 3 mm: 761907.30			
Preparative VarioPrep columns							
10 mm		762081.100			762071.100		762070.100
21 mm		762081.210			762071.210	762082.210	762070.210
VP guard columns **		10 x 8 mm: 762097.80		10 x 16 mm: 762097.160			

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® C_{18} Gravity-SB hydrophobic phase with polar selectivity · USP L1

Key feature

- Hydrophobic C_{18} phase with distinct polar selectivity, ideal for method development, better retention of early eluting substances
- Excellent performance under highly aqueous conditions
- Suitable for LC/MS due to low bleeding characteristics

Technical data

- Monomeric octadecyl modification, extensive endcapping
- Pore size 110 Å; available particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 13 %; pH stability 1–9

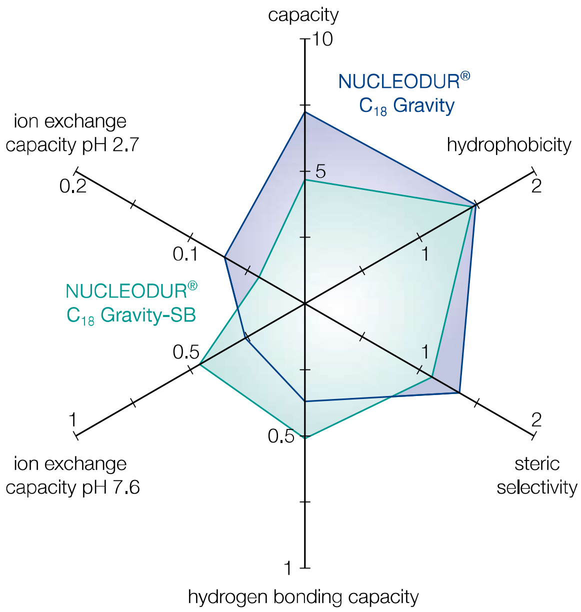
Recommended application

- Overall sophisticated analytical separations, especially for polar compounds, e.g., antibiotics, water-soluble vitamins, organic acids

NUCLEODUR® C_{18} Gravity-SB excels with a relatively high hydrophobicity – similar to C_{18} Gravity – while simultaneously showing distinctive polar selectivity, without having polar embedded groups or polar endcapping. As a result the column displays better retention of early eluting analytes and high performance under strongly aqueous conditions. Additionally the column is suitable for LC/MS due to low bleeding characteristics. These features are achieved through side chains (isobutyl) of the monomeric C_{18} phase.

In the TANAKA plot the NUCLEODUR® Gravity-SB shows similar hydrophobicity than the Gravity, however with a reduced capacity. The ion exchange capacity under basic conditions (pH 7.6) is high, which favors good retention of early eluting, polar substances.

Due to the broad selectivity and stability the base deactivated NUCLEODUR® C_{18} Gravity-SB is versatile applicable, especially for polar analytes like nucleobases or pesticides the column shows good separation efficiency.



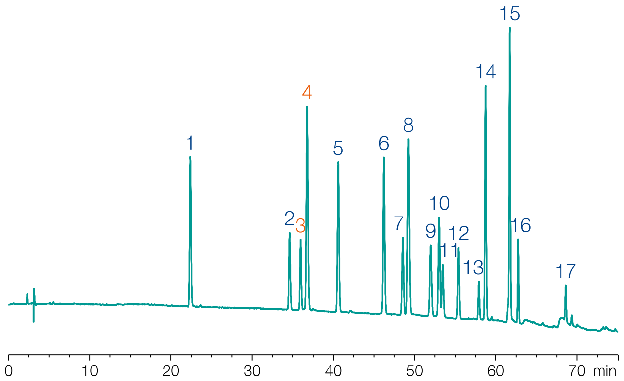
Pesticide mix (Ehrenstorfer, 17 components)

MN Appl. No. 127330

Column:	EC 250/4.6 NUCLEODUR® C_{18} Gravity-SB, 3 µm
Eluent:	A) acetonitrile
	B) 5 mmol/L NH_4Ac;
	10–37.5 % A in 50 min, 37.5–75 % A in 25 min
Flow rate:	1.1 mL/min
Temperature:	35 °C
Detection:	UV, 230 nm
Injection:	3 µL

Peaks:

1. Desethylatrazine	7. Chlortoluron	13. Metazachlor
2. Metoxuron	8. Atrazine	14. Sebuthylazin
3. Hexazinone	9. Monolinuron	15. Terbuthylazine
4. Simazine	10. Isoproturon	16. Linuron
5. Cyanazine	11. Diuron	17. Metolachlor
6. Methabenzthiazuron	12. Metobromuron	



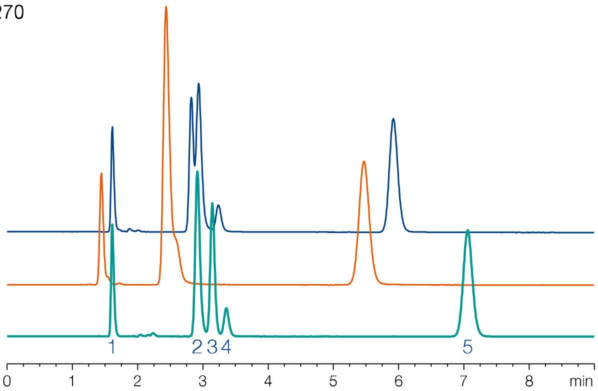
Good separation of the critical pair hexazinone/simazine

Comparing of selectivity for nucleobases

MN Appl. No. 127270

Columns: EC 150/4.6 mm
NUCLEODUR® C_{18} Gravity-SB, 5 µm
NUCLEODUR® C_{18} Gravity, 5 µm
NUCLEODUR® C_{18} Pyramid, 5 µm
Eluent: 25 mmol/L KH_2PO_4, pH 3 – methanol (95:5, v/v)
Flow rate: 1.0 mL/min, Temperature: 20 °C
Detection: UV, 220 nm, Injection: 2.5 µL (1 mg/mL)

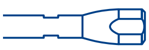
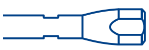
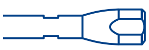
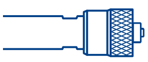
Peaks:
1. Cytosine
2. Adenine
3. Uracil
4. Guanine
5. Thymine



Better resolution of early eluting analyte

Ordering information

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Gravity-SB, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760591.20	760593.20	760595.20	760596.20		760598.20	
	3 mm	760591.30	760593.30		760596.30			
	4 mm	760591.40	760593.40		760596.40			
	4.6 mm	760591.46	760593.46		760596.46			
EC guard columns*			4 x 2 mm: 761990.20		4 x 3 mm: 761990.30			
NUCLEODUR® C_{18} Gravity-SB, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760603.20		760606.20	760607.20	760608.20	760609.20
	3 mm		760603.30		760606.30	760607.30	760608.30	760609.30
	4 mm		760603.40		760606.40	760607.40	760608.40	760609.40
	4.6 mm		760603.46	760605.46	760606.46	760607.46	760608.46	760609.46
EC guard columns*			4 x 2 mm: 761991.20		4 x 3 mm: 761991.30			
NUCLEODUR® C_{18} Gravity-SB, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760613.20		760616.20	760617.20	760618.20	760619.20
	3 mm		760613.30		760616.30	760617.30	760618.30	760619.30
	4 mm		760613.40		760616.40	760617.40	760618.40	760619.40
	4.6 mm		760613.46	760615.46	760616.46	760617.46	760618.46	760619.46
EC guard columns*			4 x 2 mm: 761992.20		4 x 3 mm: 761992.30			
Preparative VarioPrep columns								
	10 mm		762350.100			762351.100		762353.100
	21 mm		762350.210			762351.210		762353.210
	32 mm							762353.320
	40 mm						762352.400	762353.400
VP guard columns **			10 x 8 mm: 762354.80		10 x 16 mm: 762354.160		15 x 32 mm: 762355.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® C_{18} Isis phase with high steric selectivity · USP L1

Key feature

- Exceptional steric selectivity
- Outstanding surface deactivation
- Suitable for LC/MS and HPLC at pH 1–10

Technical data

- C_{18} phase with special polymeric, crosslinked surface modification; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 20 %

Recommended application

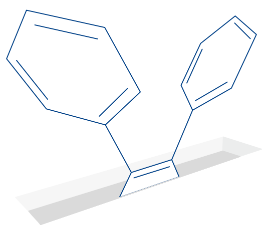
- Steroids, (*o*,*p*,*m*-)substituted aromatics, fat-soluble vitamins

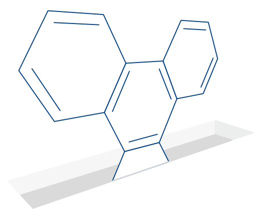
Surface modification

By use of specific C_{18} silanes and polymeric bonding technologies a dense shield of alkyl chains protects the subjacent silica matrix. Elemental analysis of NUCLEODUR® C_{18} Isis shows a carbon load of 20 %. The target crosslinking of the C_{18} chains on the surface enables the separation of compounds with similar molecular structure but different stereochemical properties. The technical term for this feature is steric selectivity.

Slot Model

Sander and Wise [5] proposed a model for the retention of aromatic compounds based on molecular shape, which is referred to as “Slot Model”. This model pictures the bonded C_{18} phase on the silica surface with slots which the analytes have to penetrate during retention. Planar molecules are able to penetrate these slots deeper than non-planar molecules of similar molecular weight and length-to-width ratio. Thus triphenylene (lower structure) is longer retained than o-terphenyl (upper structure).





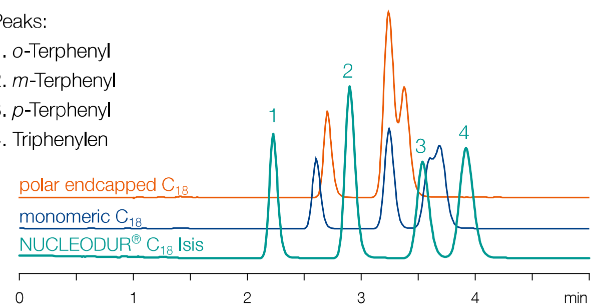
Steric selectivity

The following chromatograms reveal the improved resolution for positional isomers in a test mixture of aromatic compounds on NUCLEODUR® C_{18} Isis (green) in direct comparison with monomerically coated (blue) and polar endcapped (orange) C_{18} columns.

Steric selectivity of NUCLEODUR® C_{18} Isis

Columns:	125 x 4 mm NUCLEODUR® C_{18} Isis monomerically coated C_{18} phase polar endcapped phase C_{18} phase
Eluent:	methanol – water (90:10, v/v)
Flow rate:	1 mL/min, temperature: 35 °C
Detection:	UV, 254 nm
Injection:	5 µL

Peaks:
1. *o*-Terphenyl
2. *m*-Terphenyl
3. *p*-Terphenyl
4. Triphenylen

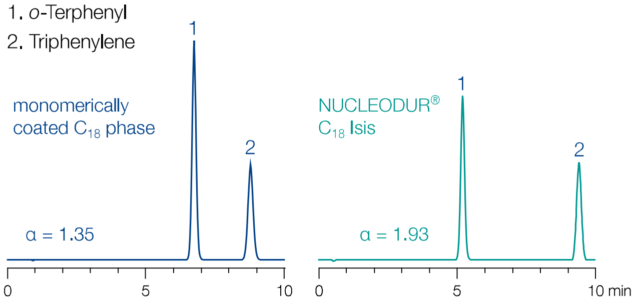


The separation of o-terphenyl and triphenylene is a good example to evaluate the selectivity of a RP column in terms of the shape of two molecules. The phenyl rings of o-terphenyl are twisted out of plane while triphenylene has a planar geometry. The separation factor α is a measure for the steric selectivity. As is shown below the α value is considerable larger on NUCLEODUR® C_{18} Isis compared to a conventional C_{18} column.

Steric selectivity of NUCLEODUR® C_{18} Isis

Columns:	125 x 4 mm
Eluent:	methanol – water (80:20, v/v)
Flow rate:	1 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	1 µL

Peaks:
1. *o*-Terphenyl
2. Triphenylene



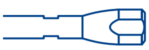
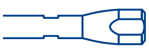
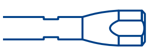
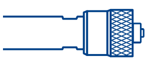
The surface bonding technology also provides improved stability features for the NUCLEODUR® C_{18} Isis phase.

Surface deactivation

The chromatography of basic analytes requires a high density of surface-bonded C_{18} silanes combined with a thorough endcapping procedure to keep silanol activity at a minimum. This ensures tailing-free elution of even strongly basic amino-containing compounds (see application 121210 at *www.mn-net.com/apps*).

Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Isis, 1.8 µm particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760406.20	760405.20	760396.20	760407.20		760409.20	
3 mm	760406.30	760405.30		760407.30			
4 mm	760406.40	760405.40		760407.40			
4.6 mm	760406.46	760405.46		760407.46			
EC guard columns*		4 x 2 mm: 761910.20		4 x 3 mm: 761910.30			
NUCLEODUR® C_{18} Isis, 3 µm particle size 3 µm							
Analytical EC columns							
2 mm		760400.20		760401.20	760402.20	760403.20	760404.20
3 mm		760400.30		760401.30	760402.30	760403.30	760404.30
4 mm		760400.40		760401.40	760402.40	760403.40	760404.40
4.6 mm		760400.46	760397.46	760401.46	760402.46	760403.46	760404.46
EC guard columns*		4 x 2 mm: 761911.20		4 x 3 mm: 761911.30			
NUCLEODUR® C_{18} Isis, 5 µm particle size 5 µm							
Analytical EC columns							
2 mm		760410.20		760415.20	760412.20	760413.20	760414.20
3 mm		760410.30		760415.30	760412.30	760413.30	760414.30
4 mm		760410.40		760415.40	760412.40	760413.40	760414.40
4.6 mm		760410.46	760416.46	760415.46	760412.46	760413.46	760414.46
EC guard columns*		4 x 2 mm: 761912.20		4 x 3 mm: 761912.30			
Preparative VarioPrep columns							
10 mm		762404.100			762405.100		762403.100
21 mm		762404.210			762405.210		762403.210
32 mm							762403.320
40 mm						762406.400	762403.400
VP guard columns **		10 x 8 mm: 762420.80		10 x 16 mm: 762420.160		15 x 32 mm: 762422.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® C_{18} Pyramid phase for highly aqueous eluents · USP L1

Key feature

- Stable in 100 % aqueous mobile phase systems
- Interesting polar selectivity features
- Excellent base deactivation; suitable for LC/MS due to low bleeding characteristics

Technical data

- Special phase with polar endcapping; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm (7 and 10 µm particles for preparative purposes on request); carbon content 14 %; pH stability 1–9

Recommended application

- Analgesics, penicillin antibiotics, nucleic acid bases, water-soluble vitamins, complexing agents, organic acids

RP-HPLC with highly aqueous mobile phases

The efforts to neutralize unwanted silanol activity often results in well base-deactivated RP phases with high carbon load, but a limited scope of selectivity beyond non-polar interactions. Polar compounds like carboxylic acids or drug metabolites show only weak retention on densely bonded RP columns due to distinct hydrophobic properties but low polar interactions. Very polar analytes require highly aqueous mobile phases for solubility and retention. Conventional reversed phase columns often display stability problems in eluent systems with high percentage of water (> 95 %) as evidenced by a sudden decrease of retention time and overall poor reproducibility. This phenomenon is described as phase collapse caused by the mobile phase expelled from the pores due to the fact, that hydrophobic RP phases are incompletely wetted with the mobile phase [6].

Different approaches can be used to increase column stability with highly aqueous mobile phase systems. The most promising concepts are incorporating a polar group in the hydrophobic alkyl chain, or using hydrophilic endcapping procedures to improve the wettability of the reversed phase modification. NUCLEODUR® PolarTec may be taken as an example for the embedded polar group strategy, in which a C_{18} silane with a polar function is successfully linked to the silica surface.

Stability features

NUCLEODUR® C_{18} Pyramid is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. The upper figure shows the retention behavior of tartaric, acetic and maleic acid under purely aqueous conditions on NUCLEODUR® C_{18} Pyramid in comparison with a conventionally bonded C_{18} phase.

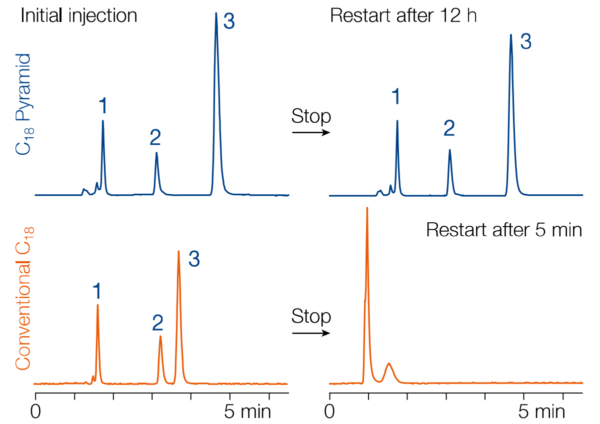
It can be shown that the retention times for NUCLEODUR® C_{18} Pyramid remain nearly unchanged between initial injection and restart after the flow has been stopped for 12 h, whilst the performance of the conventional RP column already collapsed totally after 5 min.

Stability test

MN Appl. No. 120870

Both columns:	125 x 4 mm
Eluent:	50 mmol/L KH_2PO_4, pH 2.5
Flow rate:	0.7 mL/min
Temperature:	25 °C
Detection:	UV, 210 nm
Injection:	1 µL

Peaks:
1. Tartaric acid
2. Acetic acid
3. Maleic acid



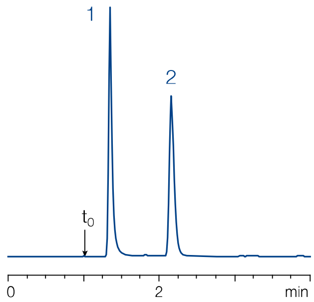
Retention characteristics

Separation of very polar compounds

MN Appl. No. 119170

Column:	125 x 4 mm NUCLEODUR® C_{18} Pyramid, 5 µm
Eluent:	0.2 % H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	22 °C
Detection:	UV, 202 nm
Injection:	2 µL

Peaks:
1. Formic acid
2. Acetic acid



The polar surface exhibits retention characteristics different from conventional C_{18} phases. Application 119170 shows the improved retention behavior of the very polar short chain organic acids, which are insufficiently retained on RP columns with predominantly hydrophobic surface properties. In addition to the exceptional polar selectivity NUCLEODUR® C_{18} Pyramid also provides adequate hydrophobic retention (see application No. 19190 at *www.mn-net.com*). The perceptible increase in polarity has no impact on the retention behavior of ionizable analytes. Even with the strongly basic compounds of the tricyclic antidepressant drug test mixture, no unwanted interactions or a so-called lack in base deactivation are observed (see application 119200 at *www.mn-net.com/apps*).

Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Pyramid, 1.8 µm particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760271.20	760272.20	760275.20	760273.20		760274.20	
3 mm	760271.30	760272.30		760273.30			
4 mm	760271.40	760272.40		760273.40			
4.6 mm	760271.46	760272.46		760273.46			
EC guard columns*		4 x 2 mm: 761915.20		4 x 3 mm: 761915.30			
NUCLEODUR® C_{18} Pyramid, 3 µm particle size 3 µm							
Analytical EC columns							
2 mm		760263.20		760264.20	760260.20	760261.20	760262.20
3 mm		760263.30		760264.30	760260.30	760261.30	760262.30
4 mm		760263.40		760264.40	760260.40	760261.40	760262.40
4.6 mm		760263.46	760259.46	760264.46	760260.46	760261.46	760262.46
EC guard columns*		4 x 2 mm: 761916.20		4 x 3 mm: 761916.30			
NUCLEODUR® C_{18} Pyramid, 5 µm particle size 5 µm							
Analytical EC columns							
2 mm		760200.20		760204.20	760201.20	760203.20	760202.20
3 mm		760200.30		760204.30	760201.30	760203.30	760202.30
4 mm		760200.40		760204.40	760201.40	760203.40	760202.40
4.6 mm		760200.46	760205.46	760204.46	760201.46	760203.46	760202.46
EC guard columns*		4 x 2 mm: 761917.20		4 x 3 mm: 761917.30			
Preparative VarioPrep columns							
10 mm		762271.100			762273.100		762272.100
21 mm		762271.210			762273.210		762272.210
32 mm							762272.320
40 mm						762269.400	762272.400
VP guard columns **		10 x 8 mm: 762291.80		10 x 16 mm: 762291.160		15 x 32 mm: 762293.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® PolarTec RP phase with embedded polar group · USP L1 and L60

Key feature

- Excellent base deactivation
- Suitable for LC/MS and 100 % aqueous eluents
- Pronounced steric selectivity

Technical data

- Phase with embedded polar group; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 17 %; pH stability 1–9

Recommended application

- Exceptional selectivity for phenols and nitrogen containing compounds, polar compounds like basic pharmaceuticals, organic acids, pesticides, amino acids, water-soluble vitamins, etc.

RP-HPLC under 100 % aqueous conditions

The dominant form of interactions of conventional C_{18} phases are nonpolar London dispersion forces. Besides nonpolar interactions phases with embedded polar groups possess the ability to show polar interactions (dipole-dipole, hydrogen bonds, π-π, etc.). These interactions enhance retention and selectivity for polar compounds like carboxylic acids, phenols and nitrogen containing compounds.

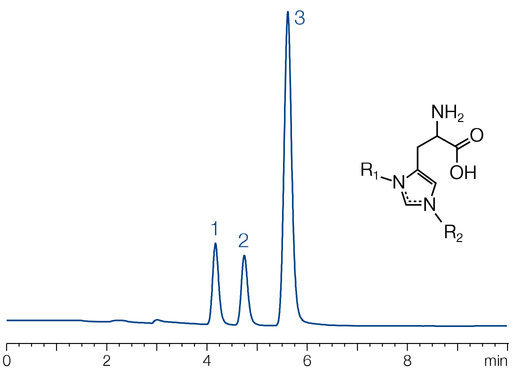
Separation of histidines

MN Appl. No. 125140

Column:	150 x 3 mm NUCLEODUR® PolarTec, 3 µm
Eluent:	1.0 mmol/L perfluoropentanoic acid in water – 0.5 mmol/L perfluoropentanoic acid in acetonitrile (99.5:0.5, v/v)
Flow rate:	0.4 mL/min
Temperature:	20 °C
Detection:	UV, 230 nm

Peaks:

1. 3-Methylhistidine	$R_1 = H, R_2 = CH_3$
2. Histidine	$R_1 = R_2 = H$
3. 1-Methylhistidine	$R_1 = CH_3, R_2 = H$



In order to increase retention for polar compounds it is often necessary to decrease the organic ratio of the mobile phase to zero. Under these conditions many conventional C_{18} phases display the so-called dewetting effect which means that the mobile phase is expelled from the pores. This phenomenon leads to a dramatic loss in retention. NUCLEODUR® PolarTec is stable in 100 % aqueous mobile phases and therefore especially suited for the separation of polar compounds like organic acids.

Due to the shielding effect of the embedded group NUCLEODUR® PolarTec shows an excellent base deactivation, which is at the top-notch of embedded polar group phases on the market. The pronounced steric selectivity (see Tanaka plot) is an additional tool for the separation of complex mixtures.

Due to low bleeding characteristics NUCLEODUR® PolarTec is also suitable for LC/MS.

Even after days or weeks of operation in purely aqueous eluents the C_{18} chains of NUCLEODUR® PolarTec are neither folded nor show any collapsing. A significant reduction of retention time cannot be observed.

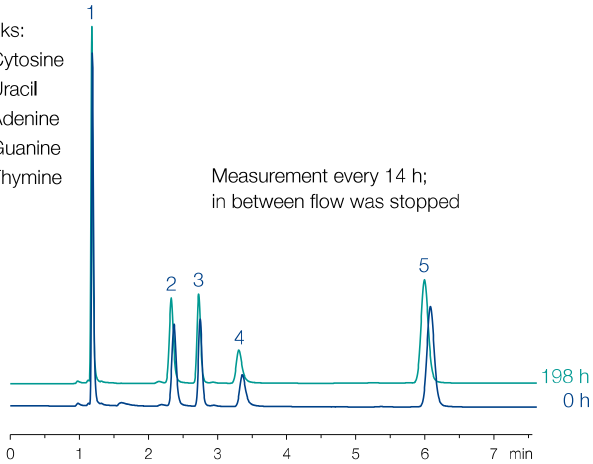
Stability of NUCLEODUR® PolarTec

MN Appl. No. 124610

Column:	150 x 3 mm NUCLEODUR® PolarTec, 3 µm
Eluent:	30 mmol/L KH_2PO_4, pH 3.0
Flow rate:	0.5 mL/min
Temperature:	30 °C
Detection:	UV, 220 nm

Peaks:

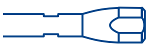
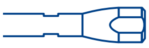
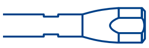
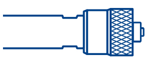
1. Cytosine
2. Uracil
3. Adenine
4. Guanine
5. Thymine



In spite of the polar character of the embedded functional group NUCLEODUR® PolarTec exhibits sufficient hydrophobic properties and is very well suited for analyzing basic compounds.

Ordering information

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® PolarTec, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760461.20	760463.20	760465.20	760466.20		760468.20	
	3 mm	760461.30	760463.30		760466.30			
	4 mm	760461.40	760463.40		760466.40			
	4.6 mm	760461.46	760463.46		760466.46			
EC guard columns*			4 x 2 mm: 761980.20		4 x 3 mm: 761980.30			
NUCLEODUR® PolarTec, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760473.20		760476.20	760477.20	760478.20	760479.20
	3 mm		760473.30		760476.30	760477.30	760478.30	760479.30
	4 mm		760473.40		760476.40	760477.40	760478.40	760479.40
	4.6 mm		760473.46	760475.46	760476.46	760477.46	760478.46	760479.46
EC guard columns*			4 x 2 mm: 761981.20		4 x 3 mm: 761981.30			
NUCLEODUR® PolarTec, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760483.20		760486.20	760487.20	760488.20	760489.20
	3 mm		760483.30		760486.30	760487.30	760488.30	760489.30
	4 mm		760483.40		760486.40	760487.40	760488.40	760489.40
	4.6 mm		760483.46	760485.46	760486.46	760487.46	760488.46	760489.46
EC guard columns*			4 x 2 mm: 761982.20		4 x 3 mm: 761982.30			
Preparative VarioPrep columns								
	10 mm		762220.100			762221.100		762223.100
	21 mm		762220.210			762221.210		762223.210
	32 mm							762223.320
	40 mm						762222.400	762223.400
VP guard columns **			10 x 8 mm: 762224.80		10 x 16 mm: 762224.160		15 x 32 mm: 762226.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® Phenyl-Hexyl productive for polar / aromatic compunds · USP L11

Key feature

- Hydrophobic phase with alternative selectivity compared to classical C_{18} modifications
- Separation principle based on 2 retention mechanisms: π-π interactions and hydrophobic interactions
- Suitable for LC/MS due to low bleeding characteristics

Technical data

- Phase with phenyl-hexyl modification and multi-endcapping; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 10 %; pH stability 1–10

Recommended application

- Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics

Phenylhexyl modified phases are an interesting alternative to classical C_{18} phases due to an excellent separation of aromatic and unsaturated compounds especially with electron withdrawing groups.

The combination of hydrophobic and polar π-π interactions result in an interesting and alternate selectivity in comparison to C_{18} and C_8 modified phases.

Through short phenylhexyl chains the NUCLEODUR® Phenyl-Hexyl is more polar than the bifunctional modified NUCLEODUR® Sphinx RP. Therefore shorter analysis times can be achieved with mixtures of structural similar aromatic and aliphatic unsaturated compounds.

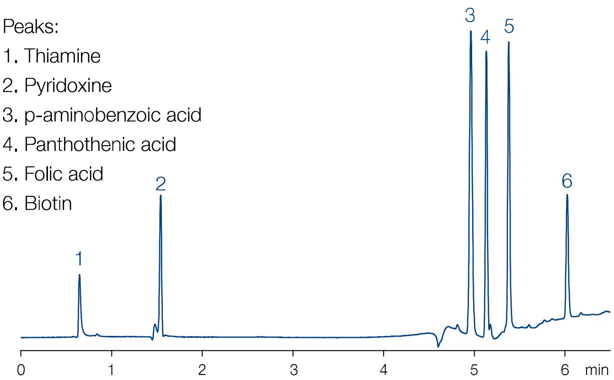
With NUCLEODUR® Phenyl-Hexyl e.g., tricyclic antidepressants or water soluble vitamins can be separated in good resolution.

Separation of water-soluble vitamins on NUCLEODUR® Phenyl-Hexyl

MN Appl. No. 125920

Column:	100 x 3 mm NUCLEODUR® Phenyl-Hexyl, 3 µm
Eluent:	A) 0.1 % phosphoric acid in water B) 0.1 % phosphoric acid in acetonitrile 0 % B for 2 min, then to 60 % B in 7 min
Flow rate:	0.56 mL/min
Temperature:	35 °C
Detection:	UV, 215 nm
Injection:	0.8 µL, 1.0 mg/mL each compound 1 mg/mL in eluent

Peaks:
1. Thiamine
2. Pyridoxine
3. p-aminobenzoic acid
4. Panthothenic acid
5. Folic acid
6. Biotin

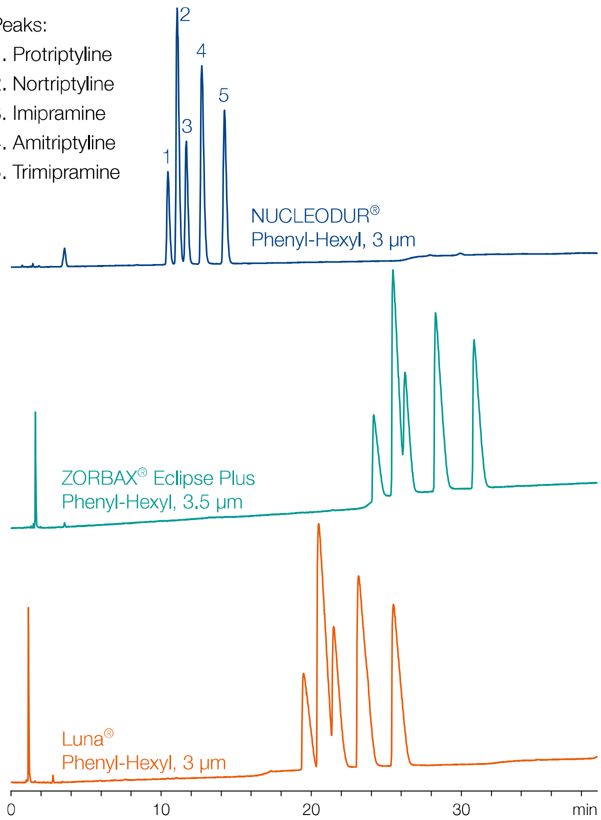


Tricyclic antidepressant (TCA)

MN Appl. No. 126020

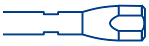
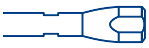
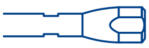
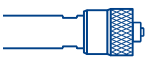
Columns:	150 x 3 mm NUCLEODUR® Phenyl-Hexyl, 3 µm Agilent ZORBAX® Eclipse Phenyl-Hexyl, 3.5 µm Phenomenex Luna® Phenyl-Hexyl, 3 µm
Eluent:	A) 0.1 % formic acid in acetonitrile B) 0.1 % formic acid in water 20–32.5 % A in 40 min
Flow rate:	0.56 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	0.2 µL, each compound 1 mg/mL in eluent

Peaks:
1. Protriptyline
2. Nortriptyline
3. Imipramine
4. Amitriptyline
5. Trimipramine



Ordering information

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® Phenyl-Hexyl, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760561.20	760563.20	760565.20	760566.20		760568.20	
	3 mm	760561.30	760563.30		760566.30			
	4 mm	760561.40	760563.40		760566.40			
	4.6 mm	760561.46	760563.46		760566.46			
EC guard columns*			4 x 2 mm: 761985.20		4 x 3 mm: 761985.30			
NUCLEODUR® Phenyl-Hexyl, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760573.20		760576.20	760577.20	760578.20	760579.20
	3 mm		760573.30		760576.30	760577.30	760578.30	760579.30
	4 mm		760573.40		760576.40	760577.40	760578.40	760579.40
	4.6 mm		760573.46	760575.46	760576.46	760577.46	760578.46	760579.46
EC guard columns*			4 x 2 mm: 761986.20		4 x 3 mm: 761986.30			
NUCLEODUR® Phenyl-Hexyl, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760583.20		760586.20	760587.20	760588.20	760589.20
	3 mm		760583.30		760586.30	760587.30	760588.30	760589.30
	4 mm		760583.40		760586.40	760587.40	760588.40	760589.40
	4.6 mm		760583.46	760585.46	760586.46	760587.46	760588.46	760589.46
EC guard columns*			4 x 2 mm: 761987.20		4 x 3 mm: 761987.30			
Preparative VarioPrep columns								
	10 mm		762210.100			762211.100		762213.100
	21 mm		762210.210			762211.210		762213.210
	32 mm							762213.320
	40 mm						762212.400	762213.400
VP guard columns **			10 x 8 mm: 762234.80		10 x 16 mm: 762234.160		15 x 32 mm: 762236.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® π² hydrophobic biphenylpropyl phase · USP L11

Key feature

- Hydrophobic phase with alternative selectivity compared to classical C_{18} modifications
- Separation principle based on 2 retention mechanisms (π-π interactions and hydrophobic interactions)
- Better retention of aromatic and unsaturated substances
- Excellent performance under highly aqueous conditions

Technical data

- Phase with biphenylpropyl modification and multi-endcapping; pore size 110 Å; particle size 5 µm; carbon content 17 %; pH stability 1.5–10

Recommended application

- Overall sophisticated analytical separations, especially aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics, steroids

Stationary HPLC phases with biphenyl ligands like NUCLEODUR® π² provide an interesting alternative to classical alkyl modified C_{18} and C_8 HPLC phases due to their remarkable orthogonal selectivity.

Furthermore the NUCLEODUR® π² provides an excellent separation performance for aromatic and unsaturated analytes by combination of hydrophobic and π-π interactions.

A unique feature is the predominant separation mechanism (π-π or hydrophobic interactions) and thus the selectivity can be controlled by selection of the eluent. In acetonitrile / water NUCLEODUR® π² shows similar retention strength then C_{18} modified phases and thereby displays a significantly stronger retention than phenyl phases. These interactions are even further enhanced in a methanol / water eluent.

NUCLEODUR® π² exceeds other aryl phases in terms of stability under strongly aqueous conditions. Therefore i.a. steroids, sulfonamides and acidic pharmaceuticals are separated in good resolution with NUCLEODUR® π². NUCLEODUR® π² is the stationary phase with the highest aromatic analyte selectivity.

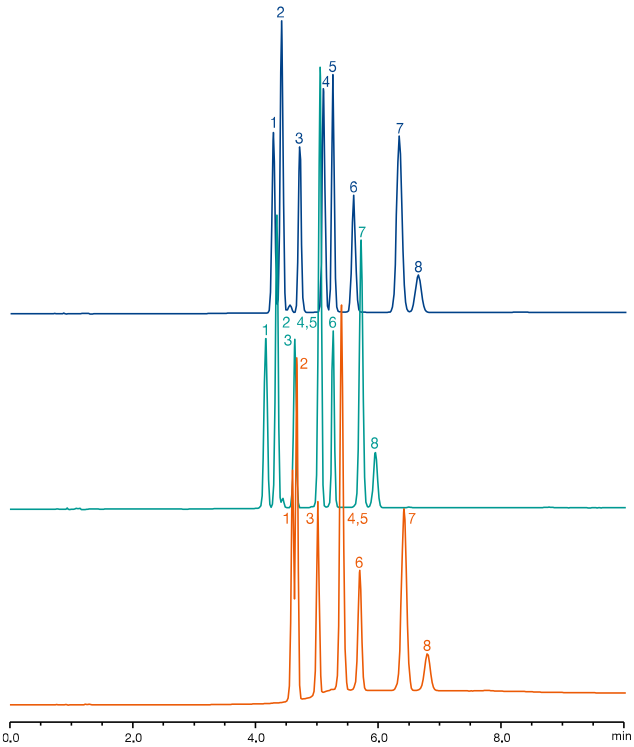
Sulfonamide antibiotics

MN Appl. No. 127920

Columns:	100 x 3 mm each NUCLEODUR® π², 5 µm Pinnacle® DB Biphenyl, 5 µm Ultra Biphenyl, 5 µm
Eluent:	A) 0.1 % TFA in water B) 0.1 % TFA in methanol 20 % B for 2 min, 20–60 % B in 2 min, 60 % B for 10 min
Flow rate:	0.56 mL/min
Temperature:	30 °C
Detection:	UV, 280 nm
Injection:	1 µL

Peaks:

1. Sulfathiazole
2. Sulfadiazine
3. Sulfachloropyridazine
4. Sulfamerazine
5. Sulfadimidine
6. Sulfamethoxazole
7. Sulfadimethoxine
8. Sulfaquinoxaline

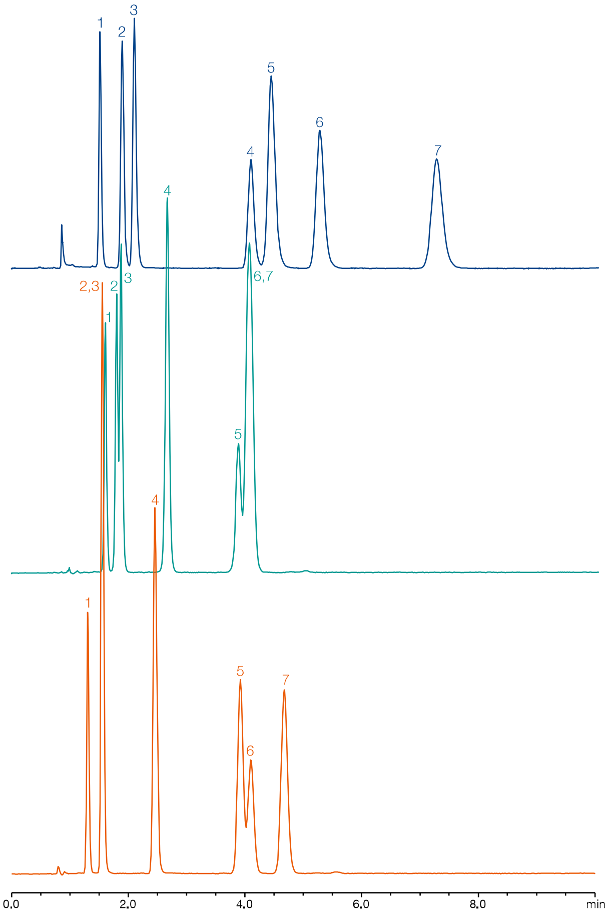


Steroids

MN Appl. No. 127910

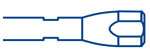
Columns: 125 x 4 mm each
NUCLEODUR® π², 5 µm
NUCLEODUR® Phenyl-Hexyl, 5 µm
NUCLEODUR® C_{18} Gravity, 5 µm
Eluent: acetonitrile – water (45:55, v/v)
Injection: 1 µL
Flow rate: 1 mL/min
Temperature: 25 °C
Detection: UV, 230 nm

Peaks:
1. Estriol
2. Hydrocortisone
3. Prednisone
4. β-Estradiol
5. Corticosterone
6. Cortisonacetate
7. Testosterone



Ordering information

Eluent in column acetonitrile – water

ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® π², 5 µm particle size 5 µm						
Analytical EC columns						
 2 mm	760620.20	760621.20	760622.20	760623.20	760624.20	760625.20
3 mm	760620.30	760621.30	760622.30	760623.30	760624.30	760625.30
4 mm	760620.40	760621.40	760622.40	760623.40	760624.40	760625.40
4.6 mm	760620.46	760621.46	760622.46	760623.46	760624.46	760625.46
EC guard columns*	4 x 2 mm: 761810.20		4 x 3 mm: 761810.30			

EC columns in packs of 1, guard columns in packs of 3.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of our column systems see page 250.

NUCLEODUR® PFP hydrophobic pentafluorophenyl phase · USP L43

Key feature

- Hydrophobic phase with alternative selectivity in comparison to classical C_{18} modifications
- Separation principle based on 4 retention mechanisms (polar interactions (H bonds), dipole-dipole, π-π, and hydrophobic interactions)
- Suitable for LC/MS due to low bleeding characteristics

Technical data

- Phase with pentafluorophenyl-propyl modification and multi-endcapping; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 8 %; pH stability 1–9

Recommended application

- Aromatic and unsaturated compounds, phenols, halogen compounds, isomers, polar compounds like pharmaceuticals, antibiotics; strong retention of basic compounds

Orthogonality in selectivity

Fluorinated stationary phases in HPLC have gained increasing interest over the last years. Most common representative of fluorinated silica phases is the pentafluorophenyl modification (PFP or F_5). Especially the orthogonal selectivity compared to traditional alkyl phases widens the scope in analytical HPLC.

Thus NUCLEODUR® PFP offers an excellent selectivity especially for highly polar analytes like aromatic and unsaturated compounds, phenols or halogenated hydrocarbons.

While a typical C_{18} phase just provides hydrophobic interactions between stationary phase and analyte NUCLEODUR® PFP offers four different retention mechanisms: polar interactions (H bonds), dipole-dipole, π-π, and hydrophobic interactions. Especially the pronounced ion exchange capacity and distinct steric selectivity are typical for fluorinated phases.

Due to low bleeding characteristics NUCLEODUR® PFP is also suitable for LC/MS. Based on a special surface modification procedure NUCLEODUR® PFP offers highest stability also at low pH values.

NUCLEODUR® PFP offers a completely different retention behavior compared to alkyl modified silica and is often used for separations which provide insufficient results on traditional C_{18} phases.

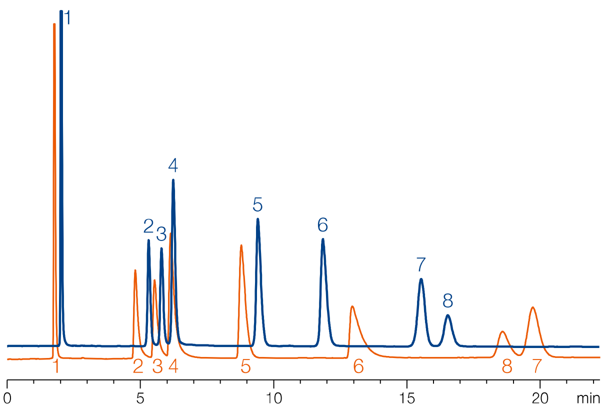
Applications in the areas of (bio-)pharma, natural compounds and environment show the broad applicability of this phase.

Separation of antihistamines

MN Appl. No. 124861

Columns:	250 x 3 mm NUCLEODUR® PFP, 5 µm
	250 x 3 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	acetonitrile – 20 mmol/L KH_2PO_4 (30:70, v/v)
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	UV, 210 nm

Peaks:
1. Maleic acid
2. Chlorpheniramine
3. Brompheniramine
4. Triprolidine
5. Diphenhydramine
6. Promethazine
7. Cetirizine
8. Hydroxyzine



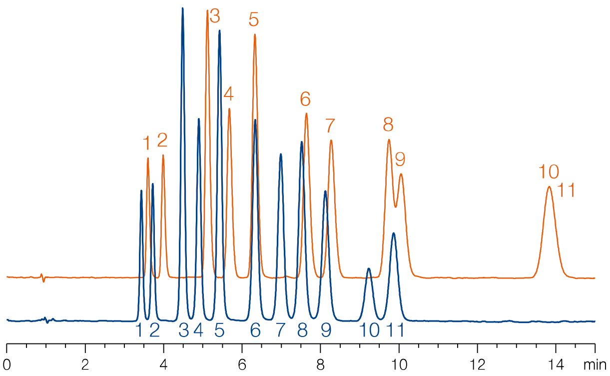
Separation of phenol isomers

MN Appl. No. 124531

Column: 125 x 4 mm NUCLEODUR® PFP, 5 µm
125 x 4 mm NUCLEODUR® C_{18} HTec, 5 µm
Eluent: acetonitrile, 0.1 % formic acid – water, 0.1 % formic acid (35:65, v/v)
Flow rate: 1 mL/min
Temperature: 35 °C
Detection: UV, 280 nm

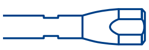
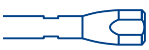
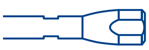
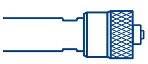
Peaks:

1. *o*-Kresol	5. 2,5-Dimethylphenol	9. 3,4-Dichlorophenol
2. *m*-Kresol	6. 2,6-Dichlorophenol	10. 2,4-Dibromophenol
3. 3,4-Dimethylphenol	7. 2,3-Dichlorophenol	11. 3,5-Dibromophenol
4. 3,5-Dimethylphenol	8. 2,4-Dichlorophenol	



Ordering information

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® PFP, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760431.20	760433.20	760435.20	760436.20		760438.20	
	3 mm	760431.30	760433.30		760436.30			
	4 mm	760431.40	760433.40		760436.40			
	4.6 mm	760431.46	760433.46		760436.46			
EC guard columns*			4 x 2 mm: 761975.20		4 x 3 mm: 761975.30			
NUCLEODUR® PFP, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760443.20		760446.20	760447.20	760448.20	760449.20
	3 mm		760443.30		760446.30	760447.30	760448.30	760449.30
	4 mm		760443.40		760446.40	760447.40	760448.40	760449.40
	4.6 mm		760443.46	760445.46	760446.46	760447.46	760448.46	760449.46
EC guard columns*			4 x 2 mm: 761976.20		4 x 3 mm: 761976.30			
NUCLEODUR® PFP, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760453.20		760456.20	760457.20	760458.20	760459.20
	3 mm		760453.30		760456.30	760457.30	760458.30	760459.30
	4 mm		760453.40		760456.40	760457.40	760458.40	760459.40
	4.6 mm		760453.46	760455.46	760456.46	760457.46	760458.46	760459.46
EC guard columns*			4 x 2 mm: 761977.20		4 x 3 mm: 761977.30			
Preparative VarioPrep columns								
	10 mm		762210.100			762211.100		762213.100
	21 mm		762210.210			762211.210		762213.210
	32 mm							762213.320
	40 mm						762212.400	762213.400
VP guard columns **			10 x 8 mm: 762214.80		10 x 16 mm: 762214.160		15 x 32 mm: 762216.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® Sphinx RP bifunctional RP phase · USP L1 and L11

Key feature

- Distinct selectivity based on well-balanced bifunctional surface coverage
- Widens the scope for method development based on additional π-π interactions
- Suitable for LC/MS due to low bleeding characteristics

Technical data

- Octadecyl and propylphenyl modified silica; pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 15 %; pH stability 1–10; high reproducibility and consistent quality

Recommended application

- Quinolone antibiotics, sulfonamides, xanthines, substituted aromatics

Alternative RP selectivity

NUCLEODUR® Sphinx RP is characterized by exceptional selectivity features generated by a well-balanced ratio of covalently bonded octadecyl and phenyl groups. The combination of classical hydrophobic with π-π interactions (aromatic ring system) expands the scope of selectivity in comparison with conventional reversed phase packings. NUCLEODUR® Sphinx RP is particularly suited for the separation of molecules containing aromatic and multiple bonds.

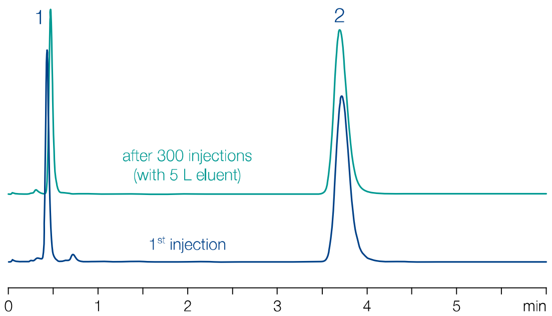
For the separation of polar compounds NUCLEODUR® Sphinx RP can be especially recommended and can also outperform many customary C_{18} phases. In addition, exhaustive endcapping steps minimize unwanted surface silanol activity and guarantee excellent peak shapes even for strong basic analytes.

Stability of NUCLEODUR® Sphinx RP at pH 10

MN Appl. No. 120900

Column:	50 x 4.6 mm NUCLEODUR® Sphinx RP, 5 µm
Eluent:	methanol – dil. NH_3, pH 10 (20:80, v/v)
Flow rate:	1.0 mL/min, temperature 30 °C
Detection:	UV, 275 nm
Injection:	3 µL

Peaks:
1. Theophylline
2. Caffeine



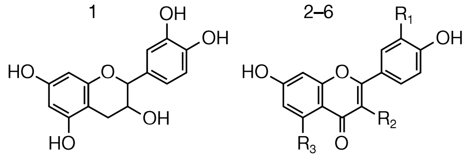
Different from standard phenyl phases, NUCLEODUR® Sphinx RP is far more stable towards hydrolysis and is also suggested for LC/MS applications. Due to the additional intermolecular interactions NUCLEODUR® Sphinx RP is an interesting replenishment to the high density bonded phases NUCLEODUR® C_8/C_{18} Gravity and the polar endcapped NUCLEODUR® C_{18} Pyramid.

Separation of flavonoids on three different NUCLEODUR® phases

MN Appl. No. 119830

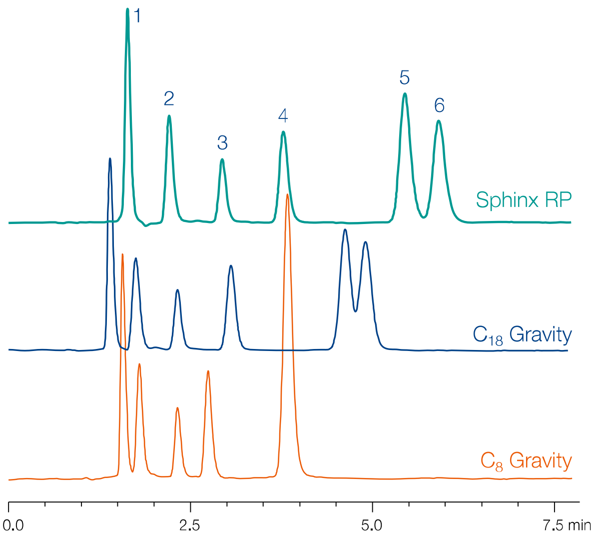
Columns:	150 x 4.6 mm
	NUCLEODUR® Sphinx RP, 5 µm
	NUCLEODUR® C_{18} Gravity, 5 µm
	NUCLEODUR® C_8 Gravity, 5 µm
Eluent:	water – methanol (40:60, v/v)
Flow rate:	1 mL/min
Temperature:	30 °C
Detection:	UV, 270 nm
Injection:	3 µL

1 2–6



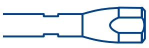
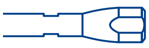
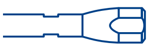
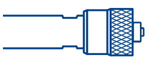
Peaks:

1. Catechin	
2. Rutin	$R_1 = R_3 = OH$, R_2 = O-Rutinose
3. Fisetin	$R_1 = R_2 = OH$, $R_3 = H$
4. Quercetin	$R_1 = R_2 = R_3 = OH$
5. Kaempferol	$R_1 = H$, $R_2 = R_3 = OH$
6. Isorhamnetin	$R_1 = OCH_3$, $R_2 = R_3 = OH$



Ordering information

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® Sphinx RP, 1.8 µm particle size 1.8 µm · UHPLC							
Analytical EC columns							
 2 mm	760821.20	760822.20	760825.20	760823.20		760824.20	
3 mm	760821.30	760822.30		760823.30			
4 mm	760821.40	760822.40		760823.40			
4.6 mm	760821.46	760822.46		760823.46			
EC guard columns*		4 x 2 mm: 761920.20		4 x 3 mm: 761920.30			
NUCLEODUR® Sphinx RP, 3 µm particle size 3 µm							
Analytical EC columns							
 2 mm		760806.20		760812.20	760807.20	760805.20	760808.20
3 mm		760806.30		760812.30	760807.30	760805.30	760808.30
4 mm		760806.40		760812.40	760807.40	760805.40	760808.40
4.6 mm		760806.46	760813.46	760812.46	760807.46	760805.46	760808.46
EC guard columns*		4 x 2 mm: 761921.20		4 x 3 mm: 761921.30			
NUCLEODUR® Sphinx RP, 5 µm particle size 5 µm							
Analytical EC columns							
 2 mm		760800.20		760809.20	760801.20	760802.20	760803.20
3 mm		760800.30		760809.30	760801.30	760802.30	760803.30
4 mm		760800.40		760809.40	760801.40	760802.40	760803.40
4.6 mm		760800.46	760815.46	760809.46	760801.46	760802.46	760803.46
EC guard columns*		4 x 2 mm: 761922.20		4 x 3 mm: 761922.30			
Preparative VarioPrep columns							
 10 mm		762372.100			762375.100		762373.100
21 mm		762372.210			762375.210		762373.210
32 mm							762373.320
40 mm						762371.400	762373.400
VP guard columns **		10 x 8 mm: 762390.80		10 x 16 mm: 762390.160		15 x 32 mm: 762392.320	

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.

NUCLEODUR® columns

NUCLEODUR® C_{18} HTec base-deactivated preparative octadecyl phase · USP L1

Key feature

- Reliable and durable standard RP phase for up-scaling to preparative scale, suited for LC/MS
- High loading capacity and excellent stability
- Outstanding base deactivation

Technical data

- High density octadecyl modification (C_{18}); pore size 110 Å; particle sizes 1.8 µm, 3 µm, 5 µm, 7 µm and 10 µm for analytical and preparative separations; carbon content 18 %, pH stability 1–11

Recommended application

- Sophisticated analytical and preparative separations of basic, neutral and acidic pharmaceuticals, derivatized amino acids, pesticides, fat-soluble vitamins, aldehydes, ketones and phenolic compounds

Preparative separations place high demands on silica based HPLC materials. Apart from excellent selectivity and base deactivation, robustness (pH, pressure stability, …) and capacity are vital criteria for optimal and efficient separation at the preparative scale.

Selectivity and base deactivation

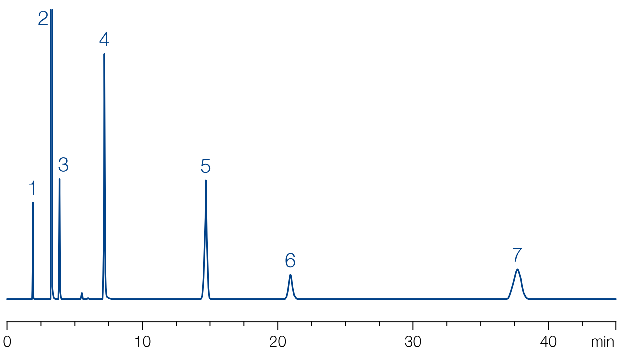
The innovative endcapping procedure leads to exceptionally good base deactivation – the Engelhardt test demonstrates superb selectivity, peak symmetry and peak shape over the entire polarity range. In addition NUCLEODUR® C_{18} HTec scores in low bleed characteristics and is therefore highly suitable for LC/MS.

Engelhardt test

MN Appl. No. 123580

Column:	250 x 4 mm NUCLEODUR® C_{18} HTec, 5 µm
Eluent:	methanol – water (49:51, v/v)
Flow rate:	1 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	5 µL

Peaks:
1. Uracil
2. Aniline
3. Phenol
4. *p*-Ethylaniline
5. *N,N*-Dimethylaniline
6. Toluene
7. Ethylbenzene



Stability and lifetime

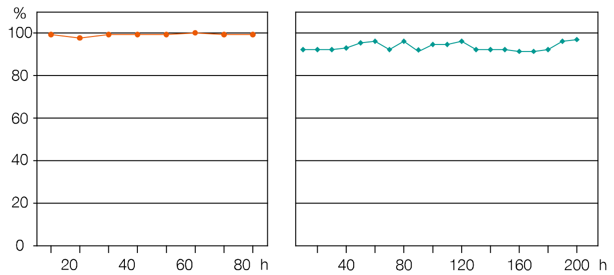
Based on fully synthetic and extremely robust totally spherical NUCLEODUR® silica, NUCLEODUR® C_{18} HTec offers outstanding mechanical rigidity and is thus the perfect choice also for self-packing of prep-columns. The special surface modification and endcapping procedure results in high chemical stability even at extreme chromatographic conditions like high flow rates, temperature or critical solvents (DMSO). Furthermore, NUCLEODUR® C_{18} HTec columns show a remarkably long lifetime in acidic (pH 1) as well as basic (pH 10) mobile phases.

pH stability test

Column:	150 x 4 mm NUCLEODUR® C_{18} HTec, 5 µm
Flow rate:	1 mL/min
Detection:	UV, 254 nm
Injection:	5 µL

• pH 1:
Eluent: acetonitrile – 1 % TFA in water (50:50, v/v); 80 °C
% initial retention of ethylbenzene
693 injections

• pH 10:
Eluent: methanol – 50 mmol/L triethylamine (25:85, v/v); 50 °C
% initial N of theophylline
1034 injections



Due to innovative surface coating procedures NUCLEODUR® C_{18} HTec offers excellent analytical separation properties and is the first choice for up-scaling to preparative column dimensions.

Up-scaling

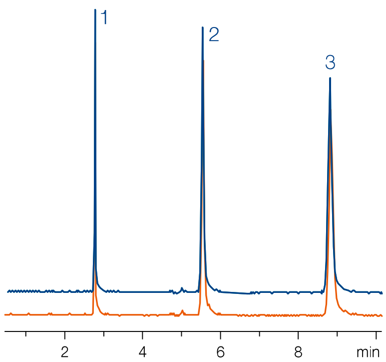
Due to highest quality standards in silica production and phase chemistry combined with optimized packing technology, NUCLEODUR® C_{18} HTec allows exceptional transferability from analytical to preparative scale with respect to different particle sizes (e.g., 5, 7 or 10 µm) as well as column dimensions (e.g., ID 4.6 to 21 mm).

Up-scaling with NUCLEODUR® C_{18} HTec

MN Appl. No. 123780

Columns:	EC 250 x 4,6 mm NUCLEODUR® C_{18} HTec, 5 µm VP 250 x 21 mm NUCLEODUR® C_{18} HTec, 5 µm
Eluent:	acetonitrile – water (80:20, v/v)
Flow rate:	1.3 mL/min / 27 mL/min
Temperature:	22 °C
Pressure:	84 bar / 109 bar
Detection:	UV, 254 nm
Injection:	3 µL / 60 µL

Peaks: (1 mg/mL each)
1. Phenol
2. Naphthalene
3. Anthracene



Capacity

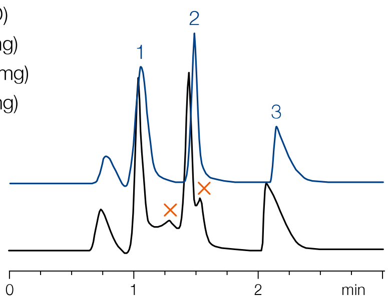
A vital criterion for efficiency in preparative HPLC is the capacity of the separation medium. NUCLEODUR® C_{18} HTec is characterized by a notably high loading capacity under both basic and acidic conditions, while competitor columns show overload effects even at lower loads (x).

Loading capacity under acidic conditions

MN Appl. No. 123890

Columns:	VP 100 x 21 mm NUCLEODUR® C_{18} HTec, 5 µm 100 x 21.2 mm AXIA™ Gemini® 5 µm C18 110 Å
Eluent:	acetonitrile – formic acid in H_2O pH 3.0 (30:70, v/v)
Flow rate:	28 mL/min
Temperature:	22 °C
Pressure:	124 bar
Detection:	UV, 254 nm

Peaks:
total load 40 mg
(sample dissolved in DMSO)
1. 4-Acetamidophenol (5 mg)
2. 2-Acetamidophenol (10 mg)
3. Acetylsalicylic acid (25 mg)



Ordering information

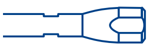
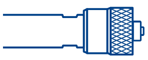
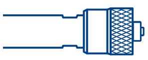
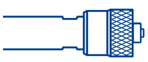
Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} HTec, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760301.20	760305.20	760304.20	760306.20		760308.20	
	3 mm	760301.30	760305.30		760306.30			
	4 mm	760301.40	760305.40		760306.40			
	4.6 mm	760301.46	760305.46		760306.46			
EC guard columns*			4 x 2 mm: 761925.20		4 x 3 mm: 761925.30			
NUCLEODUR® C_{18} HTec, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760321.20		760323.20	760324.20	760325.20	760326.20
	3 mm		760321.30		760323.30	760324.30	760325.30	760326.30
	4 mm		760321.40		760323.40	760324.40	760325.40	760326.40
	4.6 mm		760321.46	760322.46	760323.46	760324.46	760325.46	760326.46
EC guard columns*			4 x 2 mm: 761926.20		4 x 3 mm: 761926.30			

NUCLEODUR® columns

Ordering information

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} HTec, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760311.20		760313.20	760314.20	760315.20	760316.20
	3 mm		760311.30		760313.30	760314.30	760315.30	760316.30
	4 mm		760311.40		760313.40	760314.40	760315.40	760316.40
	4.6 mm		760311.46	760312.46	760313.46	760314.46	760315.46	760316.46
EC guard columns*			4 x 2 mm: 761927.20		4 x 3 mm: 761927.30			
Preparative VarioPrep columns								
	10 mm		762551.100			762554.100		762556.100
	21 mm		762551.210		762553.210	762554.210		762556.210
	32 mm				762553.320		762555.320	762556.320
	40 mm						762555.400	762556.400
	50 mm				762553.500		762555.500	762556.500
VP guard columns **			10 x 8 mm: 762591.80		10 x 16 mm: 762591.160			
			15 x 32 mm: 762592.320		15 x 50 mm: 762592.500			
NUCLEODUR® C_{18} HTec, 7 µm particle size 7 µm								
Preparative VarioPrep columns								
	10 mm		762561.100			762564.100		762566.100
	21 mm		762561.210		762563.210	762564.210		762566.210
	32 mm				762563.320		762565.320	762566.320
	40 mm						762565.400	762566.400
	50 mm				762563.500		762565.500	762566.500
VP guard columns **			10 x 8 mm: 762591.80		10 x 16 mm: 762591.160			
			15 x 32 mm: 762592.320		15 x 50 mm: 762592.500			
NUCLEODUR® C_{18} HTec, 10 µm particle size 10 µm								
Preparative VarioPrep columns								
	10 mm		762571.100			762574.100		762576.100
	21 mm		762571.210		762573.210	762574.210		762576.210
	32 mm				762573.320		762575.320	762576.320
	40 mm						762575.400	762576.400
	50 mm				762573.500		762575.500	762576.500
VP guard columns **			10 x 8 mm: 762591.80		10 x 16 mm: 762591.160			
			15 x 32 mm: 762592.320		15 x 50 mm: 762592.500			

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.
NUCLEODUR® C_{18} HTec bulk material in 7 and 10 µm for self-packing of preparative columns see page 256.

NUCLEODUR® C_{18} ec · C_8 ec · C_4 ec nonpolar phases for routine analysis · USP L1 (C_{18}) · L7 (C_8) · L26 (C_4)

Key feature

- Ideal and reliable standard RP phase for daily routine analysis and up-scaling for preparative HPLC
- Medium density Octadecyl (C_{18}) and octyl (C_8) with pore size of 110 Å with exhaustive endcapping for a wide range of applications
- Octadecyl (C_{18}) and butyl (C_4) with pore size of 300 Å for the separation of biomolecules

Technical data

- Pore size 110 Å:
  particle sizes 3 µm and 5 µm, 7 µm, 10 µm, 12 µm, 16 µm, 20 µm, 30 µm and 50 µm for preparative separations; carbon content 17.5 % for C_{18}, 10.5 % for C_8; pH stability 1–9; high reproducibility from lot to lot
- Pore size 300 Å:
  technical data and applications in chapter "HPLC column for biochemical separations" (see page 241)

Recommended application

- 110 Å:
  basic, neutral or acidic drugs; derivatized amino acids; pesticides; fat-soluble vitamins; aldehydes and ketones; phenolic compounds
- 300 Å:
  biomolecular macromolecules, like proteins and peptides

NUCLEODUR® C_{18} ec for daily routine analysis

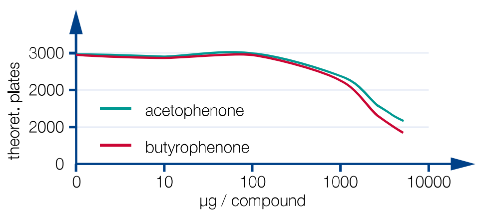
The efficiency of a separation is controlled by particle size and selectivity of the stationary phase. The exceptional surface coverage of monomeric bonded alkylsilanes, combined with an exhaustive endcapping, results in a surface with lowest silanol activity. This allows the tailing-free elution of polar compounds such as basic drugs. NUCLEODUR® C_{18} ec is available in 9 different particle sizes (3, 5, 7, 10, 12, 16, 20, 30 and 50 µm) which cover the whole range from high speed analytical HPLC up to medium and low pressure prep LC. NUCLEODUR® C_{18} ec is also an ideal tool for scale-up purposes.

Loading capacity

Loading capacity, probably the most important feature for preparative LC applications, is determined by pore size, pore volume and surface area of the packing. However, it can also be influenced by the molecular weight of the analytes. In the figure below the mass loading curve for acetophenone and butyrophenone on a NUCLEODUR® 100-20 C_{18} ec column describes the correlation between the increase of column loading and the decrease of separation efficiency.

Loading curve

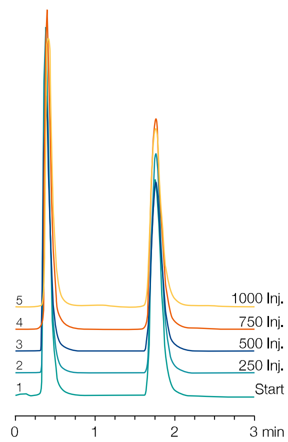
Column:	250 x 4.6 mm NUCLEODUR® 100-20 C_{18} ec
Eluent:	acetonitrile – H_2O 80:20 (v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 280 – 370 nm



pH stability of NUCLEODUR® C_{18} ec

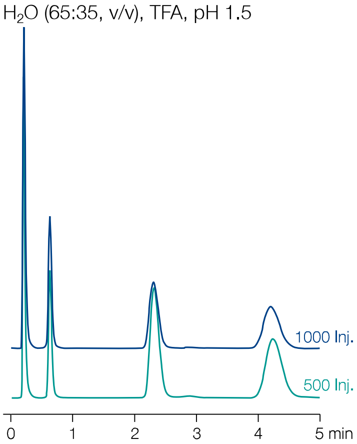
Separation of theophylline and caffeine at pH 10

Column:	30 x 3 mm NUCLEODUR® 100-5 C_{18} ec
Eluent:	methanol – aq. NH_3 (20:80, v/v), pH 10
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm



Separation of uracil, veratrol, toluene and ethylbenzene at pH 1.5

Column:	30 x 3 mm NUCLEODUR® 100-5 C_{18} ec
Eluent:	acetonitrile – H_2O (65:35, v/v), TFA, pH 1.5
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm



Chemical stability

The utmost purity of the base silica and the exceptional silane bonding chemistry minimize the risk of dissolution, or hydrolysis at pH extremes.

The chromatograms show the retention behavior at pH values of 1.5 and 10.0 for NUCLEODUR® 100-5 C_{18} ec.

NUCLEODUR® octyl phases

In addition to NUCLEODUR® C_{18} phases MACHEREY-NAGEL offers octyl modified NUCLEODUR® C_8 Gravity and NUCLEODUR® C_8 ec columns to expand the RP tool box. Based on the same spherical high purity silica the C_8 phases exhibit the same chemical and mechanical stability as the C_{18} counterparts. Indeed NUCLEODUR® C_8 Gravity can also be run at pH extremes (pH 1–11) by choosing appropriate elution parameters. Due to the shorter chain and less hydrophobic properties of the stationary phase the retention of non-polar compounds is decreased, and in consequence a reduction in time of analysis can be achieved. Moreover a stronger polar selectivity, particularly with the separation of ionizable analytes is frequently observed (as distinct from the C_{18} phases). NUCLEODUR® C_8 ec and NUCLEODUR® C_8 Gravity are most suitable for the development of new methods but also for robust routine analyses.

There are no general guidelines which could make the choice between C_8 and C_{18} phases easier but it will always be beneficial to add both phases to the existing pool of RP columns in the laboratory. Comparative studies reveal some different selectivity patterns of NUCLEODUR® C_8 ec and C_{18} ec. The separation of phenols at right shows baseline separation for 2-ethoxyphenol and dimethoxybenzene (veratrol) and in addition a reversal of the elution order of phenol and 4-methoxyphenol can be shown on the octyl phase.

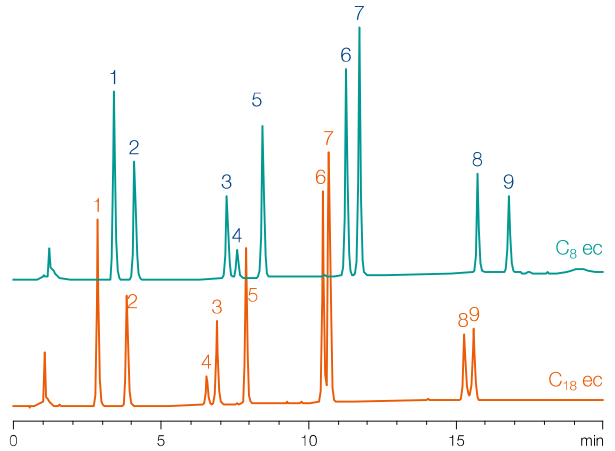
Separation of phenols

MN Appl. Nos. 120890 / 120891

Columns:	250 x 4 mm NUCLEODUR® 100-5 C_8 ec / C_{18} ec
Eluent:	A) water, B) methanol
	C_8: 20 % B (2 min) → 60 % B in 12 min
	C_{18}: 25 % B (2 min) → 65 % B in 12 min
Flow rate:	1.0 mL/min, temperature 25 °C
Detection:	UV, 275 nm, injection 10 µL

Peaks:

1. Resorcinol
2. Pyrocatechol
3. 4-Methoxyphenol
4. Phenol
5. 2-Methoxyphenol
6. 2-Ethoxyphenol
7. Veratrol
8. Biphenyl-2-ol
9. Phenetole



NUCLEODUR® phases for biochromatography

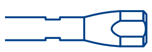
A description and applications for C_{18} and C_4 modified 300 Å NUCLEODUR® widepore materials for the separation of biopolymers, like peptids and proteins can be found in chapter "HPLC column for biochemical separations" (see page 241).

C_{18} or C_8 · the best of both worlds

- Octyl phases (C_8) show superior polar selectivity.
- Octadecyl phases (C_{18}) show superior hydrophobic selectivity.
- Hydrophobic compounds show shorter retention times on C_8 phases.

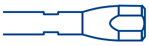
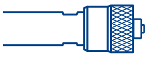
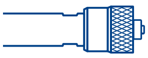
Ordering information

Eluent in column acetonitrile – water

	ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 C_{18} ec octadecyl phase, particle size 3 µm, 17.5 % C							
Analytical EC columns							
	2 mm	760050.20		760054.20	760051.20	760053.20	760052.20
	3 mm	760050.30		760054.30	760051.30	760053.30	760052.30
	4 mm	760050.40		760054.40	760051.40	760053.40	760052.40
	4.6 mm	760050.46	760046.46	760054.46	760051.46	760053.46	760052.46
EC guard columns*			4 x 2 mm: 761931.20		4 x 3 mm: 761931.30		

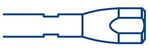
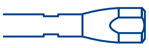
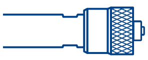
Ordering information

Eluent in column acetonitrile – water

	ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-5 C_{18} ec octadecyl phase, particle size 5 µm, 17.5 % C							
Analytical EC columns							
	2 mm	760004.20		760013.20	760001.20	760008.20	760002.20
	3 mm	760004.30		760013.30	760001.30	760008.30	760002.30
	4 mm	760004.40		760013.40	760001.40	760008.40	760002.40
	4.6 mm	760004.46	760035.46	760013.46	760001.46	760008.46	760002.46
EC guard columns*			4 x 2 mm: 761932.20		4 x 3 mm: 761932.30		
Preparative VarioPrep columns							
	10 mm	762003.100			762029.100		762022.100
	21 mm	762003.210			762029.210		762022.210
	32 mm						762022.320
	40 mm					762027.400	762022.400
VP guard columns **			10 x 8 mm: 762090.80		10 x 16 mm: 762090.160		
			15 x 32 mm: 762311.320		15 x 50 mm: 762311.500		
NUCLEODUR® 100-10 C_{18} ec octadecyl phase, particle size 10 µm, 17.5 % C							
Preparative VarioPrep columns							
	10 mm	762011.100			762302.100		762010.100
	21 mm	762011.210			762302.210		762010.210
	32 mm						762010.320
	40 mm					762303.400	762010.400
	50 mm						762010.500
VP guard columns **			10 x 8 mm: 762090.80		10 x 16 mm: 762090.160		
			15 x 32 mm: 762311.320		15 x 50 mm: 762311.500		

Ordering information

Eluent in column acetonitrile – water

	ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 C_8 ec octyl phase, particle size 3 µm, 10.5 % C							
Analytical EC columns							
	2 mm	760063.20		760059.20	760060.20		760062.20
	3 mm	760063.30		760059.30	760060.30		760062.30
	4 mm	760063.40		760059.40	760060.40		760062.40
	4.6 mm	760063.46	760064.46	760059.46	760060.46	760061.46	760062.46
EC guard columns*			4 x 2 mm: 761936.20		4 x 3 mm: 761936.30		
NUCLEODUR® 100-5 C_8 ec octyl phase, particle size 5 µm, 10.5 % C							
Analytical EC columns							
	2 mm	760700.20		760704.20	760701.20		760703.20
	3 mm	760700.30		760704.30	760701.30		760703.30
	4 mm	760700.40		760704.40	760701.40		760703.40
	4.6 mm	760700.46	760706.46	760704.46	760701.46	760702.46	760703.46
EC guard columns*			4 x 2 mm: 761937.20		4 x 3 mm: 761937.30		
Preparative VarioPrep columns							
	10 mm	762072.100			762061.100		762062.100
	21 mm	762072.210			762061.210		762062.210
	32 mm						762062.320
	40 mm					762079.400	762062.400
VP guard columns **		10 x 8 mm: 762092.80		10 x 16 mm: 762092.160		15 x 32 mm: 762321.320	

EC and VarioPrep columns in packs of 1, guard columns see previous NUCLEODUR® phases.

Guard column systems see previous NUCLEODUR® phases. For details of our column systems see page 250.
NUCLEODUR® C_{18} ec bulk material with 10–50 µm for self-packing of preparative columns see page 256.
The ordering information for C_{18} and C_4 modified 300 Å NUCLEODUR® widepore materials for the separation of biopolymers can be found in the chapter "HPLC column for biochemical separations" (see page 241).
* and ** for corresponding guard column systems see page 180.

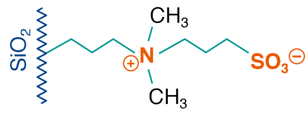
NUCLEODUR® HILIC zwitterionic phase

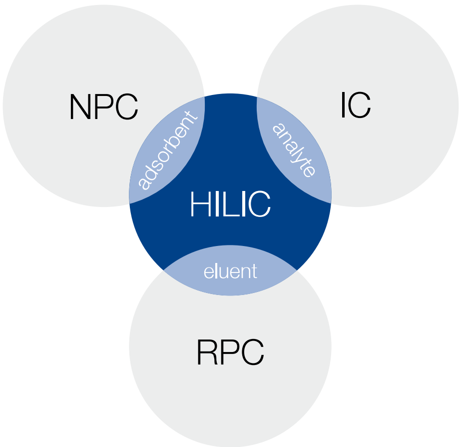
Key feature
- Ideal for reproducible and stable chromatography of highly polar analytes
- Suitable for analytical and preparative applications
- Very short column conditioning period

Technical data
- Ammonium - sulfonic acid modified silica; pore size 110 Å; particle sizes 1.8, 3 and 5 µm; carbon content 7 %; pH stability 2–8.5

Recommended application
- Hydrophilic compounds such as organic polar acids and bases, polar natural compounds, nucleosides, oligonucleotides, amino acids, peptides, water soluble vitamins

Hydrophilic interaction chromatography





Especially for polar compounds reversed phase HPLC – the most common analytical method – is often limited. Here, hydrophilic stationary phases provide an additional tool for the separation of polar analytes in HPLC.

The expression HILIC (Hydrophilic Interaction Chromatography) was firstly published by Andrew Alpert in 1990 – since then it took quite some efforts to develop robust and reproducible hydrophilic HPLC phases for HILIC chromatography [7].

HILIC combines the characteristics of the 3 major methods in liquid chromatography – reversed phase (RPC), normal phase (NPC) and ion chromatography (IC):

- Stationary phases (adsorbents) are mostly polar modifications of silica or polymers (SiOH, NH_2, Diol, (zwitter) ions, …) – like in NPC.
- Mobile phases (eluents) are mixtures of aqueous buffer systems and organic modifier like acetonitrile or methanol - like in RPC.
- Fields of application include quite polar compounds as well as organic and inorganic ions – like in IC.

Summarized: "HILIC is NP chromatography of polar and ionic compounds under RP conditions."

NUCLEODUR® HILIC is a special zwitterionic modified stationary phase based on ultra spherical NUCLEODUR® particles. The betaine character of the ammoniumsulfonic acid ligands results in total charge equalization and in an overall neutrally charged but highly polar surface

Retention characteristic

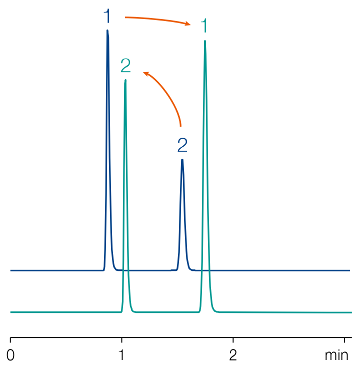
Commonly HILIC is described as partition chromatography or liquid-liquid extraction system between mobile and stationary phases. Versus a water-poor mobile phase a water-rich layer on the surface of the polar stationary phase is formed. Thus, a distribution of the analytes between these two layers will occur. Furthermore HILIC includes weak electrostatic mechanisms as well as hydrogen donor interactions between neutral polar molecules under high organic elution conditions. This distinguishes HILIC from ion exchange chromatography - main principle for HILIC separation is based on compound's polarity and degree of solvation.

Separation of uracil and naphthalene

MN Appl. Nos. 122911 / 122912

Columns: A) 125 x 4 mm NUCLEODUR® C_{18} Pyramid, 3 µm
B) 125 x 4 mm NUCLEODUR® HILIC, 3 µm
Eluent: acetonitrile – water (90:10, v/v))
Flow rate: 1.0 mL/min, temperature 25 °C
Detection: UV, 254 nm

Peaks:
1. Uracil
2. Naphthalene



More polar compounds will have stronger interaction with the stationary aqueous layer than less polar compounds – resulting in a stronger retention. Nonpolar compounds exhibit faster elution profiles due to minor hydrophobic interactions. In the separation of uracil and naphthalene the elution order is quite often inverse on HILIC columns compared to RP columns.

Stability features

Due to an advanced and unique surface modification procedure (pat. pend.) NUCLEODUR® HILIC columns provide short equilibration times – after just 20 min equilibration already the 2nd injection shows stable and reproducible results.

Beyond this, NUCLEODUR® HILIC columns are characterized by an outstanding column life time - even after nearly 800 runs the columns show no loss of pristine performance - peak shape and retention are still immaculate. Due to its high loading capacity NUCLEODUR® HILIC is absolutely suitable for preparative and semi-preparative applications.

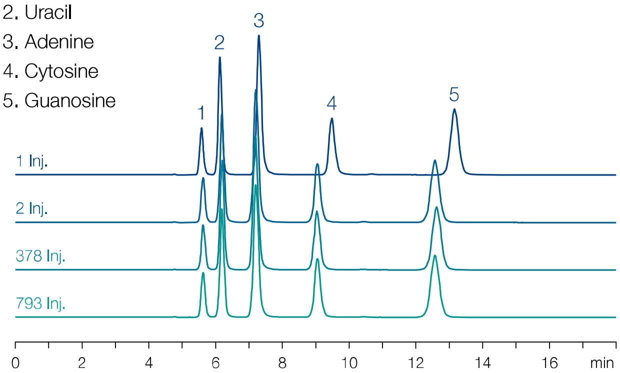
Overall NUCLEODUR® HILIC provides excellent chromatographic features and is hereby the perfect choice for separation of polar or charged compounds.

Stability and equilibration

MN Appl. No. 123100

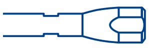
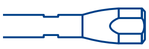
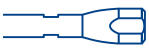
Column:	250 x 4 mm NUCLEODUR® HILIC, 5 µm
Eluent:	CH_3CN – 5 mmol/L ammonium acetate (80:20, v/v)
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm

Peaks:
1. Thymine
2. Uracil
3. Adenine
4. Cytosine
5. Guanosine



Ordering information

Eluent in column acetonitrile – water (80:20, v/v)

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® HILIC, 1.8 µm particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760521.20	760523.20	760525.20	760526.20		760528.20	
	3 mm	760521.30	760523.30		760526.30			
	4 mm	760521.40	760523.40		760526.40			
	4.6 mm	760521.46	760523.46		760526.46			
EC guard columns*			4 x 2 mm: 761960.20		4 x 3 mm: 761960.30			
NUCLEODUR® HILIC, 3 µm particle size 3 µm								
Analytical EC columns								
	2 mm		760532.20		760534.20	760531.20	760533.20	760530.20
	3 mm		760532.30		760534.30	760531.30	760533.30	760530.30
	4 mm		760532.40		760534.40	760531.40	760533.40	760530.40
	4.6 mm		760532.46		760534.46	760531.46	760533.46	760530.46
EC guard columns*			4 x 2 mm: 761961.20		4 x 3 mm: 761961.30			
NUCLEODUR® HILIC, 5 µm particle size 5 µm								
Analytical EC columns								
	2 mm		760552.20		760554.20	760551.20	760553.20	760550.20
	3 mm		760552.30		760554.30	760551.30	760553.30	760550.30
	4 mm		760552.40		760554.40	760551.40	760553.40	760550.40
	4.6 mm		760552.46		760554.46	760551.46	760553.46	760550.46
EC guard columns*			4 x 2 mm: 761962.20		4 x 3 mm: 761962.30			

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of our column systems see page 250.

NUCLEODUR® CN / CN-RP cyano-modified high purity silica phase · USP L10

Key feature

- High retention capacity especially for very polar and unsaturated compounds
- Multi-mode column (RP and NP) widens scope of selectivity
- Stable against hydrolysis at low pH (working range pH 1–8)

Technical data

- Cyanopropyl-modified high purity silica; pore size 110 Å; particle sizes 3 μm and 5 μm; carbon content 7 %; special endcapping
- High reproducibility from lot to lot; different retention characteristics in comparison to C_8 and C_{18}

Recommended application

- Tricyclic antidepressants, steroids, organic acids

Alternative bonded-phase functionality

In reversed phase HPLC it is fairly common to start with C_{18} or C_8 columns, if new methods have to be developed. However, superior polarity and selectivity properties often required for more sophisticated separations, are not always sufficiently provided by classical RP phases, which are usually characterized by a hydrophobic layer of monomeric or polymeric bonded alkylsilanes.

One approach to improve the resolution of compounds poorly separated on nonpolar stationary phases, is to change bonded-phase functionality.

The fully endcapped and highly reproducible NUCLEODUR® 100-5 CN-RP phase has cyanopropyl groups on the surface able to generate a clearly recognizable different retention behavior compared to purely alkyl-functionalized surface modifications (see figure below).

The polarity of NUCLEODUR® 100-5 CN-RP can be classified as intermediate based on multiple retention mechanisms such as dipole-dipole, π-π, and also hydrophobic interactions [8]. Therefore, this phase shows a distinct selectivity for polar organic compounds as well as for molecules containing π electron systems (e.g., analytes with double bonds, tricyclic antidepressants) [9].

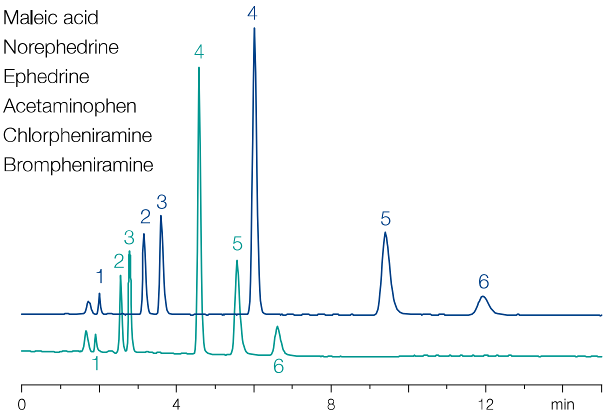
Short-chain bonded phases are sometimes suspected of revealing shortcomings in stability towards hydrolysis at low pH [10]. Application 119350 shows that even after 100 sample injections and four weeks storage at pH 1 (blue curve), neither a considerable shift in retention, nor a visible change in peak symmetry could be noticed (green curve = new column)

Separation of cold medicine ingredients on two different NUCLEODUR® phases

MN Appl. No. 119340

Columns:	250 x 4 mm NUCLEODUR® 100-5 C_{18} ec
	250 x 4 mm NUCLEODUR® 100-5 CN-RP
Eluent:	acetonitrile – 100 mmol/L sodium citrate pH 2.5 (15:85, v/v)
Flow rate:	1.0 mL/min, temperature 25 °C
Detection:	UV, 254 nm, injection 10 μL

Peaks:
1. Maleic acid
2. Norephedrine
3. Ephedrine
4. Acetaminophen
5. Chlorpheniramine
6. Brompheniramine

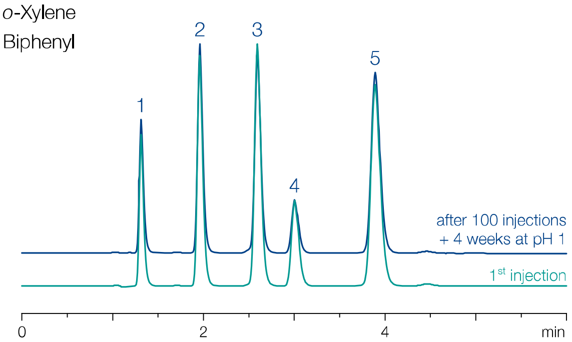


Stability of NUCLEODUR® CN-RP at pH 1

MN Appl. No. 119350

Columns:	125 x 4 mm NUCLEODUR® 100-5 CN-RP
Eluent:	acetonitrile – water, 2 % TFA pH 1 (50:50, v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm
Injection:	5 μL

Peaks:
1. Benzamide
2. Dimethyl phthalate
3. Phenetole
4. *o*-Xylene
5. Biphenyl



Multi-mode columns

Due to its polarity the cyano phase can also be run in normal phase mode. NUCLEODUR® CN columns for NP applications are shipped in *n*-heptane. The change in selectivity and order of elution for a mixture of various steroids in NP and RP mode is displayed below. The high coverage combined with a thorough endcapping makes NUCLEODUR® 100-5 CN-RP suitable for separation of ionizable compounds such as basic drugs.

Separation of steroids in normal phase and reversed phase mode

MN Appl. Nos. 119271 / 119272

Normal phase mode

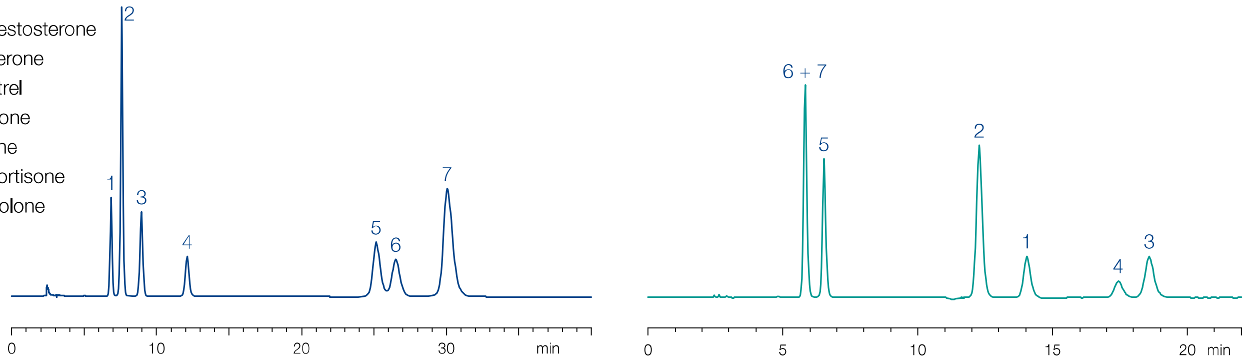
Column:	250 x 4 mm NUCLEODUR® 100-5 CN
Eluent:	*n*-heptane – 2-propanol (90:10, v/v)
Flow rate:	1.0 mL/min, temperature 25 °C
Detection:	UV, 254 nm, injection 10 μL

Reversed phase mode

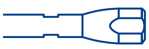
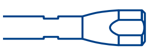
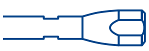
Column:	250 x 4 mm NUCLEODUR® 100-5 CN-RP
Eluent:	acetonitrile – water (25:75, v/v)

other conditions as for normal phase mode

Peaks:
1. Methyltestosterone
2. Testosterone
3. Norgestrel
4. Medrysone
5. Cortisone
6. Hydrocortisone
7. Prednisolone



Ordering information

ID	Length → 50 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 CN-RP particle size 3 μm; eluent in column acetonitrile – water				
Analytical EC columns 				
2 mm	760159.20	760157.20		
3 mm		760157.30		
4 mm			760156.40	
4.6 mm			760156.46	
EC guard columns*	4 x 2 mm: 761941.20		4 x 3 mm: 761941.30	
NUCLEODUR® 100-5 CN-RP particle size 5 μm; eluent in column acetonitrile – water				
Analytical EC columns 				
4 mm		760153.40		760152.40
4.6 mm		760153.46	760154.46	760152.46
EC guard columns*			4 x 3 mm: 761944.30	
NUCLEODUR® 100-5 CN particle size 5 μm; eluent in column *n*-heptane				
Analytical EC columns 				
4 mm		760151.40	760149.40	760150.40
4.6 mm		760151.46	760149.46	760150.46
EC guard columns*			4 x 3 mm: 761943.30	

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of our column systems see page 250.

NUCLEODUR® NH_2 / NH_2-RP amino-modified high purity silica · USP L8

Key feature

- Multi-mode columns (for RP, NP and IC)
- Stable against hydrolysis at low pH (working range pH 2–8), 100 % stable in water; suitable for LC/MS
- Widens scope of analytical HPLC into the polar range

Technical data

- Aminopropyl modified high purity silica; pore size 110 Å; particle sizes 3, 5 and 7 µm; carbon content 2.5 %; not endcapped

Recommended application

- Polar compounds under RP conditions (sugars, DNA bases), hydrocarbons under NP conditions

- Normal phase chromatography (NP) with hexane, dichloromethane or 2-propanol as mobile phase for polar compounds such as substituted anilines, esters, chlorinated pesticides
- Reversed phase chromatography (RP) of polar compounds in aqueous-organic eluent systems
- Ion exchange chromatography of anions and organic acids using conventional buffers and organic modifiers

Some compounds, especially polar substances, cannot be sufficiently resolved on C_{18} phases. Polar-modified silica phases offer alternative selectivities thus expanding the spectrum of analytical HPLC into the polar range.

Multi-mode columns

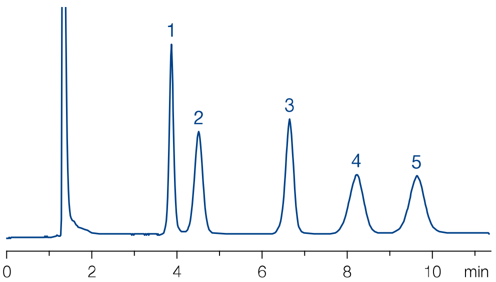
Besides cyano modifications, amino modifications belong to the most frequently used polar silica phases – both feature the important advantage, that they can be run in the RP mode using aqueous-organic eluent mixtures as well as in the NP mode, e.g., with hexane as mobile phase.

Reversed phase separation of sugars

MN Appl. No. 122160

Column: 250 x 4 mm NUCLEODUR® 100-5 NH_2-RP
Eluent: acetonitrile – water (79:21, v/v)
Flow rate: 2 mL/min
Detection: RI

Peaks:
1. Fructose
2. Glucose
3. Saccharose
4. Maltose
5. Lactose



NUCLEODUR® NH_2, too, belongs to the so-called multimode columns. It can be used for RP chromatography of polar compounds such as sugars in aqueous-organic eluent systems, for NP chromatography of substituted aromatics or chlorinated pesticides with organic mobile phases such as hexane, dichloromethane or 2-propanol, but also for ion exchange chromatography of anions and organic acids using conventional buffers and organic modifiers.

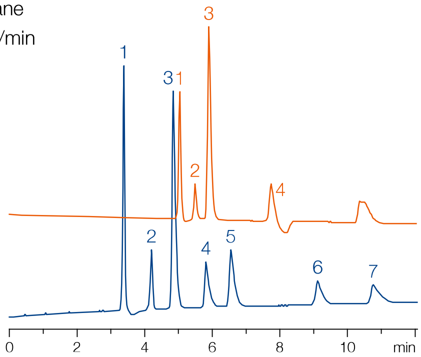
Main field of application of NUCLEODUR® NH_2 is the separation of simple and complex sugars, sugar alcohols and other hydroxy compounds under RP conditions as well as hydrocarbons under NP conditions.

Normal phase separation of middle distillates in accordance with DIN EN 12916

MN Appl. No. 122180

Columns: A) 250 x 4 mm NUCLEODUR® 100-5 NH_2
B) conventional aminopropyl phase
Eluent: heptane
Flow rate: 1 mL/min
Detection: RI

Peaks:
1. Cyclohexane
2. 1-Phenyldodecane
3. 1,2-Dimethylbenzene
4. Hexamethylbenzene
5. Naphthalene
6. Dibenzothiophene
7. 9-Methylanthracene



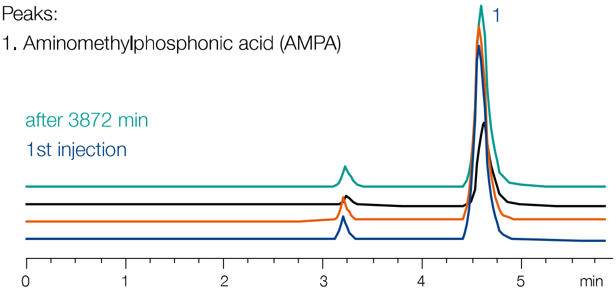
Due to the special method of surface modification NUCLEODUR® NH_2 features a pronounced stability at higher as well as at lower pH values. The following figure shows, that even after several days of exposure of the column material at pH 1.75 good separation efficiency and peak symmetry are maintained. The resulting high column life allows cost reduction due to lower column consumption.

This example shows the enhanced pH stability of NUCLEODUR® NH_2 and the outstanding suitability for the separation of total herbicides (AMPA, glyphosate, glufonisate, …) - see application 122190 in our online data base at *www.mn-net.com/apps*.

Hydrolytical resistance of NUCLEODUR® NH_2-RP

Column: 250 x 4 mm NUCLEODUR® 100-5 NH_2-RP
Eluent: acetonitrile – 50 mmol/L KH_2PO_4, pH 1.75 (50:50, v/v)
Flow rate: 0.6 mL/min
Detection: UV, 254 nm

Peaks:
1. Aminomethylphosphonic acid (AMPA)

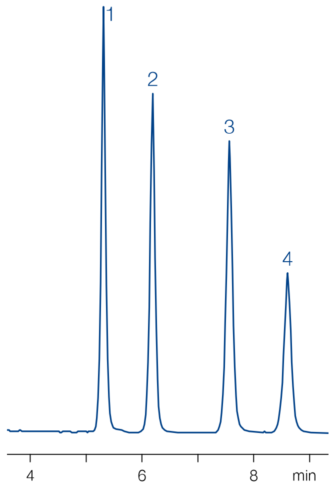


Separation of DNA bases

MN Appl. No. 122170

Column: 250 x 4 mm NUCLEODUR® 100-5 NH_2-RP
Eluent: acetonitrile – water (80:20, v/v)
Flow rate: 0.6 mL/min
Temperature: 35 °C
Pressure: 30 bar
Detection: UV, 254 nm

Peaks:
1. Thymine
2. Uracil
3. Cytosine
4. Adenine



Based on superspherical NUCLEODUR® this phase features a high pressure stability, which makes it the perfect choice for preparative separations as well as for LC/MS. Additionally, the high batch-to-batch reproducibility of NUCLEODUR® NH_2 enables reliable analyses especially for routine work.

Ordering information

	ID	Length → 100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 NH_2-RP particle size 3 µm; eluent in column acetonitrile – water					
Analytical EC columns					
	2 mm	760740.20	760741.20		
	4.6 mm			760742.46	760739.46
EC guard columns*		4 x 2 mm: 761951.20		4 x 3 mm: 761951.30	
NUCLEODUR® 100-5 NH_2-RP particle size 5 µm; eluent in column acetonitrile – water					
Analytical EC columns					
	2 mm		760730.20		760732.20
	3 mm		760730.30		760732.30
	4 mm		760730.40		760732.40
	4.6 mm		760730.46	760731.46	760732.46
EC guard columns*		4 x 2 mm: 761953.20		4 x 3 mm: 761953.30	
NUCLEODUR® 100-5 NH_2 particle size 5 µm; eluent in column *n*-heptane					
Analytical EC columns					
	4 mm		760720.40		760722.40
	4.6 mm		760720.46	760721.46	760722.46
EC guard columns*				4 x 3 mm: 761952.30	

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of our column systems see page 250.

NUCLEODUR® columns

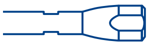
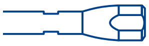
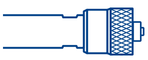
NUCLEODUR® SiOH unmodified silica for normal phase · USP L3

Key feature
- Totally spherical high purity silica
- Pressure stable up to 600 bar
- Suitable for analytical and preparative separation of polar and midpolar compounds

Technical data
- Unmodified high purity silica; pore size 110 Å; particle sizes 3 to 50 µm; pore volume 0.9 mL/g; surface area (BET) 340 m^2/g; pH stability 2–8; metal content < 10 ppm (see page 150)

Recommended application
- Polar and midpolar compounds under normal phase conditions

Ordering information
Eluent in column *n*-heptane

	ID	Length → 50 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 particle size 3 µm					
Analytical EC columns					
	4.6 mm	760170.46		760172.46	760173.46
EC guard columns*				4 x 3 mm: 761966.30	
NUCLEODUR® 100-5 particle size 5 µm					
Analytical EC columns					
	4 mm				760007.40
	4.6 mm	760023.46		760012.46	760007.46
EC guard columns*				4 x 3 mm: 761967.30	
Preparative VarioPrep columns					
	10 mm	762077.100	762078.100		762007.100
	21 mm	762077.210	762078.210		762007.210
	40 mm			762075.400	762007.400
VP guard columns *		10 x 8 mm: 762094.80		10 x 16 mm: 762094.160	
		15 x 32 mm: 762330.320			

EC and VarioPrep columns in packs of 1, guard columns see below.

Guard column systems

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966
Guard columns for VarioPrep columns with ID		**8, 10 mm**	**16, 21 mm**	**32, 40 mm**	**≥ 50 mm**	
** VP guard columns (pack of)	VP	10/8 (2)	10/16 (2)	15/32 (1)	15/50 (1)	
VP guard column holder		718251	718256	718253	718255	

For details of our column systems see page 250.
Unmodified NUCLEODUR® bulk material in 10–50 µm for self-packing of preparative columns see page 256.

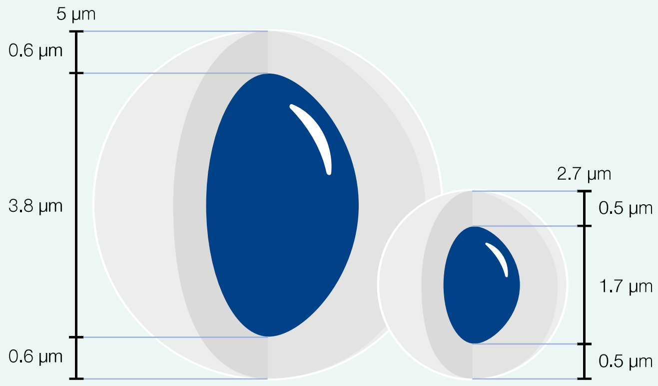
MACHEREY-NAGEL
your partner in HPLC · also online

Besides to this catalog our website provides useful information

- Applications
  Database without registration, with more than 3000 free chromatography applications for your separation task.
- Instruction manuals
  General advises for column care and individual column cleaning are available in the attached instruction manual or online.
- HPLC troubleshooting
  Sometimes during chromatographic separation unexpected effects occur. We give advise of possible reasons and how to avoid or remedy these.
- Flyers, brochures, catalogs
  Our product information is available online as PDF file at any time.

NUCLEOSHELL® core-shell silica for HPLC

Core-shell technology



Key feature

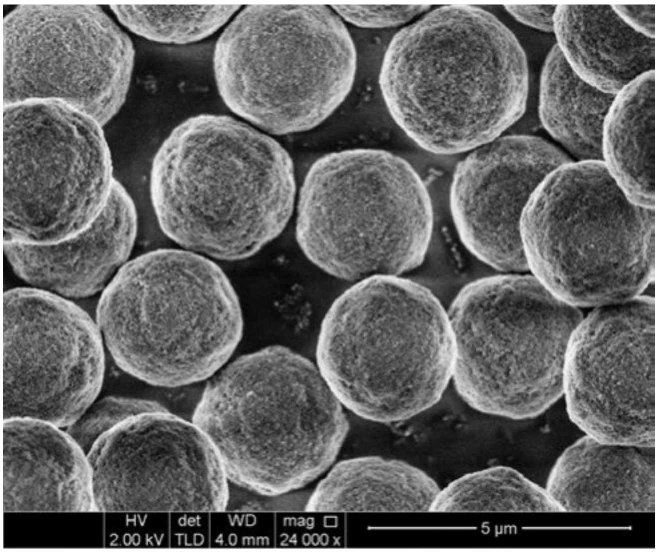
- Solid core of silicon dioxide, homogeneous shell of porous silica
- Highest efficiency compared to traditional totally porous materials
- Pore size 90 Å; particle size 2.7 µm (core 1.7 µm) and 5 µm (core 3.8 µm); specific surface 130 (2.7 µm) and 90 (5 µm) m²/g lower back pressure enables use on conventional LC systems
- Pressure stability 600 bar

Demands on HPLC separations are constantly increasing with respect to separation efficiency, detection limits, and the time requirements for each analysis.

Several approaches have been made to achieve fast separations without losing chromatographic performance. HPLC columns packed with particles < 2 µm show very high efficiencies (plates/meter) and allow the use of smaller column sizes with the positive side effect of significant solvent saving. However they generate a high back pressure of the mobile phase during column runs which requires specifically designed equipment.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_i}{k'_i+1}\right)$$

R_s = resolution, α = selectivity (separation factor), k_i' = retention
N = plate number with $N \propto 1/d_P$, d_P = particle diameter



Electron microscopic image of NUCLEOSHELL®

NUCLEOSHELL® silica particles consist of a non-porous solid core of 1.7 µm diameter and a porous outer shell of 0.5 µm thickness. Accordingly the total diameter of the particle is 2.7 µm.

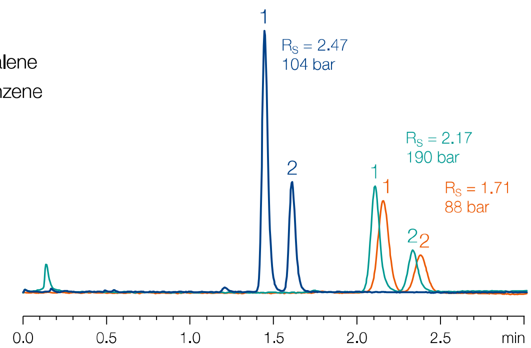
Utilizing a proprietary process of synthesis, NUCLEOSHELL® particles exhibit a distinct narrow particle size distribution (d90/d10 ~ 1.1). Columns packed with NUCLEOSHELL core shell particles feature exceptional separation efficiencies with theoretical plate numbers easily comparable to totally porous sub 2 micron particles.

Resolution R_S as function of particle size

MN Appl. No. 125270

Columns:	50 x 4 mm
	NUCLEOSHELL® RP 18, 2.7 µm
	NUCLEODUR® C_{18} Gravity, 3 µm
	NUCLEODUR® C_{18} Gravity, 1.8 µm
Eluent:	acetonitrile – water (60:40, v/v)
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm

Peaks:
1. Naphthalene
2. Ethylbenzene

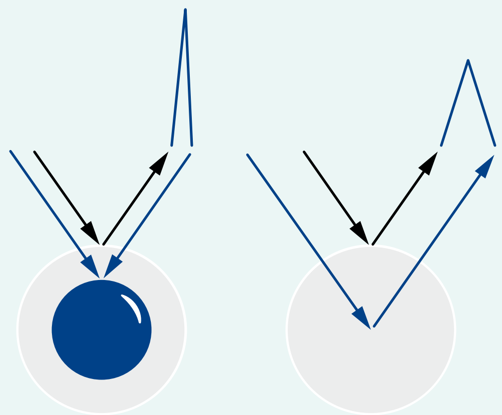


Theoretical column efficiency (optimal conditions)

Silica	d_p [µm]	L [m]	HETP [µm]	Efficiency [plates/m]	L [mm]	N	R_s	Analysis time
NUCLEOSHELL®	2.7	1	4	250 000	100	25 000	112 %	40 %
	5	1	6.5	154 000	150	23 000	115 %	60 %
NUCLEODUR®	1.8	1	4.5	222 222	100	22 000	105 %	40 %
	3	1	7.5	133 333	150	20 000	100 %	60 %
	5	1	12.5	80 000	250	20 000	100 %	100 %

Benefits of core-shell technology

Core-shell particles vs. totally porous silica



Short diffusion paths

- Fast mass transfer (term C of Van Deemter equation)
- High flow velocity without peak broadening for fast LC

Narrow particle size distribution (d_{90}/d_{10} ~ 1.1)

- Stable packing

High heat transfer

- Minimized influence of frictional heat
- Efficiency of NUCLEOSHELL® ~ 250 000 m^{-1} (HETP ~ 4 µm)

With conventional fully porous particles the mass transfer between stationary and mobile phase usually results in peak broadening at higher flow rates (C-term in van Deemter equation). The short diffusion paths in the core-shell particles reduce the dwell time of the analyte molecules in the stationary phase, so that even at high flow velocities of the mobile phase, optimal separation results can be obtained.

The van Deemter plots demonstrate how efficiency is affected by flow rate.

In comparison with fully porous silicas, core-shell particles from various manufacturers maintain the efficiency optimum (max. plates/m) over a long range of increasing linear mobile phase velocity.

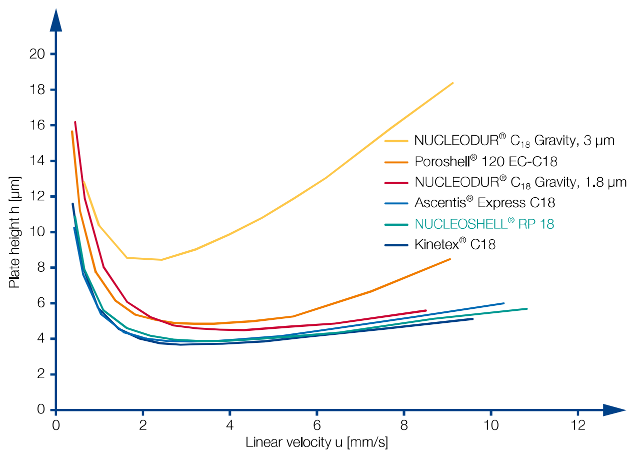
$$H = A + \frac{B}{u} + C \cdot u$$

A term = eddy-diffusion, B term = longitudinal diffusion coefficient,
C term = mass transfer coefficient

Van Deemter curves

MN Appl. No. 125500

Column: 50 x 4.6 mm
Eluent: $CH_3CN - H_2O$ (70:30, v/v)
Temperature: 25 °C
Sample: Acenaphthene



NUCLEOSHELL® core-shell silica for HPLC

In direct comparison with conventional sub 2 micron phases, NUCLEOSHELL® columns only generate about 60 % of the back pressure and can be operated with the majority of conventional HPLC systems. In order to develop the maximum performance of NUCLEOSHELL® columns, we recommend reducing extra column voids by using suitable capillaries (< 0.15 mm inner diameter) and specially adapted detector cells. Moreover detector settings should be optimized by increasing the measuring rate or by decrease of the time constant.

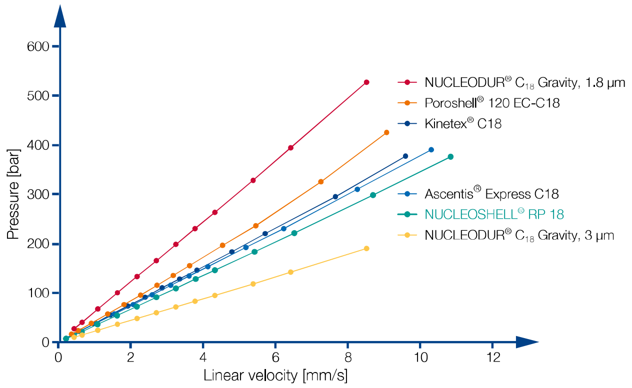
$$\Delta_P = \frac{\Phi \cdot L_C \cdot \eta \cdot u}{d_P^2}$$

Δ_P = pressure drop, Φ = flow resistance (nondimensional), LC = column length, η = viscosity, u = linear velocity, d_P = particle diameter

Pressure drop

MN Appl. No. 125510

Column: 50 x 4.6 mm
Eluent: CH_3CN – H_2O (70:30, v/v)
Temperature: 25 °C



Core-shell particle technology from MACHEREY-NAGEL is an alternate route to gain highest column efficiency and resolution in HPLC at short run time, but with moderate back pressure.

Features of NUCLEOSHELL® particles

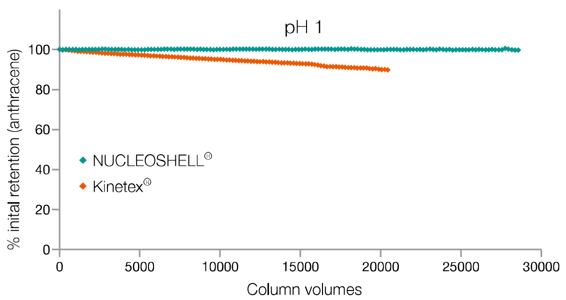
A criterion for the long-term stability of the column at pH extremes is the percentage decrease of initial retention and initial plates, respectively.

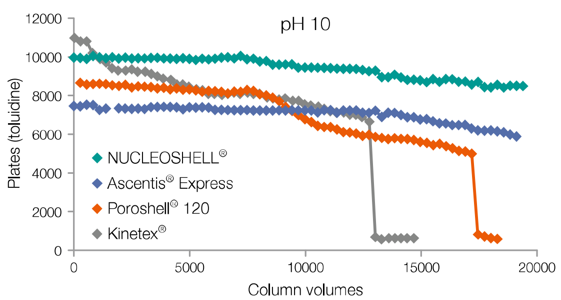
The following figure shows a column stability test of NUCLEOSHELL® RP 18 at mobile phase levels pH 1 and pH 10 compared with three competing phases.

Stability under acidic and basic conditions

MN Appl. Nos. 125520 / 125530

Columns:	50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm 50 x 4.6 mm Kinetex® 2.6 µm C18	Columns:	50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm 50 x 4.6 mm Ascentis® Express C18, 2.7 µm 50 x 4.6 mm Poroshell® 120 EC-C18 50 x 4.6 mm Kinetex® 2,6 µm C18
Eluent:	acetonitrile – 1 % TFA in water, pH 1 (50:50, v/v)	Eluent:	20 mmol/L Na borate – 10 mmol/L NaOH – methanol, pH 10 (21:49:30, v/v/v)
Flow rate:	1.3 mL/min	Flow rate:	1.5 mL/min
Temperature:	80 °C	Temperature:	40 °C
Detection:	UV, 254 nm	Detection:	UV, 220 nm
Analyt:	anthracene	Analyt:	toluidine





Columns can be operated at elevated temperatures without loss in retention, efficiency or peak symmetry.

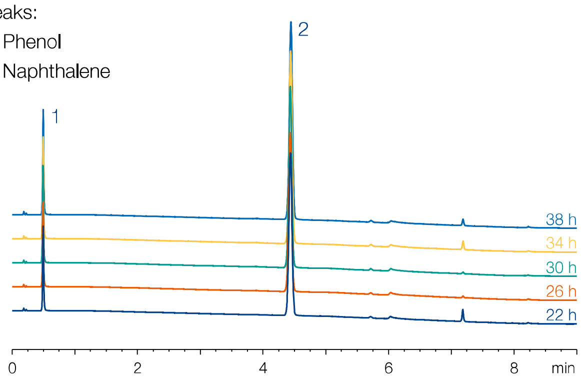
Temperature stability

MN Appl. No. 125400

Stability test:

Column:	50 x 2 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent:	A) 10 mmol/L ammonium formate – methanol (9:1, v/v) + 120 µL formic acid, ~ pH 4 B) 10 mmol/L ammonium formate – methanol (1:9, v/v) + 120 µL formic acid, ~ pH 4 0–100 % B in 7 min
Flow rate:	0.5 mL/min,
Temperature:	100 °C
Detection:	UV, 220 nm

Peaks:
1. Phenol
2. Naphthalene



Efficiency test:

Eluent:	Acetonitrile – water (60:40, v/v)
Flow rate:	0.33 mL/min;
Temperature:	25 °C
Detection:	UV, 254 nm
Analyte:	Anthracene

	HETP [µm]	Asymmetry
Start (t = 0)	5.2	0.98
End (t = 40 h)	5.2	1.01

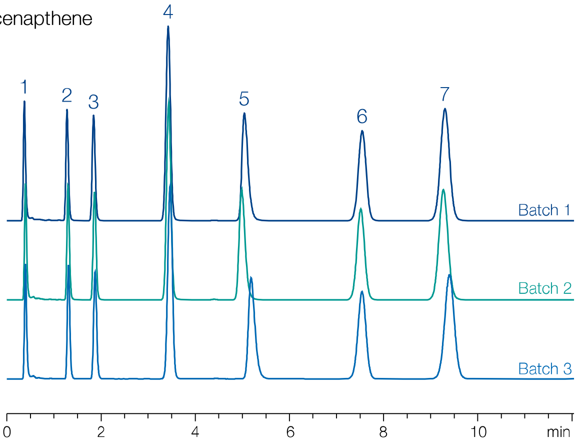
Uniformly shaped NUCLEOSHELL® particles combined with optimized bonding technology safeguard tightly packed columns for 100 % reproducible results.

Batch-to-batch reproducibility

MN Appl. No. 125410

Column:	50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent:	methanol – 25 mmol/L KH_2PO_4, pH 7 (70:30, v/v)
Flow rate:	1 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm

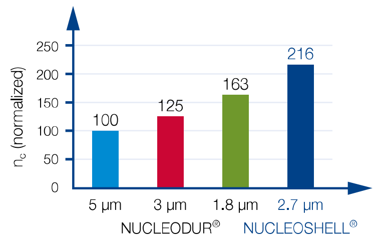
Peaks:
1. Uracil
2. Toluene
3. Ethylbenzene
4. Acenapthene
5. Amitriptyline
6. *o*-Terphenyl
7. Triphenylene



NUCLEOSHELL® core-shell silica for HPLC

Peak capacity

The peak capacity is a measure for the number of sample analytes that can be separated on HPLC columns per time unit. Narrow peaks increase the peak capacity and thus the efficiency of the analytical column.



Comparison of peak capacities

$$n_c = 1 + \left(\frac{t_g}{W}\right)$$

n_c: peak capacity
t_g: gradient time
W: peak width (at baseline)

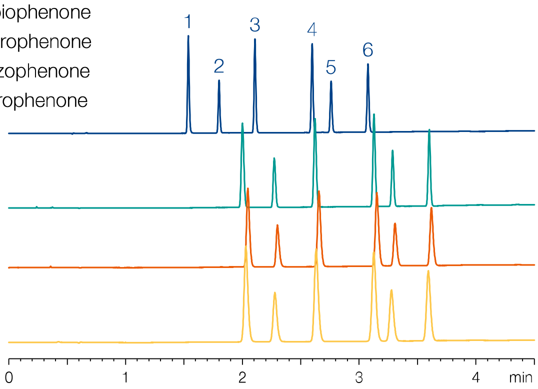
The example shows, that in comparison with totally porous NUCLEODUR® silica (1.8 µm) NUCLEOSHELL® provides 33 % higher peak capacity.

Peak capacity

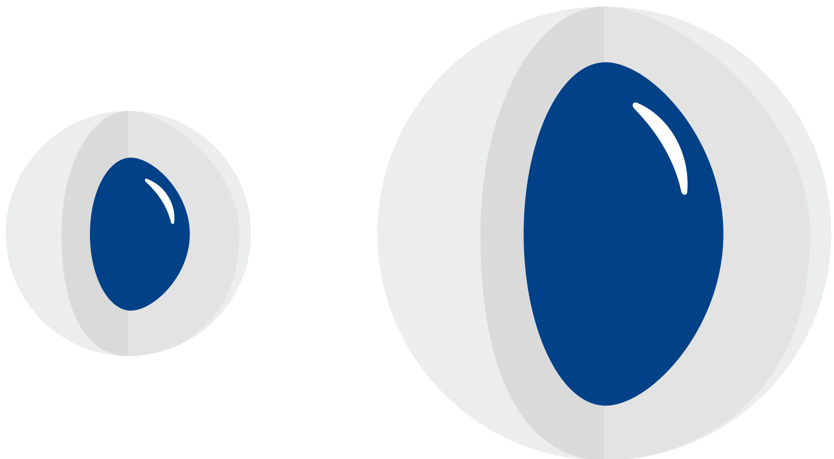
MN Appl. No. 125540

Columns:	100 x 4.6 mm each NUCLEOSHELL® RP 18, 2.7 µm NUCLEODUR® C_{18} Gravity, 1.8 µm NUCLEODUR® C_{18} Gravity, 3 µm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	A) acetonitrile, B) water, 40–100 % A in 4 min
Flow rate:	1.5 mL/min
Temperature:	25 °C
Detection:	UV, 230 nm

Peaks:
1. Acetophenone
2. Benzoin
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone



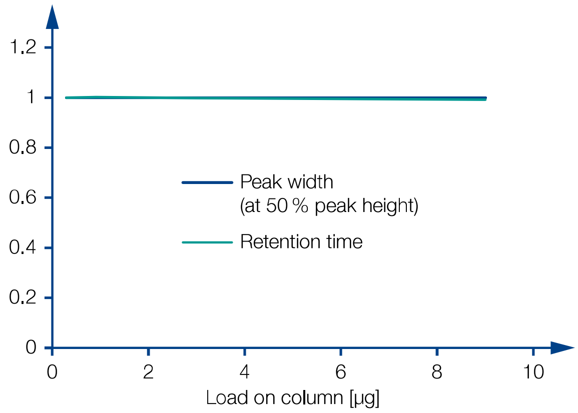
	Max. pressure [bar]	Resolution (4.5)
NUCLEOSHELL®, 2.7 µm	255	5.45
NUCLEODUR®, 1.8 µm	450	4.14
NUCLEODUR®, 3 µm	214	2.97
NUCLEODUR®, 5 µm	142	2.30



Loading capacity

NUCLEOSHELL® columns allow reliable quantification in a wide analytical detection range. Retention time and peak width at 50 % height remain constant with increasing columns load although core-shell particles are suspected of showing a slightly lower loading capacity compared to fully porous silica materials.

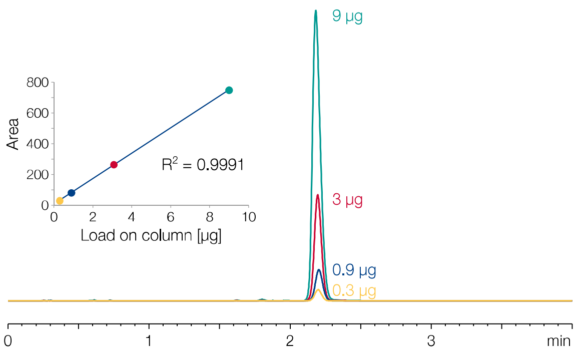
Normalized column parameters



Loading capacity

Column: 50 x 3 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent: acetonitrile – 25 mmol/L KH_2PO_4, pH 3 (70:30, v/v)
Flow rate: 0.66 mL/min
Temperature: 30 °C
Detection: UV, 285 nm

Peaks:
1. Valerophenone



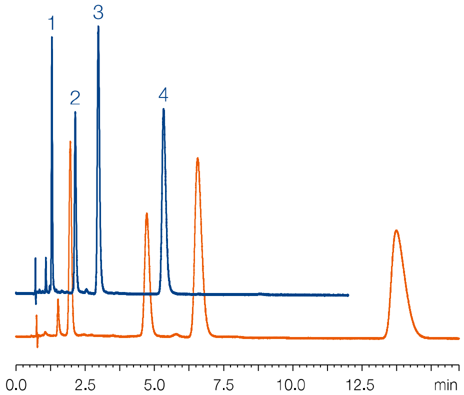
Method transfer of 5 µm particle columns

NUCLEOSHELL® is also available in 5 µm particle size to offer all benefits of core-shell technology to all applications which are bound to particle size.

Separation of cephalosporin antibiotics

MN Appl. No. 126630

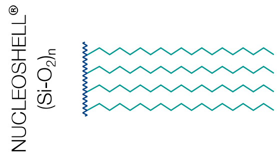
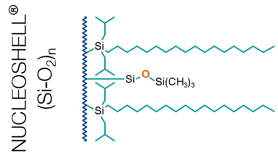
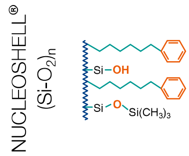
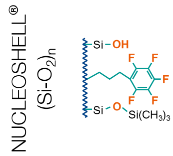
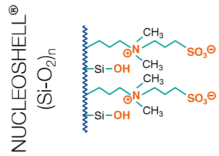
Comparison of 5 µm core-shell and totally porous phase
Columns: each 100 x 4.6 mm
A) NUCLEOSHELL® RP 18plus, 5 µm
B) NUCLEODUR® Gravity C_{18}, 5 µm
Eluent: methanol – water + 0.1 % formic acid (35:65, v/v)
Flow rate: 1.3 mL/min
Pressure: 182 bar, 219 bar
Temperature: 25 °C
Detection: UV, 254 nm
Injection: 4.0 µL



Peaks:	Ret. time [min]		Asymmetry (EP)		Plates (EP)	
	A	B	A	B	A	B
1 Cefotaxime	1.30	1.96	1.19	1.12	6800	2218
2 Cefoxitin	2.14	4.72	1.22	1.20	6599	3471
3 Cefamandole	2.97	6.57	1.24	1.25	6259	3367
4 Cefalotine	5.33	13.73	1.32	1.61	6948	3672

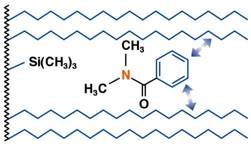
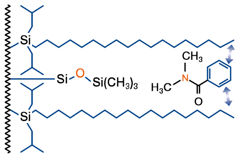
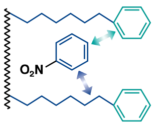
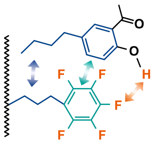
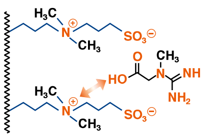
NUCLEOSHELL® phase overview

Overview of NUCLEOSHELL® HPLC phases

Phase	Specification	Page	Characteristic*	Stability	Structure
RP 18	octadecyl, multi-endcapping 7.8 % C (2.7 µm particles) 6.1 % C (5 µm particles) USP L1	200	A ●●●●● B ◖ C ●●◖	pH 1–11, suitable for LC/MS	
RP 18plus	octadecyl (monomeric), multi-endcapping 5.7 % C (2.7 µm particles) 4.4 % C (5 µm particles) USP L1	202	A ●●●● B ●●◖ C -	pH 2–9, suitable for LC/MS	
Phenyl-Hexyl	phenylhexyl, multi-endcapping 4.5 % C (2.7 µm particles) USP L11	204	A ●● B ●●● C ●	pH 1–10, suitable for LC/MS	
PFP	pentafluorophenyl, multi-endcapping ~ 3 % C (2.7 µm particles) USP L43	206	A ●● B ●●●● C ●●●●	pH 1–9, suitable for LC/MS	
HILIC	zwitterionic ammonium – sulfonic acid 1.3 % C (2.7 µm particles)	208	A ● B ●●●●● C -	pH 2–8.5, suitable for LC/MS	

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity

NUCLEOSHELL® phase overview

Application	Similar phases**	Interactions · retention mechanism	
overall sophisticated analytical separations, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants	Kinetex® C18; Cortecs® C18; Raptor® C18; Accucore® C18; Ascentis® Express C18	hydrophobic (van der Waals interactions)	
overall sophisticated analytical separations, especially for polar compounds, e.g., pharmaceuticals like antibiotics, water-soluble vitamins, organic acids	Kinetex® XB-C18; Bonshell® ASB-C18; Raptor® ARC-C18;	hydrophobic (van der Waals interactions)	
aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics	Ascentis® Express Phenyl-Hexyl; Kinetex® Phenyl-Hexyl; Accucore® Phenyl-Hexyl; Ultracore® Phenyl-Hexyl; Poroshell® Phenyl-Hexyl	π-π and hydrophobic	
aromatic and unsaturated compounds, phenols, halogenated hydrocarbons, isomers, polar compounds like pharmaceuticals, antibiotics	Kinetex® PFP; Ascentis® Express F5; Accucore® PFP	polar (H bond), dipole-dipole, π-π and hydrophobic	
hydrophilic compounds such as organic polar acids and bases, polar natural compounds	–	ionic / hydrophilic and electrostatic	

** phases which provide a similar selectivity based on chemical and physical properties

NUCLEOSHELL® RP 18 nonpolar high density phase · USP L1

Key feature
- Core-shell technology for fast and efficient HPLC
- Suitable for LC/MS and HPLC at pH extremes (pH 1–11)
- Superior base deactivation, ideal for method development

Technical data
- Octadecyl modification, multi-endcapped; pore size 90 Å, particle size 2.7 and 5 µm, carbon content 7.8 % for 2.7 µm, 6.1 % for 5 µm; pH stability 1–11; suitable for LC/MS

Recommended application
- Overall sophisticated analytical separations, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants

NUCLEOSHELL® RP 18 is based on core-shell silica. A unique derivatization process generates a homogeneous surface with a high density of bonded silanes. The following thorough endcapping suppresses any unwanted polar interactions between the silica surface and the sample, which makes NUCLEOSHELL® RP 18 particularly suitable for the separation of basic and other ionizable analytes. The extremely reduced silanol activity of the phase can be demonstrated by applying basic analytes, such as tricyclic antidepressants. The chromatogram below shows a sharp elution profile (superior resolution!) of these highly polar compounds with an excellent asymmetry value for amitriptyline of 1.12.

Tricyclic antidepressants · comparison of selectivity and resolution

MN Appl. No. 124960

Columns: 50 x 4.6 mm each
NUCLEOSHELL® RP 18, 2.7 µm
Ascentis® Express C18
Kinetex® 2.6 µm C18
Poroshell® 120 EC-C18

Eluent: methanol – acetonitrile – 25 mmol/L KH_2PO_4, pH 7 (22.5:22.5:55, v/v/v)

Flow rate: 2 mL/min

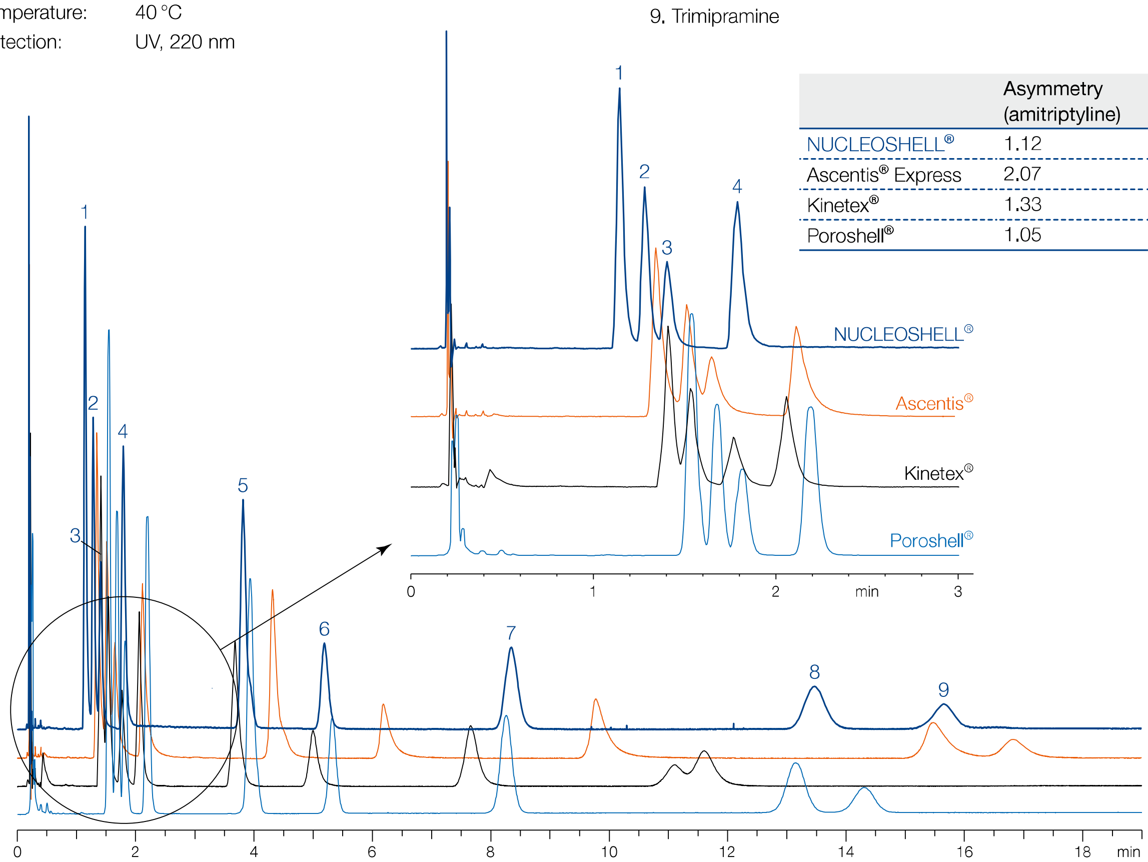
Pressure: 224 bar, 239 bar, 248 bar, 212 bar

Temperature: 40 °C

Detection: UV, 220 nm

Peaks:
1. Protriptyline
2. Desipramine
3. Maprotiline
4. Nortriptyline
5. Doxepin
6. Imipramine
7. Amitriptyline
8. Clomipramine
9. Trimipramine

	Asymmetry (amitriptyline)	Resolution (8, 9)
NUCLEOSHELL®	1.12	3.35
Ascentis® Express	2.07	1.91
Kinetex®	1.33	n.a.
Poroshell®	1.05	1.95



NUCLEOSHELL® RP 18 combines innovative silica technology and excellent surface deactivation, that outperforms conventional C_{18} silicas in terms of efficiency, resolution and speed.

Due to the applied core-shell particle design the back pressure at elevated flow rates remains at a moderate level and in many cases permits the use of existing HPLC equipment. NUCLEOSHELL® RP 18 with extended pH stability, low bleed characteristics in LC/MS applications, and overall robustness is an ideal tool for method development and routine analyses in modern HPLC.

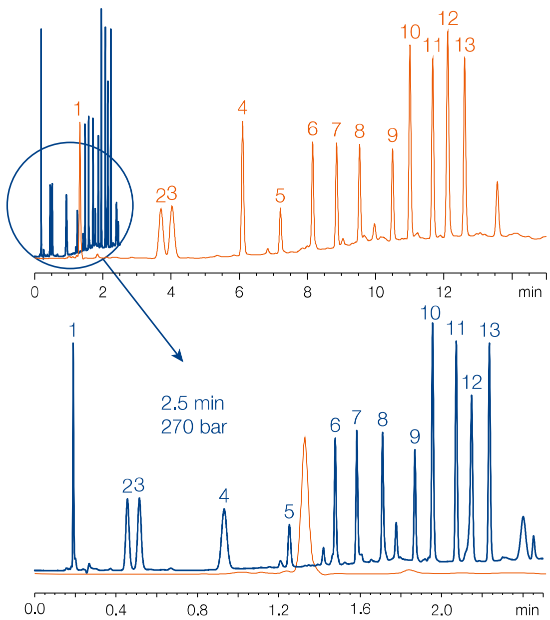
The separation of 13 β-lactam antibiotics illustrates how time of analysis can be shortened to a fractional part by using core-shell particles without loss of resolution at moderate back pressure.

13 β-lactam antibiotics in less than 3 min

MN Appl. No. 124940

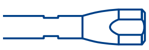
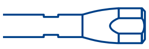
Columns:	50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm 150 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent:	A) acetonitrile B) 20 mmol/L KH_2PO_4, pH 3.5 10 % A (0,5 min) → 50 % A in 1.5 min (0.5 min 50 % A) 10 % A (3 min) → 50 % A in 9 min (3 min 50 % A)
Flow rate:	2 mL/min, 1 mL/min
Pressure:	270 bar, 110 bar
Temperature:	25 °C
Detection:	UV, 220 nm

Peaks:
1. Amoxicillin
2. Ampicillin
3. Cephalexin
4. Cefotaxime
5. Cefoxitin
6. Cefamandole
7. Cephalothin
8. Piperacillin
9. Penicillin V
10. Oxacillin
11. Cloxacillin
12. Nafcillin
13. Dicloxacillin



Ordering information

Eluent in column acetonitrile – water

	ID	Length → 50 mm	100 mm	150 mm	250 mm	EC guard columns*
NUCLEOSHELL® RP 18, 2.7 µm particle size 2.7 µm						
Analytical EC columns						
	2 mm	763132.20	763134.20	763136.20		763138.20
	3 mm	763132.30	763134.30	763136.30		763138.30
	4 mm	763132.40	763134.40	763136.40		763138.30
	4.6 mm	763132.46	763134.46	763136.46		763138.30
NUCLEOSHELL® RP 18, 5 µm particle size 5 µm						
Analytical EC columns						
	2 mm	763152.20	763154.20	763156.20	763157.20	763158.20
	3 mm	763152.30	763154.30	763156.30	763157.30	763158.30
	4 mm	763152.40	763154.40	763156.40	763157.40	763158.30
	4.6 mm	763152.46	763154.46	763156.46	763157.46	763158.30

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of the EC column system please see page 250.

NUCLEOSHELL® RP 18plus C_{18} phase with polar selectivity · USP L1

Key feature

- Based on core-shell particle technology for fast and efficient HPLC
- Hydrophobic C_{18} phase with distinct polar selectivity, ideal for method development
- Excellent performance under highly aqueous conditions

Technical data

- Monomeric octadecyl modification, multi-endcapped; pore size 90 Å, available particle sizes 2.7 µm and 5 µm, carbon content 5.7 % for 2.7 µm, 4.4 % for 5 µm; pH stability 2–9; suitable for LC/MS

Recommended application

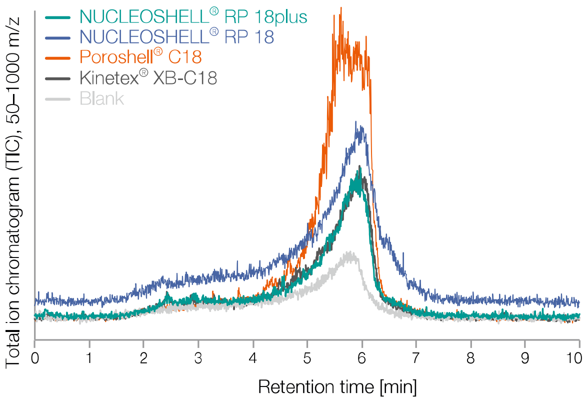
- Overall sophisticated analytical separations, especially for polar compounds, e.g., pharmaceuticals like antibiotics, water-soluble vitamins, organic acids

NUCLEOSHELL® RP 18plus is a C_{18} modified core-shell silica. Due to a monomeric bonding chemistry this HPLC phase offers hydrophobic characteristics with distinct polar selectivity. A special derivatization process generates a medium density of bonded silanes with reduced steric selectivity compared to NUCLEOSHELL® RP 18.

Bleeding characterisitics

MN Appl. No. 126640

Column:	50 x 2 mm NUCLEOSHELL® RP 18plus, 2.7 µm
Eluent:	A) 0.1 % formic acid in water
	B) 0.1 % formic acid in acetonitrile
	95 % A → 5 % A in 4.5 min (0.5 min) → 95 % A in 0.5 min (4.5 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	MS

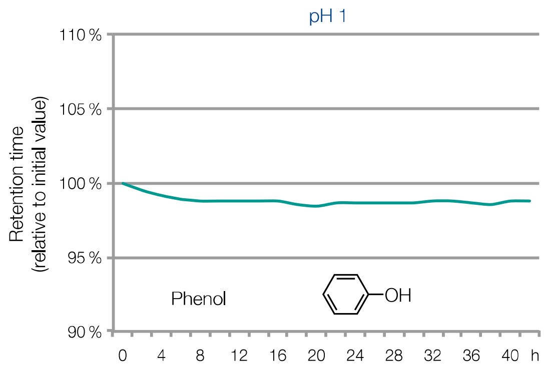


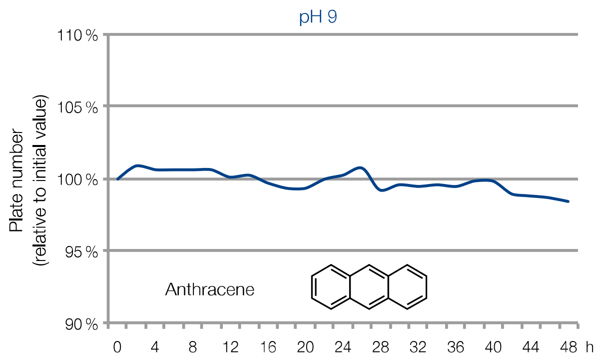
NUCLEOSHELL® RP 18plus combines superbly hydrophobic and polar selectivity – so it is a useful tool for method development in RP chromatography. Good pH stability and low bleeding characteristics make it ideal especially for LC/MS applications.

pH stability of NUCLEOSHELL® RP 18plus

MN Appl. No. 126650

Column:	100 x 4 mm NUCLEOSHELL® RP 18plus, 2.7 µm
Eluent pH 1:	1 % TFA in water - acetonitrile (50:50, v/v)
Eluent pH 9:	50 mmol/L triethylammonium acetate adjusted to pH 9
Flow rate:	for pH 1: 0.8 mL/min, for pH 9: 0.56 mL/min
Temperature:	for pH 1: 60 °C, for pH 9: 50 °C
Detection:	UV, 254 nm
Injection:	1 µL



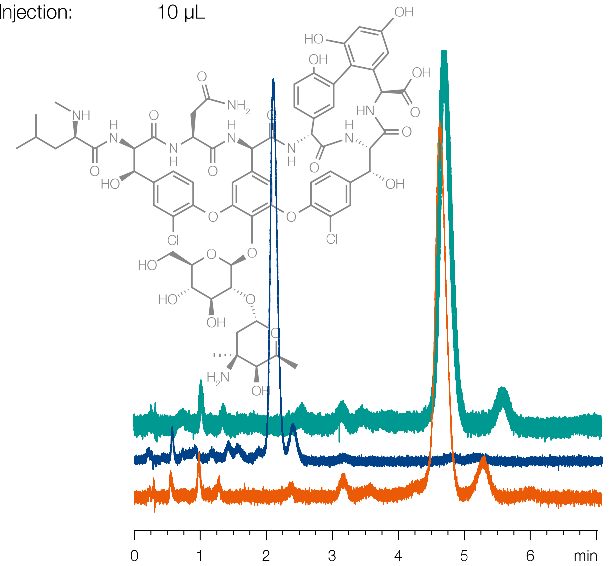


Also a comparison of retention of the glycopeptide antibiotic vancomycin on several octadecyl modified core-shell phases underlines the polar selectivity of NUCLEOSHELL® RP 18plus.

Polar selectivity shown for vancomycin

MN Appl. No. 126660

Columns:	50 x 3 mm each
	NUCLEOSHELL® RP 18plus, 2.7 µm
	NUCLEOSHELL® RP 18, 2.7 µm
	Kinetex® 2.6 µm C18
Eluent:	water – methanol – acetonitrile – glacial acetic acid (100:8:2:0.3, v/v/v/v) adjusted to pH 3.2 with sodium hydroxide solution
Flow rate:	0.9 mL/min
Temperature:	35 °C
Detection:	UV, 240 nm
Injection:	10 µL

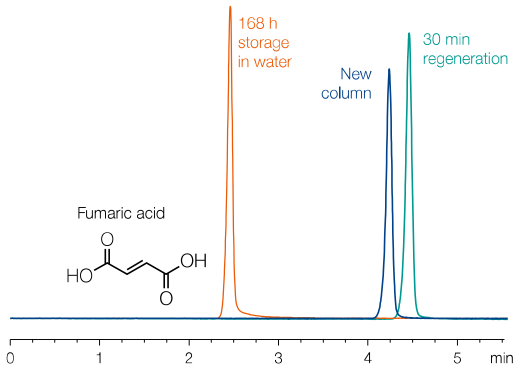


In addition NUCEOSHELL® RP 18plus provides a good stability under highly aqueous conditions. Even by long term usage or storage of the phase phase collapse and loss of retention are hardly observed. The original performance can be regained after a short regeneration procedure.

Phase collapse and regeneration

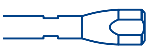
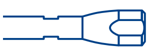
MN Appl. No. 126670

Column:	100 x 4 mm NUCLEOSHELL® RP 18plus, 2.7 µm
Eluent:	20 mmol/L KH_2PO_4, pH 2.6
Flow rate:	0.5 mL/min
Temperature:	20 °C
Detection:	UV, 215 nm
Injection:	0.5 µL



Ordering information

Eluent in column acetonitrile – water

	ID	Length → 50 mm	100 mm	150 mm	250 mm	EC guard columns*
NUCLEOSHELL® RP 18plus, 2.7 µm particle size 2.7 µm						
Analytical EC columns						
	2 mm	763232.20	763234.20	763236.20		763238.20
	3 mm	763232.30	763234.30	763236.30		763238.30
	4 mm	763232.40	763234.40	763236.40		763238.30
	4.6 mm	763232.46	763234.46	763236.46		763238.30
NUCLEOSHELL® RP 18plus, 5 µm particle size 5 µm						
Analytical EC columns						
	2 mm	763252.20	763254.20	763256.20	763257.20	763258.20
	3 mm	763252.30	763254.30	763256.30	763257.30	763258.30
	4 mm	763252.40	763254.40	763256.40	763257.40	763258.30
	4.6 mm	763252.46	763254.46	763256.46	763257.46	763258.30

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of the EC column system please see page 250.

NUCLEOSHELL® Phenyl-Hexyl nonpolar high density phase · USP L11

Key feature
- Based on core-shell particle technology for fast and efficient HPLC
- Hydrophobic phase with alternative selectivity compared to classical C_{18} modifications
- Separation principle based on 2 retention mechanisms: π-π interactions and hydrophobic interactions

Technical data
- Phenyl-Hexyl modification, multi-endcapped; pore size 90 Å, particle size 2.7 µm; carbon content 4.5 %; pH stability 1–10; suitable for LC/MS

Recommended application
- Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics

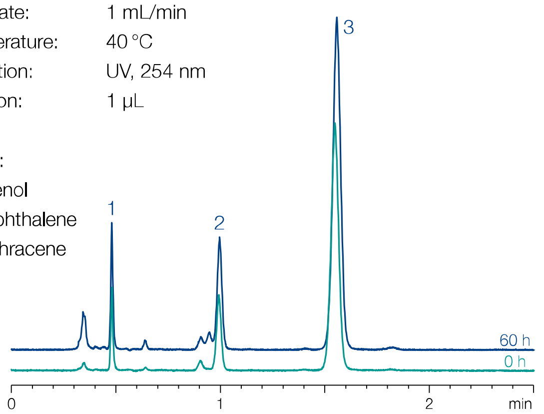
Phenyl-Hexyl modified phases offer an excellent separation efficiency especially for aromatic and unsaturated compounds with electron-withdrawing groups. The combination of hydrophobic and π-π interactions results in an alternative and interesting selectivity profile compared to C_{18} or C_8 modifications. NUCLEOSHELL® Phenyl-Hexyl is based on a unique surface bonding chemistry - therefore it is suitable for LC/MS due to low bleeding characteristics and offers high temperature stability and pH stability from 1 to 10.

Stability of NUCLEOSHELL® Phenyl-Hexyl at pH 10

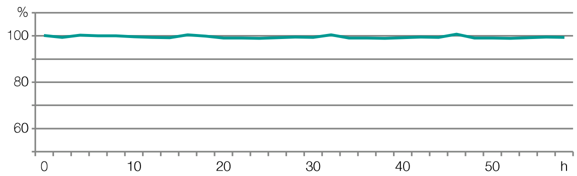
MN Appl. No. 126420

Column:	50 x 4 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
Eluent:	acetonitrile – 50 mmol/L TEA pH 10 (60:40, v/v); pH of the mixture 10.4
Flow rate:	1 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	1 µL

Peaks:
1. Phenol
2. Naphthalene
3. Anthracene



Relative plate numbers

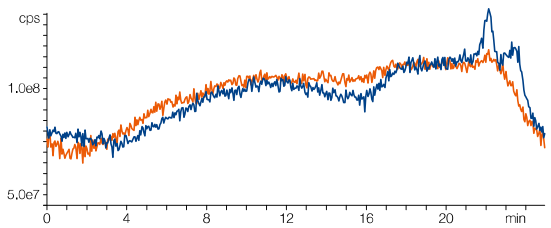


NUCLEOSHELL® Phenyl-Hexyl is a robust phase with an alternative RP selectivity for aromatic and unsaturated analytes compared to classical C_{18} / C_8 phases – it is an additional and useful tool for all chromatography users.

Bleeding characteristics of NUCLEOSHELL® Phenyl-Hexyl

MN Appl. No. 126400

Columns:	50 x 2 mm each NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm Kinetex® Phenyl-Hexyl
Eluent:	A) acetonitrile, B) water 5–95 % A in 25 min
Flow rate:	0.2 mL/min
Temperature:	25 °C
Detection:	MS



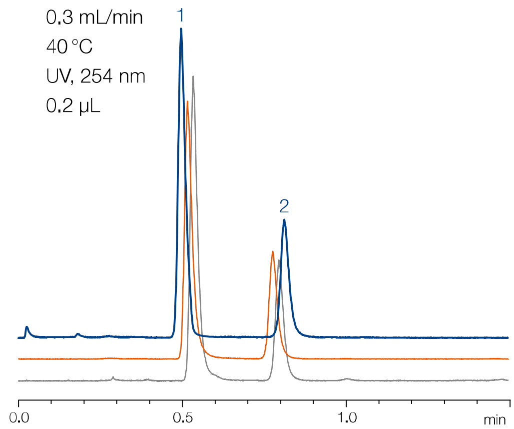
The pyridine-phenol test shows that NUCLEOSHELL® Phenyl-Hexyl provides a symmetrical peak for pyridine and higher resolution in comparison to other core-shell based Phenyl-Hexyl phases, which underlines the excellent base deactivation.

Pyridine-phenol test of NUCLEOSHELL® Phenyl-Hexyl

MN Appl. No. 126410

Columns:	50 x 2 mm each NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm Kinetex® Phenyl-Hexyl Ascentis® Express Phenyl-Hexyl
Eluent:	acetonitrile – water (70:30, v/v)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	0.2 µL

Peaks:
1. Pyridine
2. Phenol



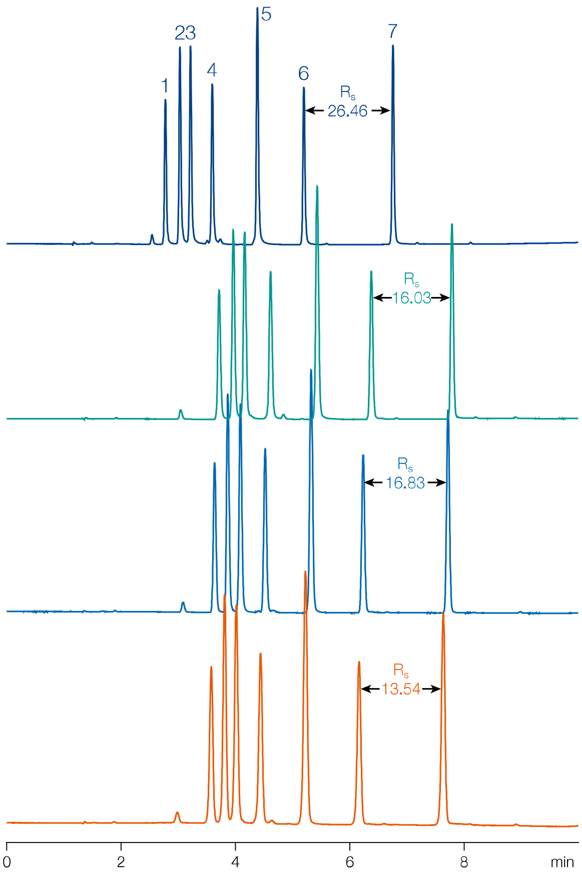
Comparing the separation of sulfonamides on NUCLEODUR® Phenyl-Hexyl with different particle sizes

MN Appl. No. 125860

Columns:	150 x 3 mm each
	NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
	NUCLEODUR® Phenyl-Hexyl, 1.8 µm
	NUCLEODUR® Phenyl-Hexyl, 3 µm
	NUCLEODUR® Phenyl-Hexyl, 5 µm
Eluent:	A) methanol
	B) 0.1 % formic acid in water
	20–80 % A in 10 min
Flow rate:	0.56 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	0.5 µL

Peaks:
1. Sulfadiazine
2. Sulfachlorpyridazine
3. Sulfapyridine
4. Sulfamerazine
5. Sulfadimidine
6. Sulfathiazole
7. Sulfadimethoxine

On NUCLEOSHELL® Phenyl-Hexyl the resolution of the last two peaks is higher than on the fully porous 1.8 µm NUCLEODUR® Phenyl-Hexyl.



The separation of sulfonamides proves the scalability from fully porous NUCLEODUR® to NUCLEOSHELL® Phenyl-Hexyl. Hereby the core-shell silica exhibits identical selectivity, narrower peaks and slightly shorter retention under the same conditions.

Thus, method transferability between NUCLEODUR® and NUCLEOSHELL® is guaranteed, either for speeding up your methods or scaling up for preparative requirements.

Ordering information

Eluent in column acetonitrile – water

ID	Length → 50 mm	100 mm	150 mm	EC guard columns*
NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm particle size 2.7 µm				
Analytical EC columns				
2 mm	763732.20	763734.20	763736.20	763738.20
3 mm	763732.30	763734.30	763736.30	763738.30
4 mm	763732.40	763734.40	763736.40	763738.30
4.6 mm	763732.46	763734.46	763736.46	763738.30

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of the EC column system please see page 250.

NUCLEOSHELL® PFP hydrophobic pentafluorophenyl phase · USP L43

Key feature

- Core-shell technology for fast and efficient HPLC
- Hydrophobic phase with alternative selectivity in comparison to classical C_{18} modifications
- Separation principle based on 4 retention mechanisms (polar interactions (H bonds), dipole-dipole, π-π, hydrophobic interactions)

Technical data

- Phase with pentafluorophenylpropyl modification, multi-endcapping; pore size 90 Å, particle size 2.7 µm; carbon content ~ 3 %; pH stability 1–9; suitable for LC/MS

Recommended application

- Aromatic and unsaturated compounds, phenols, halogen compounds, isomers, polar compounds like pharmaceuticals, antibiotics; strong retention of basic compounds

Orthogonality in selectivity

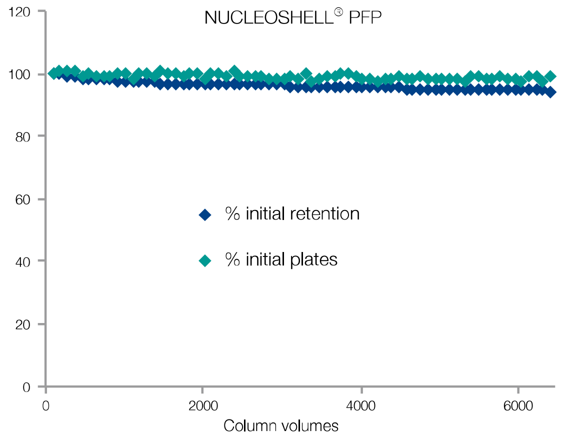
Fluorinated stationary phases in HPLC have gained increasing interest over the last years. Most common representative of fluorinated silica phases is the pentafluorophenyl modification (PFP or F_5). Especially the orthogonal selectivity compared to traditional alkyl phases widens the scope in analytical HPLC. Thus NUCLEOSHELL® PFP offers an excellent selectivity especially for highly polar analytes, aromatic and unsaturated compounds, phenols or halogenated hydrocarbons.

While a typical C_{18} phase just provides hydrophobic interactions between stationary phase and analyte NUCLEOSHELL® PFP offers four different retention mechanisms: polar interactions (H bonds), dipole-dipole interactions, π-π interactions and hydrophobic interactions. Especially the pronounced ion exchange capacity and distinct steric selectivity are typical for the character of fluorinated phases.

Stability of NUCLEOSHELL® PFP at pH 1

MN Appl. No. 125560

Columns:	100 x 4.6 mm NUCLEOSHELL® PFP, 2.7 µm 100 x 4.6 mm Kinetex® PFP, 2.6 µm F5
Eluent:	acetonitrile – 0.5 % TFA, pH 1 (50:50, v/v)
Flow rate:	1.3 mL/min
Temperature:	60 °C
Detection:	UV, 254 nm
Sample:	ethylbenzene



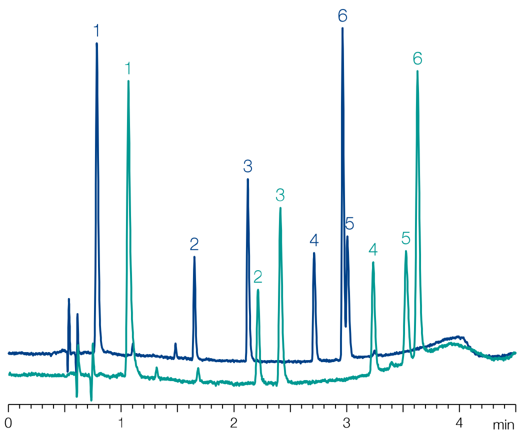
β-Blockers · orthogonal selectivity of NUCLEOSHELL® PFP

MN Appl. No. 125610

Columns:	100 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm NUCLEOSHELL® PFP, 2.7 µm
Eluent:	A) acetonitrile + 0.1 % formic acid B) 0.1 % formic acid 10–35 % A in 2.5 min, 35-50 % A in 2 min
Flow rate:	1.7 mL/min
Temperature:	25 °C
Detection:	UV, 280 nm

Peaks:

1. Atenolol	4. Labetalol
2. Pindolol	5. Alprenolol
3. Metroprolol	6. Propranolol

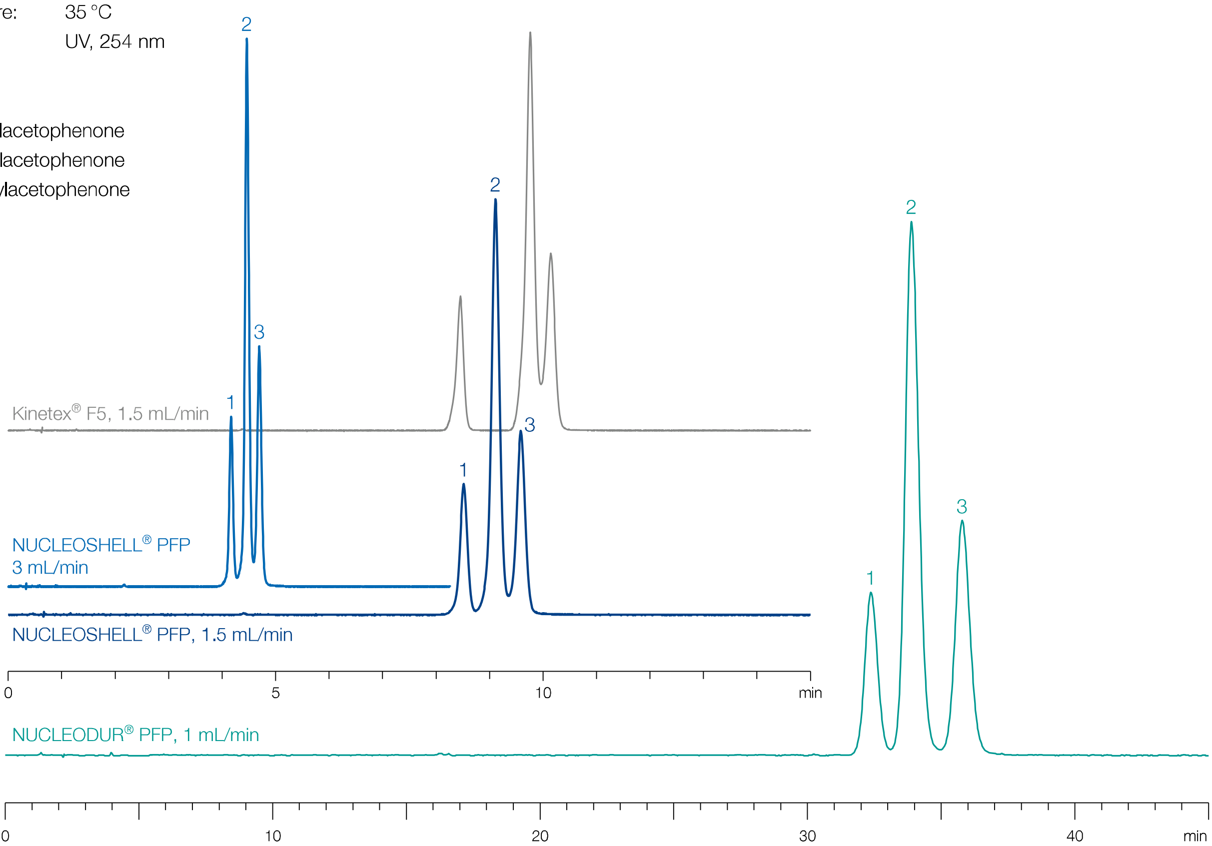


Methylacetophenones

MN Appl. No. 125590

Columns: 100 x 4.6 mm NUCLEOSHELL® PFP, 2.7 µm
250 x 4 mm NUCLEODUR® PFP, 5 µm
100 x 4.6 mm Kinetex® 2.6 µm F5
Eluent: Methanol – water (35:65, v/v)
Flow rate: 1.5 mL/min, 3 mL/min, 1 mL/min, 1.5 mL/min
Temperature: 35 °C
Detection: UV, 254 nm

Peaks:
1. *o*-Methylacetophenone
2. *p*-Methylacetophenone
3. *m*-Methylacetophenone



NUCLEOSHELL® PFP combines the benefits of core-shell technology, high stability, and orthogonal selectivity. Thus it is a useful complementary tool for highly efficient separations especially of isomers, halogenated, aromatic and / or polar compounds.

Ordering information

Eluent in column acetonitrile – water

ID	Length → 50 mm	100 mm	150 mm	EC guard columns*
NUCLEOSHELL® PFP, 2.7 µm particle size 2.7 µm				
Analytical EC columns				
2 mm	763532.20	763534.20	763536.20	763538.20
3 mm	763532.30	763534.30	763536.30	763538.30
4 mm	763532.40	763534.40	763536.40	763538.30
4.6 mm	763532.46	763534.46	763536.46	763538.30

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of the EC column system please see page 250.

NUCLEOSHELL® columns

NUCLEOSHELL® HILIC zwitterionic phase

Key feature

- Core-shell technology for fast and efficient HPLC
- Ideal for reproducible and stable chromatography of highly polar analytes
- Very short column equilibration times

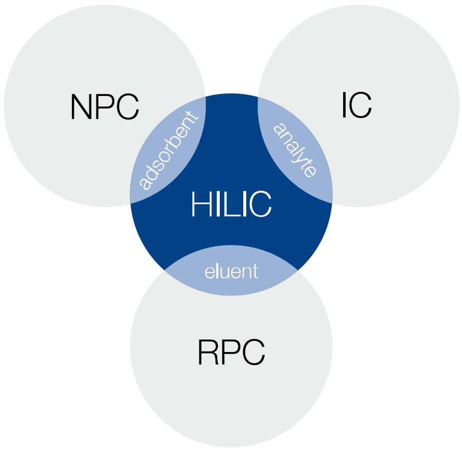
Technical data

- Ammonium - sulfonic acid modified silica; pore size 90 Å, particle size 2.7 µm; carbon content 1.3 %; pH stability 2–8.5; suitable for LC/MS

Recommended application

- Hydrophilic compounds such as polar organic acids and bases, polar natural compounds, nucleosides, oligonucleotides, amino acids, peptides, water-soluble vitamins

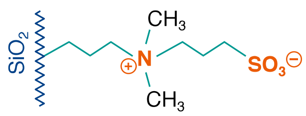
Hydrophilic interaction chromatography



Hydrophilic interaction chromatography (HILIC) is a separation technique using polar stationary phases and organic-aqueous mobile phases. A minimum water content of at least 2 % is indispensable to provide a permanent water layer between the adsorbent surface and the organic fraction of the mobile phase. The sample molecules become separated in a partition chromatography, in which polar analytes are more strongly retained than neutral, less hydrophilic compounds. Consequently, increasing the aqueous part in the mobile phase will diminish retention of the polar sample constituents. In this way HILIC behaves inverse to classical RP chromatography. The particular retention profile of HILIC enables the chromatography of very polar and often small molecules, which won't show any retention on C_8 or C_{18} reversed phases.

Ultra-fast separations at moderate back pressure

NUCLEOSHELL® HILIC is a core-shell technology based stationary phase with a covalently bonded 3-*N*,*N*-dimethylaminopropane sulfonic acid ligand (pat. p nd.). The betaine character of the strong ion-exchanger results in full charge balancing and facilitates fast equilibration times.



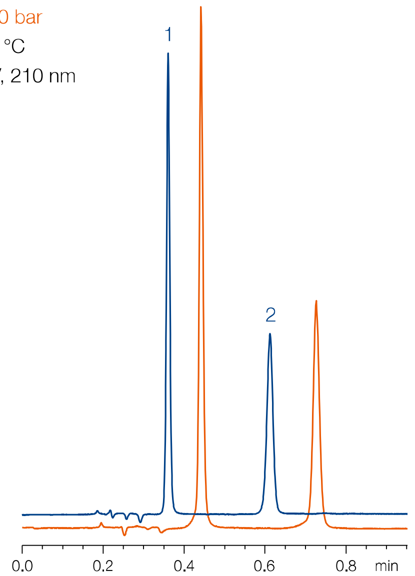
Good separation of polar compounds like the physiologically important substances creatine and creatinine can be achieved on NUCLEOSHELL® HILIC as well as on NUCLEODUR® HILIC, 1.8 µm at similar retention, but much lower back pressure.

Separation of creatine and creatinine

MN Appl. No. 124990

Columns:	50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm
	50 x 4 mm NUCLEODUR® HILIC, 1.8 µm
Eluent:	acetonitrile – 10 mmol/L ammonium acetate, pH 4.0 (90:10, v/v)
Flow rate:	1.7 mL/min
Pressure:	129 bar
	180 bar
Temperature:	25 °C
Detection:	UV, 210 nm

Peaks:
1. Creatinine
2. Creatine



The following chromatograms show the method transfer from a fully porous 3 µm HILIC phase to 2.7 µm core-shell silica with equal selectivity features.

Run time has been cut down to 1 min. Column back pressure remains modest < 400 bar, while solvent demand is reduced to less than 35 %.

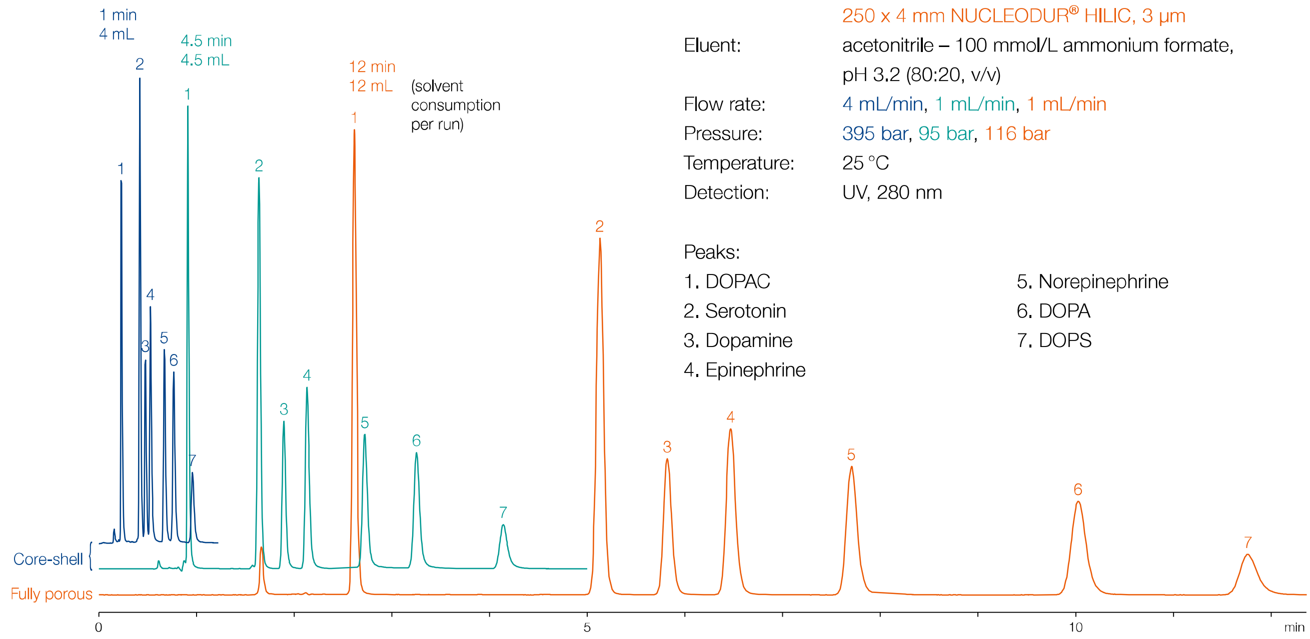
Separation of catecholamines

MN Appl. No. 125440

Columns:	100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm
	100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm
	250 x 4 mm NUCLEODUR® HILIC, 3 µm
Eluent:	acetonitrile – 100 mmol/L ammonium formate, pH 3.2 (80:20, v/v)
Flow rate:	4 mL/min, 1 mL/min, 1 mL/min
Pressure:	395 bar, 95 bar, 116 bar
Temperature:	25 °C
Detection:	UV, 280 nm

Peaks:

1. DOPAC
2. Serotonin
3. Dopamine
4. Epinephrine
5. Norepinephrine
6. DOPA
7. DOPS



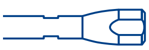
Core-shell silica: separation in 1 min pressure < 400 bar

NUCLEOSHELL® HILIC provides stable and reproducible chromatography, comprising all the benefits of a state-of-the-art core-shell silica.

Ordering information

Eluent in column acetonitrile – water

ID	Length → 50 mm	100 mm	150 mm	EC guard columns*
NUCLEOSHELL® HILIC, 2.7 µm particle size 2.7 µm				
Analytical EC columns				
2 mm	763332.20	763334.20	763336.20	763338.20
3 mm	763332.30	763334.30	763336.30	763338.30
4 mm	763332.40	763334.40	763336.40	763338.30
4.6 mm	763332.46	763334.46	763336.46	763338.30



EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

For details of the EC column system please see page 250.

MACHEREY-NAGEL Column Protection System

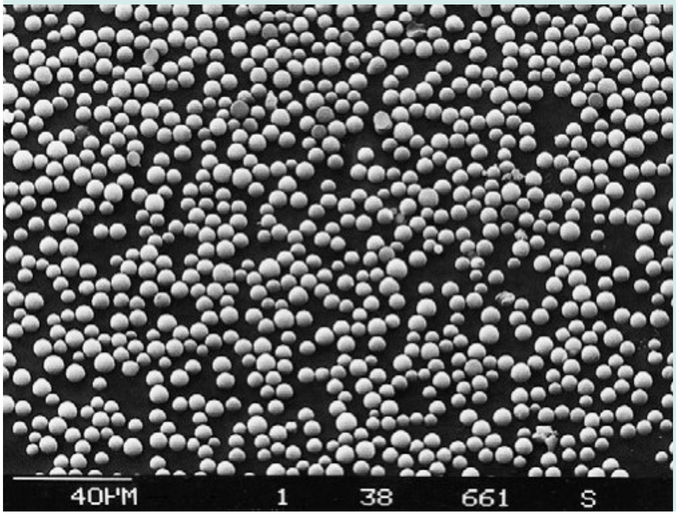
The guard column system for HPLC / UHPLC from MN

- Ideal protection for your analytical main column: significant increase in column lifetime
- Minimized void volume: suitable also for ultra fast HPLC (UHPLC)
- Special ferrules: pressure stability up to 1300 bar (18850 psi)
- Cartridges filled with NUCLEODUR®, NUCLEOSIL® and NUCLEOSHELL® HPLC adsorbents.
- Universal screw-on guard column holder system
- Suitable for all analytical HPLC columns with 1/16“ fittings

Further information on page 251.

NUCLEOSIL® standard silica for HPLC

NUCLEOSIL®



Key feature

- NUCLEOSIL® is a family of totally porous spherical silicas. They feature a very pure and uniform SiO_2 structure and have gained wide acceptance as routine chromatographic packings for very different fields of modern chromatography.
- One of the first spherical silicas used in HPLC
- Developed in the early seventies, it became a world-renowned HPLC packing
- Absolutely reliable choice for routine analyses
- Largest variety of modified HPLC silicas available
- pH stability 2–8 (for NUCLEOSIL® 100-5 C_{18} AB 1–9)
- Due to its particle sizes NUCLEOSIL® finds application in analytical as well as in preparative columns.

Benefits of NUCLEOSIL® silica

- High efficiency due to narrow particle size distribution
- High separation performance due to optimized binding techniques
- High chemical and mechanical stability
- High load capacity and recovery rates
- High reproducibility from lot to lot

Physical properties

NUCLEOSIL® is manufactured with different pore diameters (50, 100, 120, 300, 500, 1000 and 4000 Å) and particle sizes from 3 µm (only NUCLEOSIL® 50, 100 and 120) to 10 µm with very narrow fractionation. All narrow-pore NUCLEOSIL® packings are stable up to 500 bar (7 250 psi), the wide-pore NUCLEOSIL® silicas are stable up to 300 or 400 bar (4 200 or 5 600 psi).

Physical properties of unmodified NUCLEOSIL® materials

Phase	Pore size	Pore volume	Surface (BET)	Density	Pressure stability*
NUCLEOSIL® 50	50 Å	0.8 mL/g	420 m^2/g	0.45 g/mL	500 bar
NUCLEOSIL® 100	100 Å	1 mL/g	350 m^2/g	0.36 g/mL	500 bar
NUCLEOSIL® 120	120 Å	0.65 mL/g	200 m^2/g	0.55 g/mL	500 bar
NUCLEOSIL® 300	300 Å	0.8 mL/g	100 m^2/g	0.45 g/mL	400 bar
NUCLEOSIL® 500	500 Å	0.8 mL/g	35 m^2/g	0.45 g/mL	400 bar
NUCLEOSIL® 1000	1000 Å	0.8 mL/g	25 m^2/g	0.45 g/mL	300 bar
NUCLEOSIL® 4000	4000 Å	0.7 mL/g	10 m^2/g	0.48 g/mL	300 bar

* Maximum packing pressure of NUCLEOSIL® bulk packings

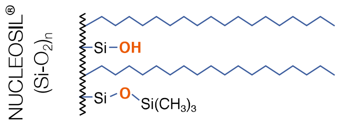
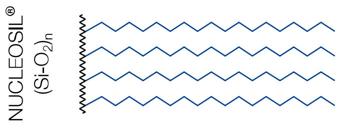
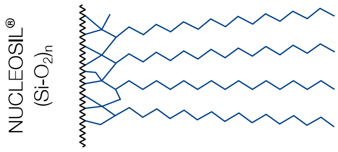
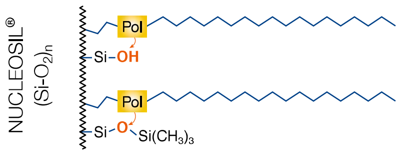
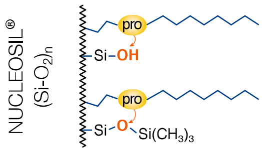
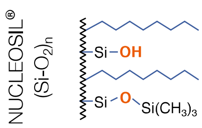
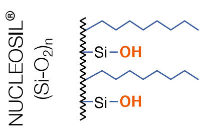
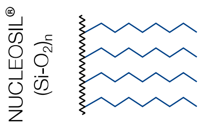
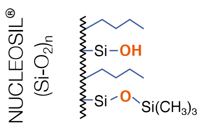
NUCLEOSIL® modifications

- NUCLEOSIL® packings are available as unmodified silica or with numerous chemically bonded phases: RP phases like C_{18} AB, C_{18} HD, C_{18} Nautilus, C_{18}, C_{18} ec, Protect I, C_8 HD, C_8 ec, C_8, C_4, C_2 and C_6H_5 separate mainly by hydrophobic interactions (van der Waals forces). The less polar the sample molecules, the more they are retained – the more polar the sample, the weaker are the hydrophobic interactions and consequently the retention times are shorter.
- Phases with chemically bonded polar groups such as CN, NH_2, $N(CH_3)_2$, OH show selective separation properties. Due to the availability of different functional groups it is possible to vary the chemical characteristics of the surface and consequently the adsorption characteristics of the stationary phase.
- Silica-based ion exchangers (NUCLEOSIL® SA and SB) are stable from pH 2 to 8 and do not swell. Compared to resin-based ion exchangers they offer the advantage of constant permeability, even when the ionic strength and/or pH of the eluent are changed. The separation can be influenced by
  – the type of buffer
  – the ionic strength and
  – the pH value.

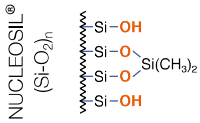
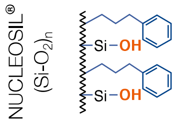
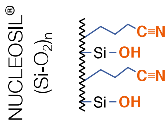
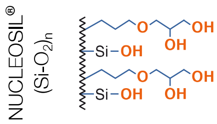
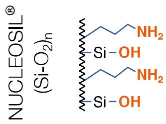
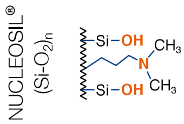
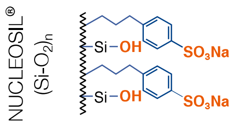
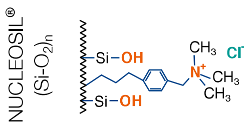
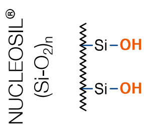
A tabular overview of NUCLEOSIL® phases can be found on page 212.

NUCLEOSIL® phase overview

Overview of NUCLEOSIL® HPLC phases

Phase	Specification	Page	Stability	Interactions	Structure
NUCLEOSIL® RP-Phasen					
C_{18}	octadecyl phase, medium density modification, endcapping 15 % C · USP L1	214	pH 2–8	hydrophobic (van der Waals) interactions slight residual silanol interactions	
C_{18} HD	octadecyl phase, high density monomeric modification, endcapping 20 % C · USP L1	214	pH 2–9	hydrophobic (van der Waals) interactions	
C_{18} AB	octadecyl phase, special crosslinked modification, endcapping 25 % C · USP L1	214	pH 1–9	steric and hydrophobic interactions	
C_{18} Nautilus	octadecyl phase, embedded polar group, endcapping 16 % C · USP L60	214	pH 2–8 up to 100 % H_2O	hydrophobic and polar interactions	
Protect I	special RP phase, protective polar group, monomeric modification, endcapping 11 % C	216	pH 2–8 up to 100 % H_2O	hydrophobic and polar interactions	
C_8 ec	octyl phase, medium density modification, endcapping 9 % C · USP L7	217	pH 2–8	hydrophobic (van der Waals) interactions slight residual silanol interactions	
C_8	octyl phase, no endcapping 8.5 % C · USP L7	217	pH 2–8	hydrophobic (van der Waals) interactions noticeable residual silanol interactions	
C_8 HD	octyl phase, high density modification, endcapping 13 % C · USP L7	218	pH 2–8	hydrophobic (van der Waals) interactions	
C_4	butyl phase, medium density modification, endcapping ~ 2 % C · USP L26	219	pH 2–8	hydrophobic (van der Waals) interactions residual silanol interactions	

Overview of NUCLEOSIL® HPLC phases

Phase	Specification	Page	Stability	Interactions	Structure
C_2	dimethyl phase 3.5 % C · USP L16	219	pH 2–8	hydrophobic (van der Waals) interactions noticeable residual silanol interactions	
C_6H_5	phenyl phase, no endcapping 8 % C · USP L11	220	pH 2–8	π–π interactions and hydrophobic interactions noticeable residual silanol interactions	
Polar NUCLEOSIL® phases and NUCLEOSIL® ion exchangers					
CN / CN-RP	cyano (nitrile) phase USP L10	222	pH 2–8	π–π, polar and hydrophobic interactions	
OH (Diol)	diol · USP L20	220	pH 2–8	polar interactions (hydrogen bonds)	
NH_2 / NH_2-RP	amino · USP L8	221	pH 2–8	polar and hydrophobic interactions, weak ion exchange interactions	
$N(CH_3)_2$	dimethylamino	221	pH 2–8	polar and hydrophobic interactions, weak ion exchange interactions	
SA	sulfonic acid, strongly acid cation exchanger (SCX) USP L9	223	pH 2–8	strong ion exchange interactions	
SB	quaternary ammonium, strongly basic anion exchanger (SAX) USP L14	223	pH 2–8	strong ion exchange interactions	
SiOH	unmodified spherical silica USP L3	224	pH 2–8	polar	

NUCLEOSIL® octadecyl phases (C_{18})

NUCLEOSIL® standard octadecyl phases · USP L1

$-(CH_2)_{17}-CH_3$

Technical data

- Nonpolar phases
- pH stability at 20 °C: 2–8
- carbon content depending on pore size (see table)
- Corresponding NUCLEODUR® phases see C_{18} ec page 181

NUCLEOSIL® C_{18} HD · USP L1

$-(CH_2)_{17}-CH_3$

Technical data

- Nonpolar hydrophobic high density phases; monomeric modification
- pH stability 2–9
- Carbon content 20 %
- Corresponding NUCLEODUR® phases see C_{18} Gravity page 158

NUCLEOSIL® C_{18} AB · USP L1

$-(CH_2)_{17}-CH_3$

Technical data

- Crosslinked hydrophobic phase; polymeric modification; inert towards acidic and basic substances with high affinity for silica
- pH stability 1–9
- Carbon content 25 %; distinct steric selectivity
- Corresponding NUCLEODUR® phases see C_{18} Isis page 164

NUCLEOSIL® C_{18} Nautilus · USP L60

$-(CH_2)_{17}-CH_3$

Technical data

- Stable in 100 % aqueous eluents
- Carbon content 16 %
- Interesting polar selectivity features; very good base deactivation
- Corresponding NUCLEODUR® phases see C_{18} PolarTec page 168

All NUCLEOSIL® octadecyl phases are endcapped.
Custom-packed columns with different column dimensions are available on request.

Ordering information

Eluent in column acetonitrile – water

ID	Length → 100 mm	125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 50-5 C_{18} ec particle size 5 µm, pore size 50 Å, endcapped, 14.5 % C					
Analytical EC columns					
4.6 mm				720098.46	721473.30
NUCLEOSIL® 100-3 C_{18} particle size 3 µm, pore size 100 Å, endcapped, 15 % C					
Analytical EC columns					
4 mm		720150.40		720133.40	721022.30
4.6 mm	720841.46	720150.46	720949.46	720133.46	721022.30
NUCLEOSIL® 100-5 C_{18} particle size 5 µm, pore size 100 Å, endcapped, 15 % C					
Analytical EC columns					
2 mm		720002.20		720014.20	721074.20
3 mm		720002.30		720014.30	721074.30
4 mm	720141.40	720002.40	720120.40	720014.40	721074.30
4.6 mm	720141.46	720002.46	720120.46	720014.46	721074.30

Ordering information

Eluent in column acetonitrile – water

	ID	Length → 100 mm	125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-7 C_{18} particle size 7 µm, pore size 100 Å, endcapped, 15 % C						
Analytical EC columns						
	4 mm				720018.40	
	4.6 mm		720951.46	720110.46	720018.46	
NUCLEOSIL® 100-10 C_{18} particle size 10 µm, pore size 100 Å, endcapped, 15 % C						
Analytical EC columns						
	4 mm				720023.40	
	4.6 mm		720701.46	720140.46	720023.46	
NUCLEOSIL® 120-3 C_{18} particle size 3 µm, pore size 120 Å, endcapped, 11 % C						
Analytical EC columns						
	4 mm	720149.40	720040.40		720055.40	721075.30
	4.6 mm	720149.46	720040.46	720740.46	720055.46	721075.30
NUCLEOSIL® 120-5 C_{18} particle size 5 µm, pore size 120 Å, endcapped, 11 % C						
Analytical EC columns						
	4 mm		720051.40		720041.40	721070.30
	4.6 mm		720051.46	720730.46	720041.46	721070.30
NUCLEOSIL® 120-7 C_{18} particle size 7 µm, pore size 120 Å, endcapped, 11 % C						
Analytical EC columns						
	4 mm				720042.40	
NUCLEOSIL® 120-10 C_{18} particle size 10 µm, pore size 120 Å, endcapped, 11 % C						
Analytical EC columns						
	4 mm				720043.40	
	4.6 mm				720043.46	
NUCLEOSIL® 100-3 C_{18} HD particle size 3 µm, pore size 100 Å, 20 % C						
Analytical EC columns						
	4 mm		720191.40			721196.30
	4.6 mm		720191.46	720193.46		721196.30
NUCLEOSIL® 100-5 C_{18} HD particle size 5 µm, pore size 100 Å, 20 % C						
Analytical EC columns						
	4 mm		720296.40		720280.40	721072.30
	4.6 mm		720296.46	720294.46	720280.46	721072.30
NUCLEOSIL® 100-5 C_{18} AB particle size 5 µm, pore size 100 Å, 25 % C						
Analytical EC columns						
	4 mm		720935.40		720936.40	721073.30
	4.6 mm		720935.46	720305.46	720936.46	721073.30
NUCLEOSIL® 100-3 C_{18} Nautilus particle size 3 µm, pore size 100 Å, 16 % C						
Analytical EC columns						
	4 mm		720472.40			721649.30
	4.6 mm		720472.46	720471.46		721649.30
NUCLEOSIL® 100-5 C_{18} Nautilus particle size 5 µm, pore size 100 Å, 16 % C						
Analytical EC columns						
	4 mm		720430.40		720431.40	721133.30
	4.6 mm		720430.46	720432.46	720431.46	721133.30

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® octadecyl phases (C_{18}) wide pore octadecyl phases · USP L1

$-(CH_2)_{17}-CH_3$

Technical data

- Many biologically interesting molecules can not be separated using conventional narrow pore silicas with pore sizes of about 100 Å. This is why MACHEREY-NAGEL offers a complete line of wide pore packings with pore sizes of 300, 500, 1000 and 4000 Å.
- These materials can also be used for size exclusion chromatography (SEC).

All NUCLEOSIL® octadecyl phases are endcapped.
Custom-packed columns with different column dimensions are available on request.

Ordering information

Eluent in column acetonitrile – water

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 300-5 C_{18} particle size 5 µm, pore size 300 Å, endcapped, 6.5 % C		
Analytical EC columns		
4 mm	720065.40	721085.30
4.6 mm	720065.46	721085.30
NUCLEOSIL® 500-7 C_{18} particle size 7 µm, pore size 500 Å, endcapped, 2 % C		
Analytical EC columns		
4.6 mm	720074.46	
NUCLEOSIL® 1000-7 C_{18} particle size 7 µm, pore size 1000 Å, endcapped, ~ 1 % C		
Analytical EC columns		
4.6 mm	720077.46	

EC columns in packs of 1, guard columns in packs of 3.

VarioPrep preparative HPLC columns with NUCLEOSIL® packing material on request.

NUCLEOSIL® 100 Protect I special RP phase with protective polar group

Technical data

- RP phase with pronounced hydrophilic properties
- Monomeric coating
- Endcapped
- Carbon content 11 %

Ordering information

Eluent in column acetonitrile – water

ID	Length → 125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 Protect I particle size 5 µm, pore size 100 Å				
Analytical EC columns				
4 mm	720175.40		720170.40	721157.30
4.6 mm	720175.46	720174.46	720170.46	721157.30

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® octyl phases (C_8) NUCLEOSIL® standard octyl phases · USP L7

$-(CH_2)_7-CH_3$

Technical data

- Nonpolar phases for RP and ion-pairing chromatography
- Endcapped and non-endcapped modifications available; pH stability at 20 °C: 2–8
- Carbon content depending on pore size (see table)

Recommended application

- Separation of moderately to highly polar (water-soluble) compounds: steroids, nucleosides, cyclodextrins, pharmacological plant constituents
- Corresponding NUCLEODUR® phases see C_8 ec page 183

Ordering information

Eluent in column acetonitrile – water

ID	Length → 125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_8 ec particle size 5 µm, pore size 100 Å, endcapped, 9 % C				
Analytical EC columns				
4.6 mm			720165.46	721096.30
NUCLEOSIL® 100-5 C_8 particle size 5 µm, pore size 100 Å, not endcapped, 8.5 % C				
Analytical EC columns				
4 mm	720001.40		720013.40	721194.30
4.6 mm	720001.46	720990.46	720013.46	721194.30
NUCLEOSIL® 100-7 C_8 particle size 7 µm, pore size 100 Å, not endcapped, 8.5 % C				
Analytical EC columns				
4.6 mm			720017.46	
NUCLEOSIL® 100-10 C_8 particle size 10 µm, pore size 100 Å, not endcapped, 8.5 % C				
Analytical EC columns				
4 mm			720022.40	
4.6 mm			720022.46	
NUCLEOSIL® 120-3 C_8 particle size 3 µm, pore size 120 Å, not endcapped, 6.5 % C				
Analytical EC columns				
4 mm	720071.40			721093.30
4.6 mm	720071.46	720214.46		721093.30
NUCLEOSIL® 120-5 C_8 particle size 5 µm, pore size 120 Å, not endcapped, 6.5 % C				
Analytical EC columns				
4 mm	720050.40		720052.40	721095.30
4.6 mm	720050.46	720735.46	720052.46	721095.30
NUCLEOSIL® 300-5 C_8 particle size 5 µm, pore size 300 Å, not endcapped, ~ 3 % C				
Analytical EC columns				
4.6 mm			720062.46	721061.30

EC columns in packs of 1, guard columns in packs of 3.

Custom-packed columns with different column dimensions are available on request.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® columns

NUCLEOSIL® octyl phases (C_8) NUCLEOSIL® C_8 HD · USP L7

$-(CH_2)_7-CH_3$

Technical data

- Nonpolar high density phases; monomeric modification; endcapped; carbon content 13 %
- Corresponding NUCLEODUR® phases see C_8 Gravity page 158

Recommended application

- Separation of moderate to strong polar (water soluble) analytes like steroids, cyclodextrines, pharmalogical plant ingredients

Ordering information

Eluent in column acetonitrile – water

ID	Length → 125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_8 HD particle size 5 µm, pore size 100 Å				
Analytical EC columns				
4 mm			720196.40	721071.30
4.6 mm		720194.46	720196.46	721071.30

EC columns in packs of 1, guard columns in packs of 3.

Custom-packed columns with different column dimensions are available on request.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

Beside analytical HPLC columns we also produce VarioPrep columns (see page 252) for preparative applications.

NUCLEOSIL® butyl phases (C_4) · USP L26

$-(CH_2)_3-CH_3$

Technical data

- Endcapped phases for RP and ion-pairing chromatography
- pH stability at 20 °C: 2–8; carbon content ~ 2 %
- Retention times are shorter than on C_8 and C_{18} phases

Recommended application

- For separation of macromolecules and hydrophobic substances
- For butyl phases for biochemical separations please refer to page 241

Ordering information

Eluent in column acetonitrile – water

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 120-5 C_4 particle size 5 µm, pore size 120 Å		
Analytical EC columns		
4.6 mm	720096.46	721083.30
NUCLEOSIL® 300-5 C_4 particle size 5 µm, pore size 300 Å		
Analytical EC columns		
4 mm	720059.40	721916.30
4.6 mm	720059.46	721916.30

EC columns in packs of 1, guard columns in packs of 3.

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

NUCLEOSIL® dimethyl phase (C_2) · USP L16

$-(CH_3)_2$

Technical data

- Non-endcapped phase for RP and ion-pairing chromatography
- pH stability at 20 °C: 2–8; carbon content 3.5 %
- Retention times are much shorter than for the other RP phases

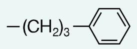
Ordering information

Eluent in column acetonitrile – water

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-7 C_2 particle size 7 µm, pore size 100 Å		
Analytical EC columns		
4.6 mm	720089.46	721030.30

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® phenyl phases (C_6H_5) · USP L11



Technical data

- Relatively nonpolar, non-endcapped phases for RP and ion pairing chromatography
- Polarity similar to C_8, but with different selectivity for PAHs, polar aromatics, fatty acids etc.
- pH stability at 20 °C: 2–8; carbon content 8 %

Recommended application

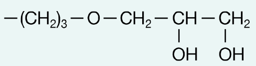
- Separation of moderately polar compounds

Ordering information

Eluent in column acetonitrile – water

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_6H_5 particle size 5 µm, pore size 100 Å, not endcapped		
Analytical EC columns		
4.6 mm	720956.46	721137.30
NUCLEOSIL® 100-7 C_6H_5 particle size 7 µm, pore size 100 Å, not endcapped		
Analytical EC columns		
4 mm	720019.40	
4.6 mm	720019.46	

NUCLEOSIL® diol phases · USP L20



Technical data

- Dihydroxypropyl modified silica for RP and NP chromatography
- Less polar than unmodified silica, very easily wettable with water
- pH stability at 20 °C: 2-8; carbon content 5 %

Ordering information

Eluent in column is *n*-heptane. When using an eluent which is not miscible with *n*-heptane (e.g., water), it is necessary to rinse the column with THF first.

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 OH (Diol) particle size 5 µm, pore size 100 Å		
Analytical EC columns		
4.6 mm	720143.46	721142.30

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® amino phases · USP L8

$-(CH_2)_3-NH_2$

Technical data

- Aminopropyl modified polar silica phase; pH stability at 20 °C: 2–8; carbon content 3.5 %
- Corresponding NUCLEODUR® phases see page 188

Recommended application

Multi-mode chromatography

- NP chromatography with hexane, dichloromethane or 2-propanol as mobile phase for polar compounds such as substituted anilines, esters, chlorinated pesticides
- RP chromatography of polar compounds like carbohydrates in aqueous-organic eluent systems
- Anion exchange chromatography of anions and organic acids using common buffers (e.g., acetate or phosphate) in conjunction with organic modifiers (e.g., acetonitrile)

Ordering information

Eluent in column is *n*-heptane (except for NH_2 RP). When using an eluent which is not miscible with *n*-heptane (e.g., water), it is necessary to rinse the column with THF first.

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 NH_2 particle size 5 µm, pore size 100 Å; eluent in column *n*-heptane		
Analytical EC columns		
4.6 mm	720095.46	721020.30
NUCLEOSIL® 100-5 NH_2-RP particle size 5 µm, pore size 100 Å; eluent in column acetonitrile – water (80:20)		
Analytical EC columns		
4.6 mm	720095.46RP	721155.30
NUCLEOSIL® 100-10 NH_2 particle size 10 µm, pore size 100 Å; eluent in column *n*-heptane		
Analytical EC columns		
4.6 mm	720025.46	

NUCLEOSIL® dimethylamino phase

$-(CH_2)_3-N(CH_3)_2$

Technical data

- Weakly basic anion exchanger, pH stability at 20 °C: 2–8; carbon content 4 %

Recommended application

- Separation of many anions; can also be used in a similar way as the NH_2 phase

Ordering information

Eluent in column is *n*-heptane. When using an eluent which is not miscible with *n*-heptane (e.g., water), it is necessary to rinse the column with THF first.

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 $N(CH_3)_2$ particle size 5 µm, pore size 100 Å		
Analytical EC columns		
4.6 mm	720994.46	721158.30

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

NUCLEOSIL® columns

NUCLEOSIL® cyano phases · USP L10

$-(CH_2)_3-CN$

Technical data

- Polar to midpolar cyano (nitrile) modified silica
- pH stability at 20 °C: 2–8; carbon content 5 % for 100 Å pores, ~ 3 % for 120 Å pores
- Corresponding NUCLEODUR® phases see page 186

Recommended application

Reversed phase and normal phase chromatography

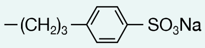
- Normal phase:
  with low-polarity solvents for many compounds, which can also be separated on unmodified silica, however, due to the rapid equilibration much more suitable for gradient separations
- Reversed phase:
  with different selectivity than C_{18}, C_8 or phenyl modified packings

Ordering information

Eluent in column (except for NUCLEOSIL® 100-5 CN-RP) is *n*-heptane. When using an eluent which is not miscible with *n*-heptane (e.g., water), it is necessary to rinse the column with THF first.

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 CN particle size 5 µm, pore size 100 Å; eluent in column *n*-heptane		
Analytical EC columns		
4 mm	720090.40	721078.30
4.6 mm	720090.46	721078.30
NUCLEOSIL® 100-5 CN-RP particle size 5 µm, pore size 100 Å; eluent in column acetonitrile – water		
Analytical EC columns		
4 mm	720205.40	721039.30
4.6 mm	720205.46	721039.30
NUCLEOSIL® 100-10 CN particle size 10 µm, pore size 100 Å; eluent in column *n*-heptane		
Analytical EC columns		
4 mm	720024.40	
4.6 mm	720024.46	
NUCLEOSIL® 120-7 CN particle size 7 µm, pore size 120 Å; eluent in column *n*-heptane		
Analytical EC columns		
4 mm	720057.40	
4.6 mm	720057.46	

NUCLEOSIL® SA phases · USP L9



Technical data

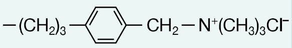
- Strongly acidic cation exchanger (SCX) with benzenesulfonic acid modification
- Capacity ~ 1 meq/g; pH stability at 20 °C: 2–8; carbon content 6.5 %

Ordering information

Eluent in column 0.15 mol/L $(NH_4)_2HPO_4$, pH 5

ID	Length → 125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 SA particle size 5 µm, pore size 100 Å				
Analytical EC columns				
4 mm			720097.40	721024.30
4.6 mm	720709.46	720182.46	720097.46	721024.30
NUCLEOSIL® 100-10 SA particle size 10 µm, pore size 100 Å				
Analytical EC columns				
4.6 mm			720028.46	

NUCLEOSIL® SB phases · USP L14



Technical data

- Strongly basic anion exchanger (SAX) with quaternary ammonium modification
- Capacity ~ 1 meq/g; pH stability at 20 °C: 2–8; carbon content 10 %

Ordering information

Eluent in column 0.15 mol/L $(NH_4)_2HPO_4$, pH 5

ID	Length → 125 mm	150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 SB particle size 5 µm, pore size 100 Å				
Analytical EC columns				
4 mm			720996.40	721025.30
4.6 mm	720989.46	720183.46	720996.46	721025.30
NUCLEOSIL® 100-10 SB particle size 10 µm, pore size 100 Å				
Analytical EC columns				
4.6 mm			720029.46	

NUCLEOSIL® columns

NUCLEOSIL® SiOH unmodified silica · USP L3

Technical data

- Spherical silica, pH stability 2–8
- For physical properties of unmodified NUCLEOSIL® materials please see page 211.
- Maximum working pressure for the EC columns listed below is 400 bar.

Ordering information

Eluent in column is *n*-heptane. When using an eluent which is not miscible with *n*-heptane (e.g., water), it is necessary to rinse the column with THF first.

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 50-5 particle size 5 µm, pore size 50 Å		
Analytical EC columns		
4.6 mm	720093.46	721167.30
NUCLEOSIL® 100-5 particle size 5 µm, pore size 100 Å		
Analytical EC columns		
4.6 mm	720099.46	721518.30

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.

LiChrospher® packings manufactured by E. Merck (D)

Phase	USP	Particle size	Pore size	Modification	Endcapped	Carbon content
LiChrospher® 100 RP 18, 5 µm	L1	nom. 5 µm	100 Å	Octadecyl	–	21 %
LiChrospher® 100 RP 18 ec, 5 µm	L1	nom. 5 µm	100 Å	Octadecyl	+	21 %
LiChrospher® 60 RP select B, 5 µm	L7	nom. 5 µm	60 Å	Octyl	+	12 %
All phases as packed ChromCart® cartridges						
ChromCart® columns require the CC connecting kit (REF 721690).						

Ordering information

Eluent in column acetonitrile – water

ID	Length → 125 mm	150 mm	250 mm	Guard columns*
LiChrospher® 100 RP 18, 5 µm particle size 5 µm, pore size 100 Å				
2 mm	728031.20		728032.20	728053.30
3 mm	728031.30		728032.30	728053.30
4 mm	728031.40		728032.40	728053.40
4.6 mm	728031.46	728033.46	728032.46	728053.40
LiChrospher® 100 RP 18 ec, 5 µm particle size 5 µm, pore size 100 Å				
2 mm	728034.20		728035.20	728054.30
3 mm	728034.30		728035.30	728054.30
4 mm	728034.40		728035.40	728054.40
4.6 mm	728034.46	728036.46	728035.46	728054.40
LiChrospher® 60 RP select B, 5 µm particle size 5 µm, pore size 100 Å				
2 mm	728037.20		728038.20	728055.30
3 mm	728037.30		728038.30	728055.30
4 mm	728037.40		728038.40	728055.40
4.6 mm	728037.46	728039.46	728038.46	728055.40

* can directly be used with the CC connecting kit (REF 721690).

8 mm ChromCart® guard column cartridges in packs of 3, all other columns in packs of 1.

Phase overview for special separations

Overview

Separation / mechanism	Recommended column	Specification of the phase	Page
Environmental analysis			
Anion exchange chromatography of inorganic anions	NUCLEOGEL® Anion I	Strongly basic polymer-based anion exchanger	230
	NUCLEOSIL® Anion II	Strongly basic silica-based anion exchanger	
RP chromatography of PAHs	NUCLEODUR® C_{18} PAH	NUCLEODUR® polymer-coated with C_{18} groups USP L1	227
	NUCLEOSIL® 100-5 C_{18} PAH	NUCLEOSIL® 100 polymer-coated with C_{18} groups USP L1	229
Enantiomer separation			
Polar and π-π interactions	NUCLEOCEL *DELTA*	Silica-based modified cellulose phases USP L40	233
Formation of inclusion complexes	NUCLEODEX α-PM, β-PM, γ-PM and β-OH	Silica-based permethylated and underivatized cyclodextrin phases USP L45	231
Enantioselective binding to chiral protein surface structures	RESOLVOSIL BSA-7	Silica-based protein phase (BSA)	234
Ligand exchange	NUCLEOSIL® CHIRAL-1	Covalently bonded amino acid – Cu(II) complexes USP L32	235
Charge-transfer, dipole-dipole interactions and others	NUCLEOSIL® CHIRAL-2 NUCLEOSIL® CHIRAL-3	Silica-based brush type phases USP L36	236
Separation of biological macromolecules			
Anion exchange chromatography of oligonucleotides and nucleic acids	NUCLEOGEN® DEAE	Silica-based DEAE anion exchanger	237
Anion exchange chromatography of peptides, large proteins and oligonucleotides	NUCLEOGEL® SAX	Polymer-based strongly basic anion exchanger USP L23	240
Cation exchange chromatography of proteins, peptides and carbohydrates	NUCLEOGEL® SCX	Polymer-based strong cation exchanger USP L22	240
Reversed phase chromatography of proteins, peptides and oligonucleotides	NUCLEOSIL® MPN	Monomerically bonded alkyl chains on silica USP L1 / USP L26	243
	NUCLEOSIL® PPN	Polymerically bonded alkyl chains on silica USP L1	244
	NUCLEOGEL® RP 300	Polystyrene – divinylbenzene polymer USP L21	245
Reversed phase chromatography of small molecules	NUCLEOGEL® RP 100	Small pore macroporous PS-DVB polymer USP L21	245
Food analysis · sugars			
RP chromatography of mono- and oligosaccharides	NUCLEOSIL® Carbohydrate	Silica-based special amino phase USP L8	246
Separation of sugars, alcohols, org. acids based on ion exclusion, ion exchange, size exclusion, ligand exchange, NP and RP effects	NUCLEOGEL® SUGAR 810 H, Ca	Resins with sulfonic acid modification in different ionic forms H form USP L17 / Ca form L19 / Pb form L34 / Na form L58	247
Separation of sugars, alcohols, org. acids based on steric exclusion, ligand exchange and partition effects	NUCLEOGEL® SUGAR Ca, Na, Pb NUCLEOGEL® ION 300 OA		248
Gel permeation chromatography (GPC)			
Water-insoluble compounds	NUCLEOGEL® GPC	Polystyrene – divinylbenzene polymer	249

NUCLEODUR® C_{18} PAH special octadecyl phase for PAH analysis · USP L1

Technical data

· Base material NUCLEODUR® silica, particle sizes 1.8 and 3 µm, pore size 110 Å; polymeric coating

Recommended application

· Allows efficient gradient separation of the 16 PAHs according to EPA

Analysis of 16 EPA PAHs with or without acetonitrile

MN Appl. Nos. 123820 / 123830

Separation with acetonitrile

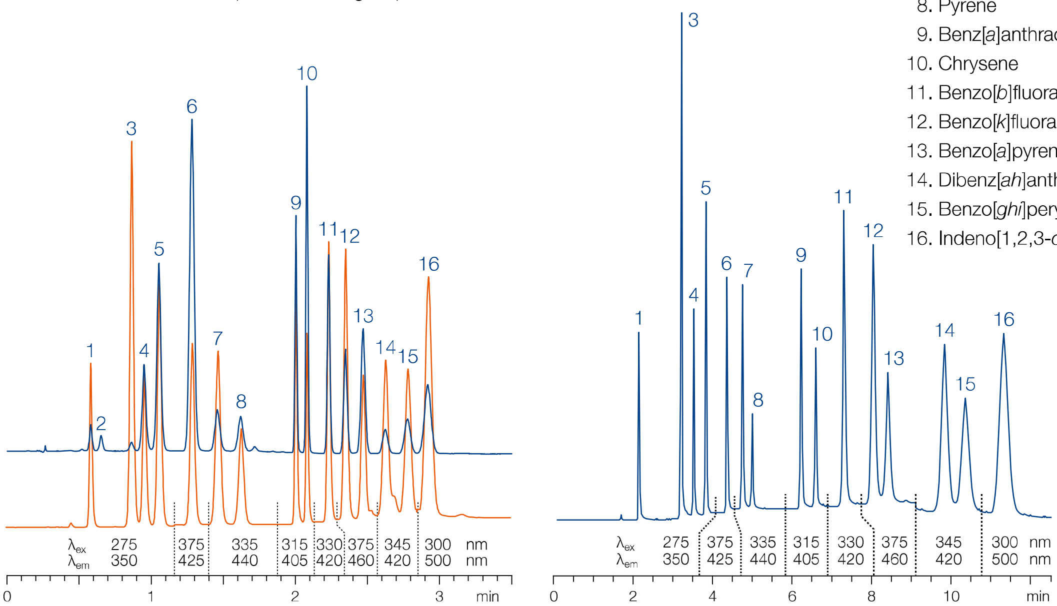
Column:	100 x 4 mm NUCLEODUR® C18 PAH, 3 µm
Eluent:	A) methanol – water (80:20, v/v) B) acetonitrile 2–20 % B in 1.2 min, 20–100 % B in 0.5 min, 100 % B for 2.5 min, 100–2 % B in 0.4 min
Flow rate:	2.5 mL/min, temperature 35 °C
Detection:	UV, 254 nm fluorescence (see chromatogram)

Separation without acetonitrile

Column:	125 x 4 mm NUCLEODUR® C18 PAH, 3 µm
Eluent:	A) water B) methanol 65–97 % B in 6 min, 97 % B for 5 min, 97–65 % B in 0.5 min
Flow rate:	2 mL/min, temperature 35 °C
Detection:	fluorescence (see chromatogram)

Peaks:

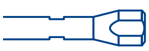
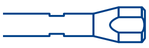
1. Naphthalene
2. Acenaphthylene (not detectable by fluorescence)
3. Acenaphthene
4. Fluorene
5. Phenantrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[*a*]anthracene
10. Chrysene
11. Benzo[*b*]fluoranthene
12. Benzo[*k*]fluoranthene
13. Benzo[*a*]pyrene
14. Dibenz[*ah*]anthracene
15. Benzo[*ghi*]perylene
16. Indeno[1,2,3-*cd*]pyrene



Detection of separated PAHs with UV (250–280 nm), diode array or fluorescence detection at different wavelengths for excitation and emission (acenaphthylene cannot be analyzed with fluorescence detection).

Ordering information

Eluent in column acetonitrile – water (70:30, v/v)

	ID	Length → 100 mm	125 mm	150 mm	250 mm	EC guard columns*
NUCLEODUR® C_{18} PAH, 1.8 µm particle size 1.8 µm · UHPLC						
Analytical EC columns						
	2 mm	760773.20				761970.20
	3 mm	760773.30				761970.30
	4 mm	760773.40				761970.30
NUCLEODUR® C_{18} PAH, 3 µm particle size 3 µm						
Analytical EC columns						
	3 mm	760783.30	760784.30	760785.30	760786.30	761971.30
	4 mm	760783.40	760784.40	760785.40	760786.40	761971.30

Guard column system

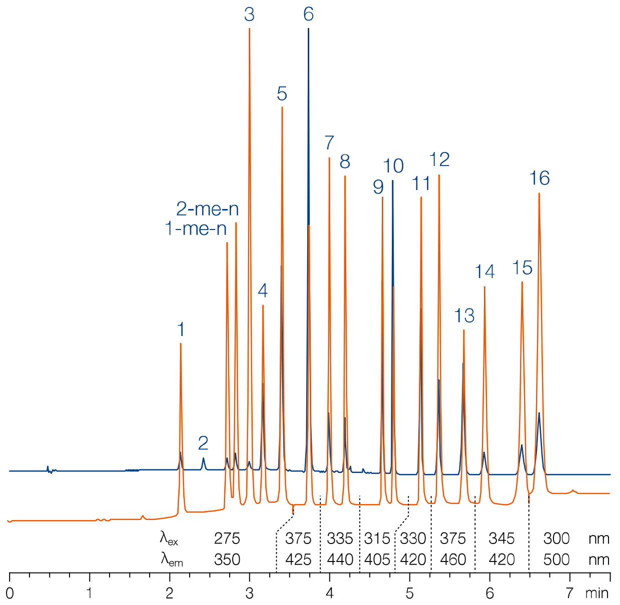
Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

Separation of 18 PAHs on NUCLEODUR® C_{18} PAH

MN Appl. No. 123840

Column:	125 x 4 mm NUCLEODUR® C_{18} PAH, 3 µm
Eluent:	A) methanol – water (70:30, v/v); B) acetonitrile 0–20 % B in 1.5 min, 20–50 % B in 1.5 min, 50–100 % B in 1.0 min, 100 % B for 3 min, 100–0 % B in 0.5 min
Flow rate:	1.5 mL/min
Temperature:	35 °C
Injection:	UV: 1 µL,
Fluorescence:	0.5 µL
Detection:	UV, 254 nm fluorescence (see chromatogram)

Peaks:
(concentrations 10 ng/µL per compound)
1.–16. see page 227
1-me-n: 1-methylnaphthalene
2-me-n: 2-methylnaphthalene

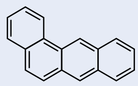


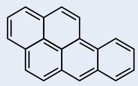
Analysis of polycyclic aromatic hydrocarbons (PAHs) by HPLC

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. PAHs are natural components of coal or gas. They are delivered to our environment by pyrolysis (incomplete burning) of organic materials like coal, oil, fuel, wood, tobacco, … and hence can be found globally. Today most PAHs accrue from anthropogenic processes – but also natural origins (forest fire) are possible. Regarding to past pollutions an important impact had production of coke and gas from black coal. Waste products (e.g., tar) from coking or gas plants are often origin of serious ground water pollutions.

Since a number of PAHs (e.g., benzo[a]pyrene, 3-methylcholanthrene and benzanthracene) have been proven to be carcinogenic, control of the PAH content of food, water and soil is an important task for routine analysis. For choice and limiting values of the polycyclics we refer to the governmental regulations, which exist in many countries (e.g., EPA method 610 of the United States Environmental Protection Agency).

PAHs can be determined by different chromatographic techniques (TLC, GC, HPLC). Thus the 6 PAHs according to German drinking water specification (TVO) can, e.g., be analyzed by TLC (see German Standard DIN 38 409), while a much larger number of polycyclic aromatics can be determined by GC or HPLC.


Benzo[a]anthracen


Benzo[a]pyren

HPLC columns for PAH analysis

For PAH analyses we have developed specially modified C_{18} phases based on NUCLEODUR® and NUCLEOSIL® which allow efficient gradient separation of 16 PAHs according to EPA. Detection of the separated PAHs can be achieved by UV (250–280 nm), with diode array or with fluorescence detection at different wavelengths for excitation and emission. Acenaphthylene cannot be analyzed with fluorescence detection. For cost-effective routine PAH analysis we recommend applications using methanol instead of acetonitrile as eluent. For rapid analysis NUCLEODUR® C_{18} PAH (3 µm) in short columns (100 mm) provides excellent results at high flow rates. Hereby separation of 16 PAHs according to EPA can be achieved in less than 3 min.

Tightened regulations require determination of 2 additional PAHs (1- and 2-methylnaphthalene) – so we developed highly efficient methods for 18 PAHs on the NUCLEODUR® C_{18} PAH.

NUCLEOSIL® 100-5 C_{18} PAH special octadecyl phase for PAH analysis · USP L1

Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å; polymeric coating
- Detection of the separated PAH with UV (250–280 nm), diode array or fluorescence detection at different wavelengths for excitation and emission (acenaphthylene cannot be analyzed with fluorescence detection)

Recommended application

- Efficient gradient separation of the 16 PAHs according to EPA

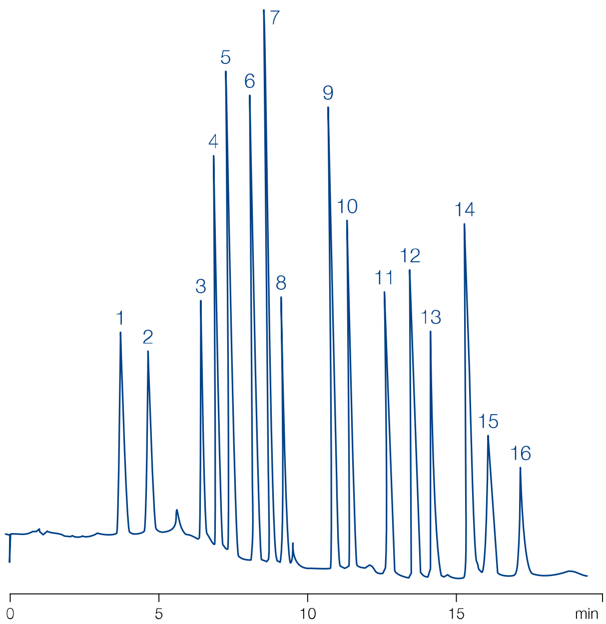
Separation of the PAH standard according to EPA (REF 722393)

MN Appl. No. 115040

Column:	150 x 4 mm NUCLEOSIL® 100-5 C_{18} PAH
Eluent:	A) methanol – water (80:20) B) acetonitrile – tetrahydrofuran (93:7) 0–100 % B in 10 min, 5 min 100 % B
Flow rate:	1 mL/min
Pressure:	140 bar
Temperature:	20 °C
Detection:	UV, 260 nm

Peaks: (10 µg/mL each in acetonitrile)

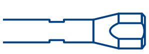
1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenz[ah]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene



Ordering information

Eluent in column acetonitrile – water 70:30

ID	Length → 150 mm	250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_{18} PAH particle size 5 µm, pore size 100 Å			
Analytical EC columns			
2 mm		720117.20	721168.20
3 mm	720923.30	720117.30	721168.30
4 mm	720923.40	720117.40	721168.30
4.6 mm		720117.46	721168.30



PAH standard according to EPA for HPLC

Analytical EC columns		
PAH standard for HPLC	16 PAH according to EPA method 610 in acetonitrile (1 mL) for composition see chromatogram above	722393

Guard column system

Guard columns for EC columns with ID		2 mm	3 mm	4 mm	4.6 mm	Guard column holder
* Column Protection System (pack of)	EC	4/2 (3)	4/3 (3)	4/3 (3)	4/3 (3)	718966

EC columns in packs of 1, guard columns in packs of 3. For details of our column systems see page 250.
[#] This product contains harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

Anion columns for analysis of inorganic anions

NUCLEOGEL® Anion I

Technical data

- Strongly basic polymer-based anion exchanger, particle size 10 µm; pH stability 1–14
- Eluent in column 4 mmol/L salicylate buffer pH 7.8
- Contrary to the silica-based phase also suited for fluoride analysis

NUCLEOSIL® Anion II

Technical data

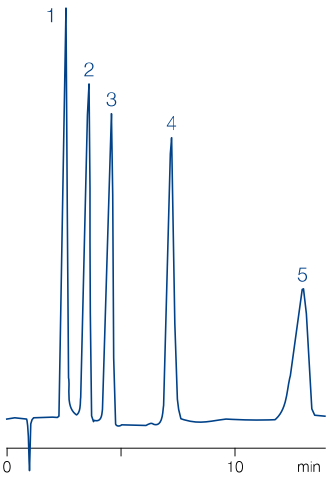
- Base material NUCLEOSIL® silica, particle size 10 µm, pore size 300 Å strongly basic anion exchanger, exchange capacity 50 µeq/g, pH stability 2–7.5
- Eluent in column 0.15 mol/L $(NH_4)_2HPO_4$ buffer pH 5.2 recommended buffer concentration for separation of inorganic anions: 2 mmol/L phthalate
- Preferred method of detection: conductivity or negative UV detection

Separation of an anion standard

MN Appl. No. 106440

Column: 250 x 4 mm NUCLEOSIL® Anion II
Eluent: 2 mmol/L potassium hydrogen phthalate, pH 5.7
Flow rate: 2 mL/min
Detection: UV, 280 nm

Peaks:
1. H_2PO^{4-}
2. Cl^-
3. NO^{2-}
4. NO^{3-}
5. SO_4^{2-}

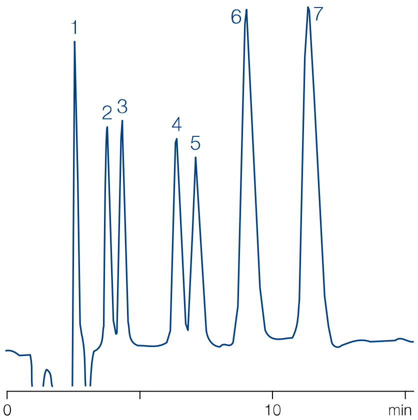


Separation of inorganic anions

MN Appl. No. 115050

Column: 120 x 4.6 mm NUCLEOGEL® Anion I
Eluent: 4 mmol/L salicylic acid – Tris pH 7.8
Flow rate: 1 mL/min
Detection: UV, 254 nm

Peaks:
1. F^-
2. Cl^-
3. NO^{2-}
4. Br^-
5. NO_3^-
6. PO_4^{3-}
7. SO_4^{2-}

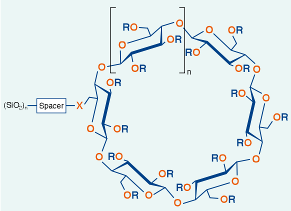


Ordering information

ID	Length → 120 mm	250 mm	Guard columns*
NUCLEOGEL® Anion I eluent 4 mmol/L salicylate buffer pH 7.8			
Analytical Valco type columns			
4.6 mm	719533		719543
NUCLEOSIL® Anion II eluent 0.15 mol/L $(NH_4)_2HPO_4$ buffer pH 5.2			
Analytical EC columns			
4 mm		720094.40	721169.30

* NUCLEOGEL® Anion I Valco type guard columns cartridges are 21 x 4 mm, require guard column holder C, REF 719538, see page 250 (columns in packs of 1, guard columns in packs of 2)
NUCLEOSIL® Anion II guard columns are used with the Column Protection System (REF 718966, see page 251).

NUCLEODEX columns enantiomer separation based on cyclodextrins



NUCLEODEX β-OH β-cyclodextrin (R = H; n = 2) · USP L45

Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å modified cyclodextrins as chiral selectors
- Separation based on hydrogen bonds and dipole interactions between functional groups of the analyte and hydroxyl groups of the cyclodextrin
- Examples for successful enantiomer separations: chlorthalidone and other compounds, which require free hydroxyl groups for enantioselective interactions
- Eluent in column CH_3OH – 0.1 % TEAA pH 4 (55:45)

NUCLEODEX α-PM permethylated α-cyclodextrin (R = CH_3; n = 1)

Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å modified cyclodextrins as chiral selectors
- Examples for successful enantiomer separations: mecoprop and dichlorprop as free carboxylic acids, trans-stilbene oxide, styrene oxide
- Eluent in column CH_3OH – 50 mmol/L phosphate pH 3 (70:30)

NUCLEODEX β-PM permethylated β-cyclodextrin (R = CH_3; n = 2) · USP L45

Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å modified cyclodextrins as chiral selectors
- Examples for successful enantiomer separations: mephobarbital (prominal), pesticide derivatives mecoprop methyl and dichlorprop methyl
- Eluent in column CH_3OH – 0.1 % TEAA pH 4 (65:35)

NUCLEODEX γ-PM permethylated γ-cyclodextrin (R = CH_3; n = 3)

Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å modified cyclodextrins as chiral selectors
- Examples for successful enantiomer separations: steroids or other larger molecules
- Eluent in column CH_3OH – 0.1 % TEAA pH 4 (55:45)

Recommended application

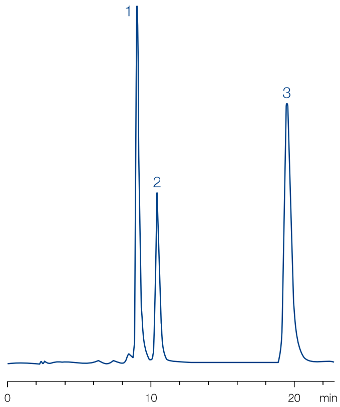
- NUCLEODEX phases are especially suited for the control of optical purity, but also for semipreparative separations and for the analysis of positional and cis-trans isomers.
- For numerous separations on NUCLEODEX phases please visit our website: *www.mn-net.com/apps*

Separation of the positional isomers of nitroaniline

MN Appl. No. 101420

Column:	200 x 4 mm NUCLEODEX β-OH
Eluent:	methanol – 0.1 % triethylammonium acetate pH 4.0 (50:50, v/v)
Flow rate:	0.7 mL/min
Pressure:	180 bar
Detection:	UV, 254 nm
Injection:	1 µL

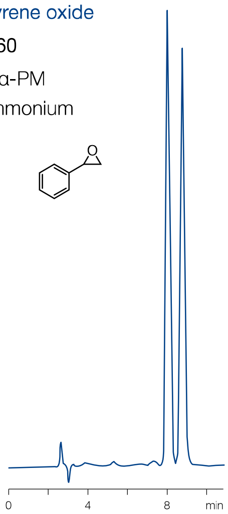
Peaks:
1. *m*-Nitroaniline
2. *o*-Nitroaniline
3. *p*-Nitroaniline



Enantiomer separation of styrene oxide

MN Appl. No. 106160

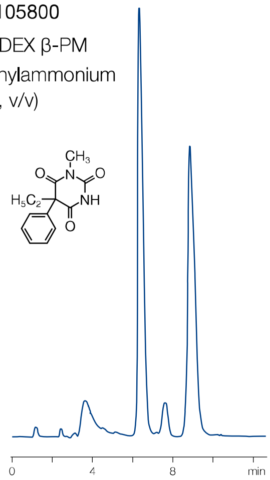
Column:	200 x 4 mm NUCLEODEX α-PM
Eluent:	methanol – 0.1 % triethylammonium acetate pH 4.0 (60:40, v/v)
Flow rate:	0.7 mL/min
Pressure:	160 bar
Detection:	UV, 230 nm
Injection:	2 µL



Enantiomer separation of mephobarbital

MN Appl. No. 105800

Column:	200 x 4 mm NUCLEODEX β-PM
Eluent:	methanol – 0.1 % triethylammonium acetate pH 4.0 (55:45, v/v)
Flow rate:	0.7 mL/min
Pressure:	180 bar
Detection:	UV, 254 nm
Injection:	1 µL

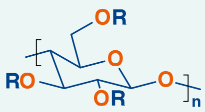


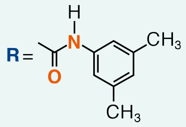
Ordering information

ID	Length → 200 mm	EC guard columns*
NUCLEODEX β-OH eluent methanol – 0.1 % TEAA pH 4 (55:45)		
Analytical EC columns		
4 mm	720124.40	721171.30
NUCLEODEX α-PM eluent methanol – 50 mmol/L phosphate pH 3 (70:30)		
Analytical EC columns		
4 mm	720127.40	721469.30
NUCLEODEX β-PM eluent methanol – 0.1 % TEAA pH 4 (65:35)		
Analytical EC columns		
4 mm	720125.40	721176.30
NUCLEODEX γ-PM eluent methanol – 0.1 % TEAA pH 4 (55:45)		
Analytical EC columns		
4 mm	720752.40	721178.30
NUCLEODEX CC screening kit		
contains one CC 30/4 each with NUCLEODEX β-OH, α-PM, β-PM and γ-PM as well as one CC column holder 30 mm	721920	

* EC 4/3 guard columns for EC columns with 4 mm ID require the Column Protection System guard column holder (REF 718966, see page 251). Columns and guard columns in packs of 1.

NUCLEOCEL *DELTA* enantiomer separation based on a cellulose derivative · USP L40





Technical data

- Base material silica, chiral selector cellulose tris-(3,5-dimethylphenylcarbamate)
  High resolution type (S) with 5 µm particle size, allows use of shorter columns (150 mm) for faster separations, pressure stability up to ~150 bar (2000 psi), pH stability 1–9

NUCLEOCEL *DELTA* for normal phase applications: eluent in column *n*-heptane – 2-propanol (90:10, v/v) typical eluents are heptane – propanol mixtures

NUCLEOCEL *DELTA*-RP for reversed phase applications: eluent in column acetonitrile – water (40:60, v/v) designed for use either in polar organic mode or with eluents containing high concentrations of chaotropic salts such as perchlorate

Recommended application

- Pharmaceutically active compounds, chiral pollutants (e.g., herbicides, PCB), chiral compounds in food (dyes, preservatives), chiral catalysts and bioorganic compounds

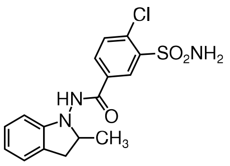
Similar phases: Chiralcel® OD, Kromasil® CelluCoat™, Eurocel® 01, Lux™ Cellulose-1

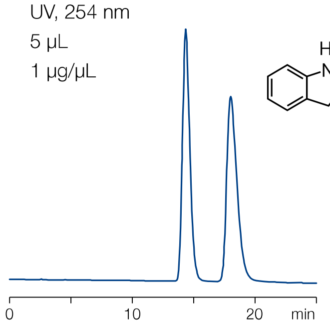
Enantiomer separation of indapamide

MN Appl. No. 121230

Column:	250 x 4,6 mm NUCLEOCEL *DELTA-RP S*
Eluent:	acetonitrile – water (40:60, v/v)
Flow rate:	0.5 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
Injection:	5 µL
Concentration:	1 µg/µL

α = 1.3
R_s = 2.6



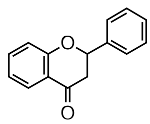


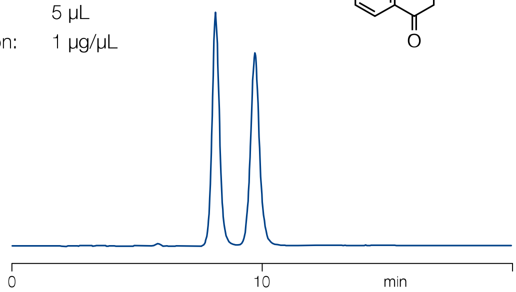
Enantiomer separation of flavanone

MN Appl. No. 121260

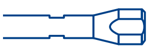
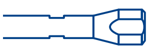
Column:	250 x 4,6 mm NUCLEOCEL *DELTA S*
Eluent:	*n*-heptane – 2-propanol (90:10, v/v)
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm
Injection:	5 µL
Concentration:	1 µg/µL

α = 1.29
R_s = 2.6





Ordering information

	ID	Length → 150 mm	250 mm	EC guard columns*
NUCLEOCEL *DELTA S*, 5 µm eluent n-heptane – 2-propanol (90:10, v/v)				
Analytical EC columns				
	4.6 mm		720445.46	721185.30
NUCLEOCEL *DELTA-RP S*, 5 µm eluent acetonitrile – water (40:60, v/v)				
Analytical EC columns				
	4.6 mm	720451.46	720450.46	721186.30

* EC 4/3 guard column cartridges are used for EC columns of 4.6 mm ID with the Column Protection System guard column holder (REF 718966, see page 251). Columns and guard columns in packs of 1.

RESOLVOSIL BSA-7 protein phase for enantiomer separation · USP L75

Technical data

- Base material NUCLEOSIL® silica, particle size 7 µm, pore size 300 Å chiral selector bovine serum albumin (BSA)
- Separation based on selective interaction of proteins with low molecular compounds, i.e. principles of bioaffinity, including hydrophobic interactions (similar to a true reversed phase), interactions of polar groups and steric effects

Recommended application

- Amino acid derivatives, aromatic amino acids, aromatic sulfoxides, barbiturates, benzodiazepinones, benzoin and benzoin derivatives, β-blockers, coumarin derivatives, and for monitoring stereoselective microbial and enzymatic conversions

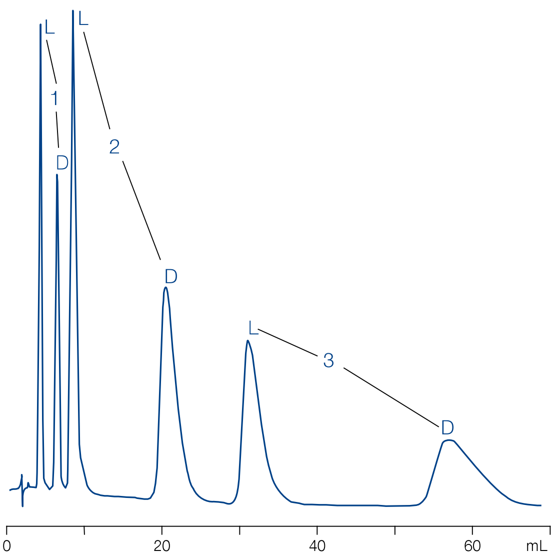
Enantiomer separation of *N*-benzoyl-*D*,*L*-amino acids

MN Appl. No. 105450

S. Allenmark et al. in "Affinity chromatography and biological recognition" (I. Chaiken, M. Wilchek, and I. Parikh. Eds.), Academic Press, New York, 1983, 259–260

Column:	150 x 4 mm RESOLVOSIL BSA-7
Eluent:	50 mmol/L phosphate buffer pH 6.5 + 1 % 1-propanol
Flow rate:	0.70 mL/min
Detection:	UV, 225 nm

Peaks:
1. Serine
2. Alanine
3. Phenylalanine



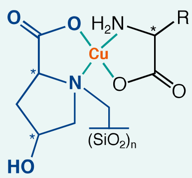
Ordering information

Eluent in column 0.1 mol/L phosphate buffer pH 7.5, 2 % 1-propanol

ID	Length → 150 mm	EC guard columns*
RESOLVOSIL BSA-7		
Analytical EC columns		
4 mm	720046.40	721402.30

* EC 4/3 guard columns for EC columns with 4 mm ID require the Column Protection System guard column holder (REF 718966, see page 251). Columns and guard columns in packs of 1.

NUCLEOSIL® CHIRAL-1 enantiomer separation based on ligand exchange · USP L32



Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 120 Å chiral selector L-hydroxyproline – Cu^{2+} complexes
- Principal interaction mode:
- formation of ternary mixed-ligand complexes with Cu(II) ions; differences in the stability of the diastereomeric complexes cause chromatographic separation

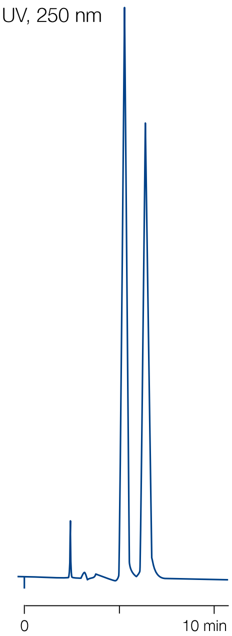
Recommended application

- Enantiomers with two polar functional groups with the correct spacing such as α-amino acids, α-hydroxycarboxylic acids (e.g., lactic acid), *N*-alkyl-α-amino acids etc.

D,L-alanine enantiomers

MN Appl. No. 105410

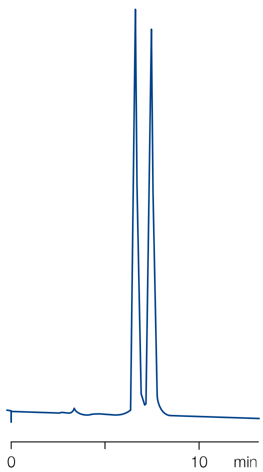
Column:	250 x 4 mm NUCLEOSIL® CHIRAL-1
Eluent:	0.5 mmol/L $CuSO_4$
Flow rate:	1 mL/min
Pressure:	60 bar
Temperature:	60 °C
Detection:	UV, 250 nm



D,L-threonine enantiomers

MN Appl. No. 105410

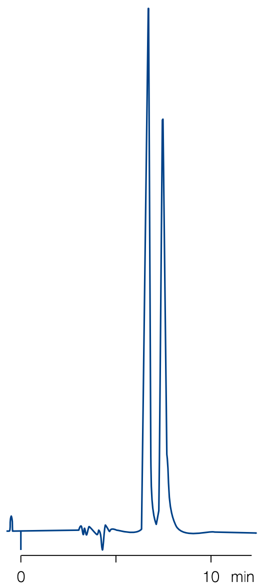
Column:	250 x 4 mm NUCLEOSIL® CHIRAL-1
Eluent:	0.25 mmol/L $CuSO_4$
Flow rate:	0.8 mL/min
Pressure:	65 bar
Temperature:	60 °C
Detection:	UV, 240 nm



Lactic acid enantiomers

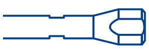
MN Appl. No. 105560

Column:	250 x 4 mm NUCLEOSIL® CHIRAL-1
Eluent:	0.5 mmol/L $CuSO_4$
Flow rate:	0.8 mL/min
Temperature:	60 °C
Detection:	UV, 240 nm
Injection:	1 µL



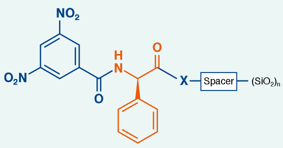
Ordering information

Eluent in column 0.5 mmol/L copper sulfate solution

	ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® CHIRAL-1			
Analytical EC columns			
	4 mm	720081.40	721188.30

* EC 4/3 guard columns for EC columns with 4 mm ID require the Column Protection System guard column holder (REF 718966, see page 251). Columns and guard columns in packs of 1.

NUCLEOSIL® CHIRAL-2 · CHIRAL-3 enantiomer separation in organic eluent systems · USP L36



Technical data

- Base material NUCLEOSIL® silica, particle size 5 µm, pore size 100 Å chiral selector for NUCLEOSIL® CHIRAL-2 is *N*-(3,5-dinitrobenzoyl)-D-phenylglycine, for CHIRAL-3 the optical antipode is used, "brush type" phases
- Principle interaction modes: charge-transfer interactions, hydrogen bonds, dipole-dipole interactions and steric effects

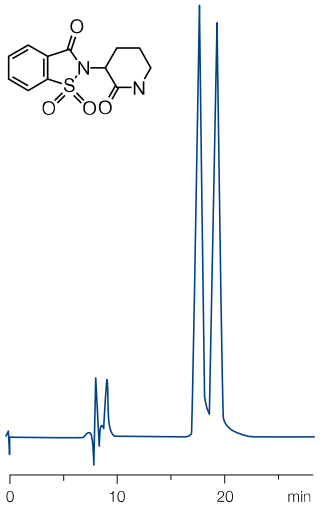
Recommended application

- analysis of stereoisomers such as separation of enantiomers and diastereomers, control of optical purity of plant protectives (pesticides, e.g., propionic acid derived herbicides) pharmaceuticals etc. and for product control in chiral organic syntheses
- For control of optical purity of a substance, the columns NUCLEOSIL® CHIRAL-2 and NUCLEOSIL® CHIRAL-3 allow to select conditions such that the minor enantiomer, present as an impurity, is eluted before the main peak. Overlapping peaks are avoided. This makes an exact quantification of the impurity much easier.

Enantiomer separation of *D*,*L*-supidimide

MN Appl. No. 105690

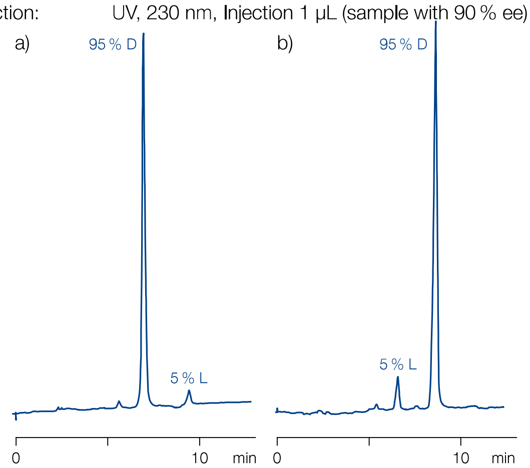
Column:	250 x 4 mm NUCLEOSIL® CHIRAL-2
Eluent:	tetrahydrofuran – *n*-heptane (10:3, v/v)
Flow rate:	1.0 mL/min
Detection:	UV, 220 nm



Control of optical purity of mecoprop methyl

MN Appl. No. 111360

Columns:	a) 250 x 4 mm NUCLEOSIL® CHIRAL-2
	b) 250 x 4 mm NUCLEOSIL® CHIRAL-3
Eluent:	*n*-heptane – 2-propanol – TFA (100:0.05:0.05, v/v/v)
Flow rate:	1 mL/min, ambient temperature
Detection:	UV, 230 nm, Injection 1 µL (sample with 90 % ee)



Ordering information

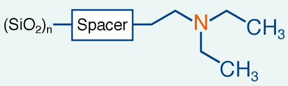
Eluent in column *n*-heptane – 2-propanol – TFAA (100:0.05:0.05, v/v/v)

	ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® CHIRAL-2			
Analytical EC columns			
	4 mm	720088.40	721190.30
NUCLEOSIL® CHIRAL-3			
Analytical EC columns			
	4 mm	720350.40	721190.30

Guard columns for NUCLEOSIL® CHIRAL-2 and CHIRAL-3 are identical.

* EC 4/3 guard columns for EC columns with 4 mm ID require the Column Protection System guard column holder (REF 718966, see page 251). EC columns and EC guard columns in packs of 1.

NUCLEOGEN® columns anion exchange chromatography of nucleic acids



NUCLEOGEN® 60-7 DEAE pore size 60 Å

Technical data

- Base material silica, particle size 7 µm; DEAE anion exchanger
- For the separation of oligonucleotides up to chain lengths of 40 bases with recoveries > 95 % capacity 200 A_{260}/mL (~ 300 A_{260} for a 125 x 4 mm ID column, 1875 A_{260} for a 125 x 10 mm ID column)
- Preparative separations possible when using higher flow rates and longer gradient times

NUCLEOGEN® 500-7 DEAE pore size 500 Å

Technical data

- Base material silica, particle size 7 µm; DEAE anion exchanger
- For the separation of tRNA, 5S RNA, viroids and messenger RNA in the intermediate molecular weight range (25–1 000 kDa) with recoveries > 95 %
- Capacity 730 A_{260} for a 125 x 6 mm ID column, 1940 A_{260} for a 125 x 10 mm ID column

NUCLEOGEN® 4000-7 DEAE pore size 4000 Å

Technical data

- Base material silica, particle size 7 µm; DEAE anion exchanger
- For the separation of plasmids, DNA restriction fragments, ribosomal RNA, messenger RNA and viral RNA, i.e. very high molecular weight nucleic acids (e.g., 1–50 MDa)
- Capacity 120 A_{260} for a 125 x 6 mm ID column, 350 A_{260} for a 125 x 10 mm ID column

For more separations of deoxyoligonucleotides, plasmids and DNA restriction fragments visit our website *www.mn-net.com/apps*

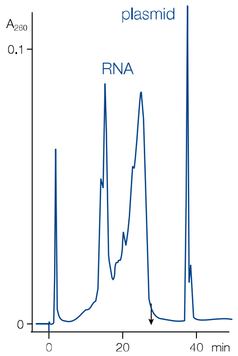
Separation of plasmid pBR 322

MN Appl. No. 107480

M. Colpan, D. Riesner, private communication

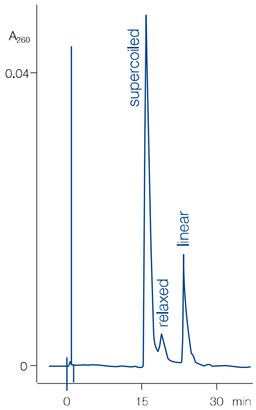
A) isolation of plasmid DNA from a crude cell lysate

Sample:	5 µg plasmid pBR 322 containing cleared lysate from *E. coli*
Column:	125 x 6 mm NUCLEOGEN® 4000-7 DEAE
Eluent:	A) 20 mmol/L K phosphate buffer pH 6.9; 5 mol/L urea
	B) eluent A + 1.5 mol/L KCl
	20–100 % B in 50 min;
	arrow = ionic strength of 850 mmol/L
Flow rate:	1.0 mL/min, 70 bar, ambient temperature
Detection:	UV, 260 nm



B) separation of supercoiled plasmid from relaxed and linear forms

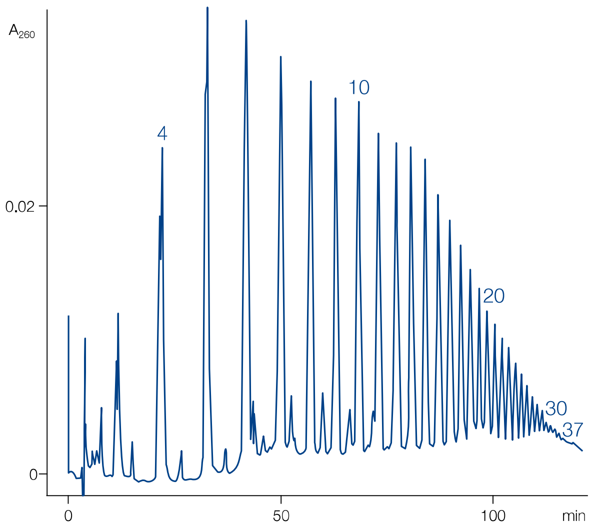
Sample:	plasmid pBR 322, supercoiled, relaxed and linear
Column:	125 x 6 mm NUCLEOGEN® 4000-7 DEAE
Eluent:	A) 20 mmol/L K phosphate buffer pH 6.8; 6 mol/L urea
	B) eluent A + 2 mol/L KCl
	42–100 % B in 230 min
Flow rate:	1.5 mL/min, 45 bar, ambient temperature



Separation of oligo(rA)$_n$

MN Appl. No. 115180

Column:	125 x 4 mm NUCLEOGEN® 60-7 DEAE
Eluent:	A) 20 mmol/L phosphate buffer, pH 5.5, 5 mol/L urea
	B) buffer A + 1 mol/L KCl
	0–100 % B in 200 min
Flow rate:	2 mL/min
Pressure:	110 bar
Temperature:	ambient
Detection:	UV, 260 nm

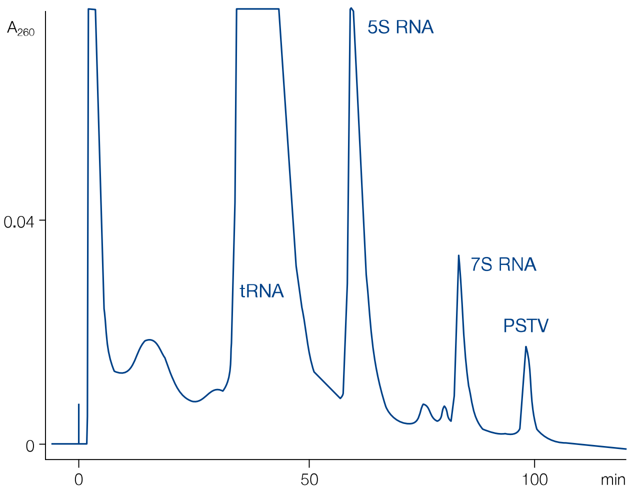


Preparative separation of a crude RNA extract of viroid (PSTV) infected tomato plants

MN Appl. No. 107490

D. Riesner, BioEngineering 1 (1988) 42–48

Column:	125 x 6 mm NUCLEOGEN® 500-7 DEAE
Eluent:	A) `250 mmol/L KCl, 20 mmol/L phosphate buffer, pH 6.6, 5 mol/L urea
	B) 1 mol/L KCl, 20 mmol/L phosphate buffer, pH 6.6, 5 mol/L urea
	0–50 % B in 120 min, 50–100 % B in 250 min
Flow rate:	3 mL/min
Pressure:	40 bar, ambient temperature
Detection:	260 nm



Ordering information

Eluent in column methanol

ID	Length → 125 mm	Guard columns*
NUCLEOGEN® 60-7 DEAE particle size 7 µm, pore size 60 Å		
Analytical EC columns		
4 mm	736596.40	736400.40
Preparative VarioPrep columns		
10 mm	736597.100	736400.40
NUCLEOGEN® 500-7 DEAE particle size 7 µm, pore size 500 Å		
Analytical Valco type columns		
6 mm	736598	736400.40
Preparative VarioPrep columns		
10 mm	736599.100	736400.40
NUCLEOGEN® 4000-7 DEAE particle size 7 µm, pore size 4000 Å		
Analytical Valco type columns		
6 mm	736601	736400.40
Preparative VarioPrep columns		
10 mm	736602.100	736400.40

* NUCLEOGEN® guard columns are 30 mm long and require the CC column holder 30 mm (REF 721823).
Columns in packs of 1, guard columns in packs of 2.

NUCLEOGEL® SAX anion exchange of biological macromolecules · USP L23

Technical data

- Polymer-based strongly basic anion exchanger -$N^+(CH_3)_3$, gel matrix quaternized PEI; particle size 8 µm, pore size 1000 Å
- pH working range 1–13, max. working pressure 200 bar

Recommended application

- Purification of peptides, large proteins and oligonucleotides, high capacity for proteins even at pH 10

NUCLEOGEL® SCX cation exchange of biological macromolecules · USP L22

Technical data

- Polymer-based strongly acidic cation exchanger -SO_3^-, hydrophilic gel matrix; particle size 8 µm, pore size 1000 Å
- pH working range 1–13, max. working pressure 200 bar

Recommended application

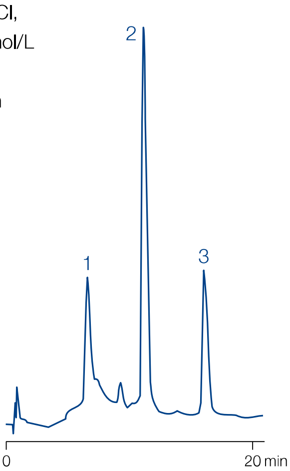
- Proteins, peptides and carbohydrates with high isoelectric point

Separation of hen's egg white

MN Appl. No. 115200

Sample:	frozen egg white was thawed, filtered and diluted 1:8 with eluent A
Column:	50 x 4.6 mm NUCLEOGEL® SAX 1000-8
Eluent:	A) 0.01 mol/L Tris-HCl, pH 7.5; B) A + 0.5 mol/L NaAc, pH 7.5; 0–100 % B in 20 min
Flow rate:	1 mL/min
Inj.volumen:	50 µL
Detection:	UV, 280 nm

Peaks:
1. Conalbumin
2. Ovalbumin
3. not identified

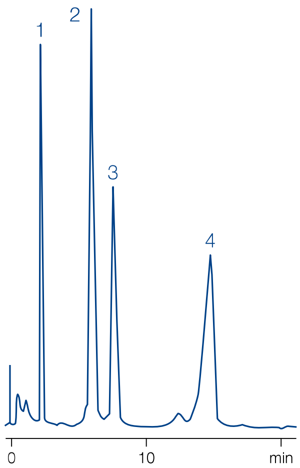


Separation of protein standards

MN Appl. No. 108261

Column:	50 x 4.6 mm NUCLEOGEL® SCX 1000-8
Eluent:	A) 0.02 mol/L KH_2PO_4, pH 6.0; B) A + 0,5 mol/L NaCl, pH 6.0; 0–100 % B in 20 min
Flow rate:	1 mL/min
Detection:	UV, 280 nm

Peaks:
1. Myoglobin
2. α-Chymotrypsinogen A
3. Cytochrome C
4. Lysozyme



Ordering information

Eluent in column 0.1 mol/L Na_2SO_4 + 0.2 % NaN_3

ID	Length → 50 mm	Guard columns*
NUCLEOGEL® SAX pore size 1000 Å		
Analytical Valco type columns		
4.6 mm	719469	719600
NUCLEOGEL® SCX pore size 1000 Å		
Analytical Valco type columns		
4.6 mm	719475	719540

* NUCLEOGEL® SAX and SCX Valco type guard columns measure 5 x 3 mm and require the guard column holder B, REF 719539 (see page 250)
Columns in packs of 1, guard columns in packs of 2.

NUCLEODUR® 300 C_{18} ec · C_4 ec wide pore silica for biochromatography · USP L1 (C_{18}) · USP L26 (C_4)

Key feature

- Reliable wide pore RP phases for daily routine analysis
- Medium density octadecyl or butyl modification with exhaustive endcapping
- Ideal phases for separation of biomolecules

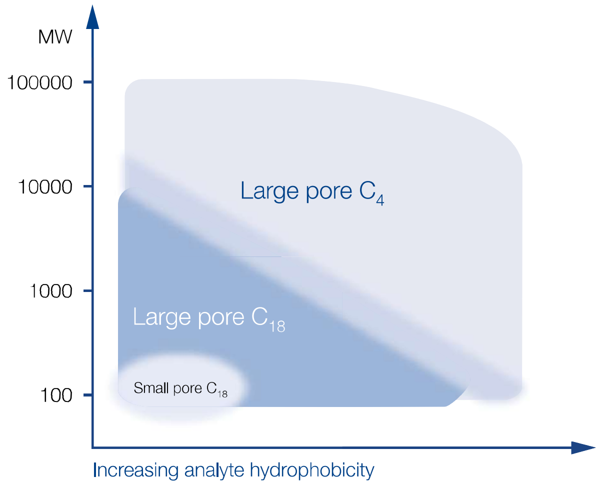
Technical data

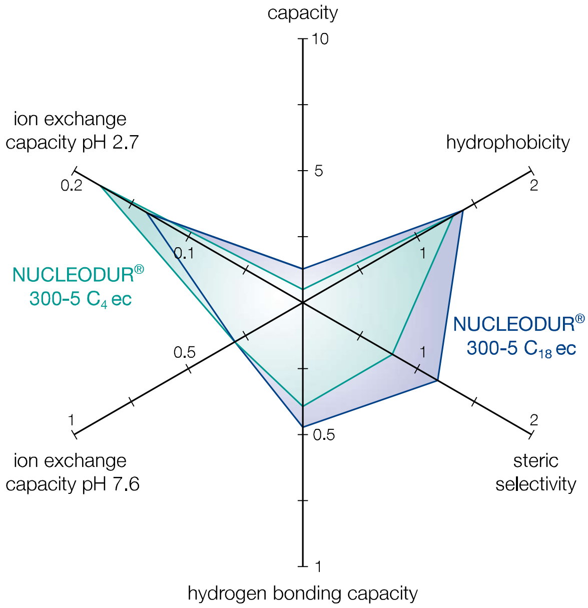
- Pore size 300 Å; particle size 5 µm, carbon content 4 % for C_{18}, 2.5 % for C_4; pH stability 1–9; high reproducibility from lot to lot

Recommended application

- Biological macromolecules like proteins or peptides

Column selection by analyte characteristics





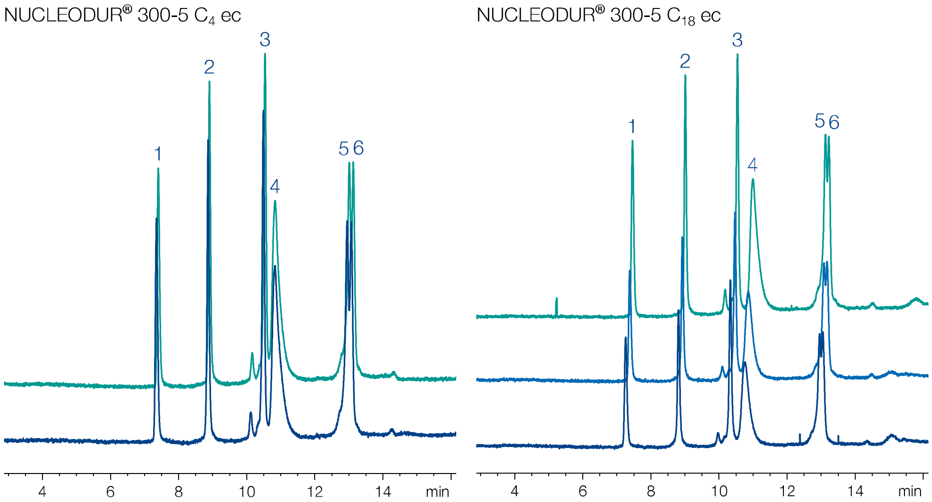
Tanaka plots of NUCLEODUR® wide pore phases

Batch-to-batch reproducibility of NUCLEODUR® 300-5 C_4 ec and NUCLEODUR® 300-5 C_{18} ec

MN Appl. Nos. 126551 / 126552

Columns:	250 x 4 mm
Eluent:	A) 0.1 % TFA in water B) 0.08 % TFA in acetonitrilel 20–60 % B in 15 min
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV, 280 nm

Peaks:
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. β-Lactoglobulin
6. β-Lactoglobulin 2



Comparison of narrow and wide pore NUCLEODUR®
for the separation of proteins

MN Appl. No. 126590

Columns: 250 x 4,6 mm NUCLEODUR® 300-5 C_{18} ec
250 x 4,6 mm NUCLEODUR® C_{18} Gravity, 5 µm

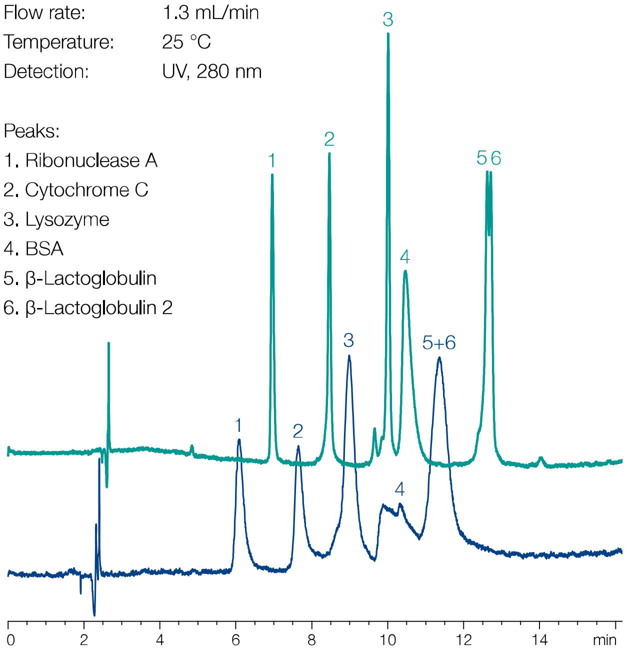
Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
20–65 % B in 15 min
(3 min 65 % B)

Flow rate: 1.3 mL/min

Temperature: 25 °C

Detection: UV, 280 nm

Peaks:
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. β-Lactoglobulin
6. β-Lactoglobulin 2



Sharper peaks of larger molecules on wide pore material

Tryptic digest of cytochrome C

MN Appl. No. 126600

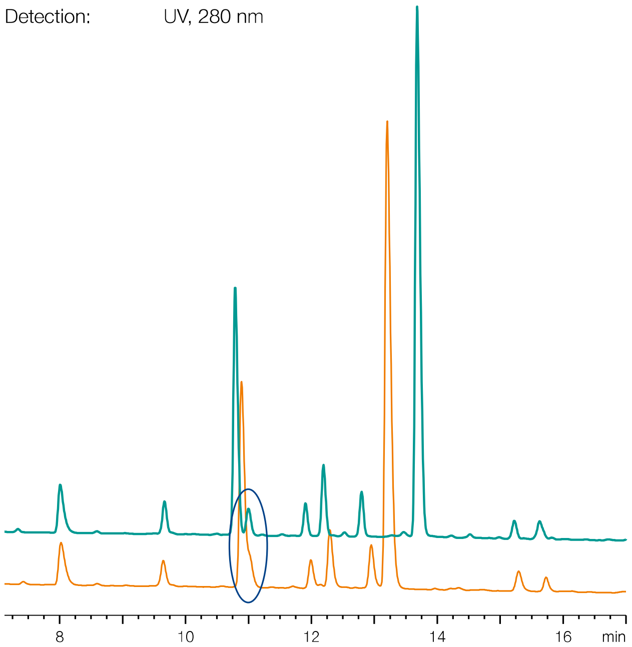
Columns: 250 x 4,6 mm NUCLEODUR® 300-5 C_{18} ec
250 x 4,6 mm Jupiter® C_{18}, 5 µm

Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
5–40 % B in 15 min (1 min 40 % B)

Flow rate: 1.3 mL/min

Temperature: 30 °C

Detection: UV, 280 nm



Less tailing and better separation on NUCLEODUR® 300 C_{18} ec

Ordering information

Eluent in column acetonitrile – water

ID	Length → 100 mm	125 mm	150 mm	250 mm	EC guard columns*
NUCLEODUR® 300-5 C_{18} ec octadecyl phase, particle size 5 µm, pore size 300 Å, endcapped, 4 % C					
Analytical EC columns					
2 mm	760183.20	760184.20	760185.20	760186.20	761988.20
3 mm	760183.30	760184.30	760185.30	760186.30	761988.30
4 mm	760183.40	760184.40	760185.40	760186.40	761988.30
4.6 mm	760183.46	760184.46	760185.46	760186.46	761988.30
NUCLEODUR® 300-5 C_4 ec butyl phase, particle size 5 µm, pore size 300 Å, endcapped, 2.5 % C					
Analytical EC columns					
2 mm	760193.20	760194.20	760195.20	760196.20	761989.20
3 mm	760193.30	760194.30	760195.30	760196.30	761989.30
4 mm	760193.40	760194.40	760195.40	760196.40	761989.30
4.6 mm	760193.46	760194.46	760195.46	760196.46	761989.30

* EC guard columns require the Column Protection System guard column holder (REF 718966, see page 251).
EC columns in packs of 1, guard columns in packs of 3.

NUCLEOSIL® MPN RP chromatography of biological macromolecules

NUCLEOSIL® 100-5 C_{18} MPN · USP L1

Key feature
- Octadecyl phase, particle size 5 µm; pore size 100 Å
- Dynamic protein binding capacity per g packing: 6 mg BSA, 110 mg cytochrome C
- pH working range 2–8, max. working pressure 250 bar

Technical data
- Silica-based reversed phase materials with monomerically bonded alkyl chains, brush type structure predominantly hydrophobic forces with a small portion of hydrophilic interactions
- Maximum separation efficiency can be achieved when the injected protein mass does not exceed 1–2 % of the maximum protein loading capacity.

NUCLEOSIL® 300-5 C_4 MPN · USP L26

Key feature
- Butyl phase, particle size 5 µm, pore size 300 Å
- Dynamic protein binding capacity per g packing: 14 mg BSA, 27 mg cytochrome C especially suited for the purification of larger, hydrophobic peptides and very different proteins
- pH working range 2–8, max. working pressure 250 bar

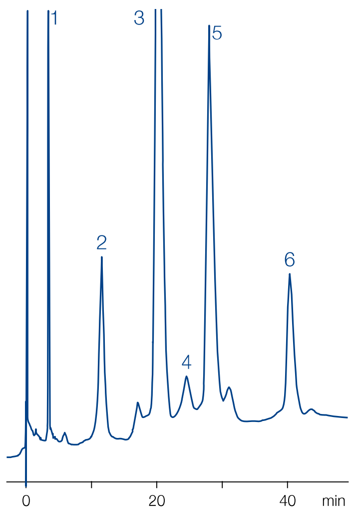
Technical data
- Silica-based reversed phase materials with monomerically bonded alkyl chains, brush type structure predominantly hydrophobic forces with a small portion of hydrophilic interactions
- Maximum separation efficiency can be achieved when the injected protein mass does not exceed 1–2 % of the maximum protein loading capacity.

Separation of haemoglobin chains

MN Appl. No. 108240

Column:	250 x 4 mm NUCLEOSIL® 300-5 C_4 MPN
Eluent:	A) 20 % acetonitrile, 80 % water, 0.1 % TFA
	B) 60 % acetonitrile, 40 % water, 0.1 % TFA
	40–60 % B in 60 min
Flow rate:	1 mL/min
Detection:	UV, 220 nm

Peaks:
1. Hem
2. β-globin
3. α-globin
4. $^{A}\gamma^{T}$-globin
5. $^{G}\gamma$-globin
6. $^{A}\gamma^{I}$-globin



Ordering information

Eluent in column methanol

	ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_{18} MPN			
Analytical EC columns			
	4 mm	720231.40	
NUCLEOSIL® 300-5 C_4 MPN			
Analytical EC columns			
	4 mm	720245.40	721119.30

* EC guard columns require the Column Protection System guard column holder (REF 718966, see page 251). Columns in packs of 1, guard columns in packs of 2.

NUCLEOSIL® PPN RP chromatography of biological macromolecules

NUCLEOSIL® 100-5 C_{18} PPN · USP L1

Key feature

- Octadecyl phase, particle size 5 µm, pore size 100 Å, dynamic protein binding capacity per g packing: 8 mg BSA, 64 mg cytochrome C; suited for the separation of peptides and proteins up to about 40 kD, also suited for basic peptides

Technical data

- Silica-based reversed phase materials with polymerically bonded alkyl chains; exclusively hydrophobic interactions
- pH working range 1–9, max. working pressure 250 bar

NUCLEOSIL® 500-5 C_{18} PPN · USP L1

Key feature

- Octadecyl phase, particle size 5 µm, pore size 500 Å, dynamic protein binding capacity per g packing: 22 mg BSA, 40 mg cytochrome C; especially suited for large peptides and medium-size hydrophilic proteins

Technical data

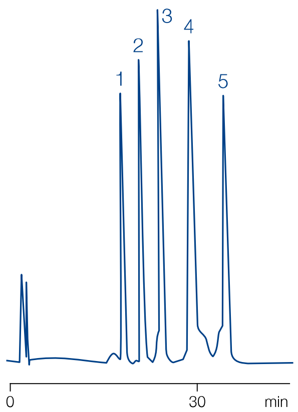
- Silica-based reversed phase materials with polymerically bonded alkyl chains; exclusively hydrophobic interactions
- pH working range 1–9, max. working pressure 250 bar

Separation of a protein standard

MN Appl. No. 108220

Column:	125 x 4 mm NUCLEOSIL® 100-5 C_{18} PPN
Eluent:	A) 0.1 % TFA in H_2O
	B) 0.08 % TFA in CH_3CN
	20–60 % B in 10 min
Flow rate:	1.0 mL/min
Detection:	UV, 280 nm

Peaks:
1. Ribonuclease
2. Cytochrome C
3. Lysozyme
4. β-Lactoglobulin
5. Ovalbumin

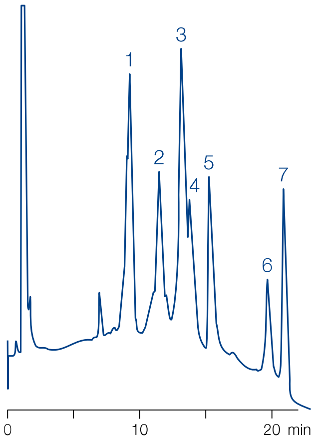


Separation of pancreatic secretion of piglets

MN Appl. No. 108280

Column:	125 x 4 mm NUCLEOSIL® 500-5 C_{18} PPN
Eluent:	A) 0.1 % TFA in H_2O
	B) 0.08 % TFA in CH_3CN
	30–50 % B in 14 min, then 50–65 % B in 6 min
Flow rate:	1 mL/min
Detection:	UV, 215 nm

Peaks:
1. Trypsin + trypsinogen
2. Proelastase
3. Lipase + α-Chymotrypsin
4. Chymotrypsinogen
5. α-Amylase
6., 7. Procarboxypeptidase



Ordering information

Eluent in column methanol

	ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® 100-5 C_{18} PPN particle size 5 µm, pore size 100 Å			
Analytical EC columns			
	4 mm	720252.40	721567.30
NUCLEOSIL® 500-5 C_{18} PPN particle size 5 µm, pore size 500 Å			
Analytical EC columns			
	4 mm	720258.40	721924.30

* EC guard columns require the Column Protection System guard column holder (REF 718966, see page 251).
Columns in packs of 1, guard columns in packs of 2.

NUCLEOGEL® RP columns RP columns for biochemical applications · USP L21

Technical data

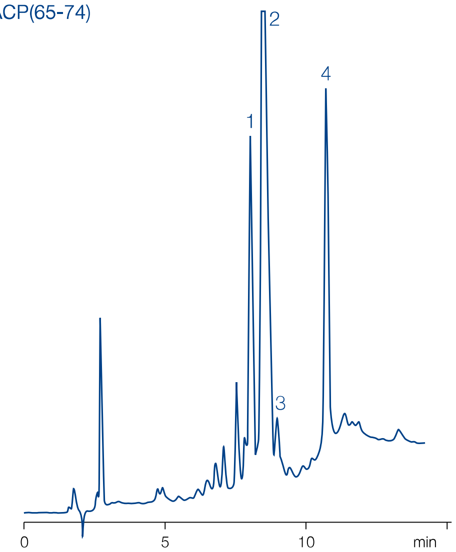
- Polystyrene resin cross-linked with divinylbenzene, available particle sizes 5 μm and 8 μm, available pore sizes 100 Å and 300 Å
- pH working range 1–13, max. working pressure 180 bar
- Small pore columns for reversed phase separation of small molecules such as pharmaceuticals with basic properties, e.g., organic heterocycles; also suited for separation of nucleosides and nucleotides up to 5000 Da; allow gradient as well as isocratic elution
- Wide pore columns are especially recommended for large biomolecules higher background hydrophobicity compared to silica phases

Analysis of the synthetic acyl carrier protein ACP(65-74)

MN Appl. No. 108500

Column: 150 x 4.6 mm NUCLEOGEL® RP 100-8
Eluant: A) 0.1 % TFA in acetonitrile – water (1:99, v/v)
B) 0.1 % TFA in acetonitrile – water (99:1, v/v)
10–60 % B in 20 min
Flow rate: 1 mL/min
Detection: UV, 220 nm

Peaks:
1. ACP(66-74)(H-Gln)
2. ACP(65-74)
3. ACP(66-74)(Glp)
4. Thioanisole



Ordering information

Eluent in column acetonitrile – water

ID	Length → 50 mm	150 mm	250 mm	Guard columns*
NUCLEOGEL® RP 100-5 particle size 5 μm, pore size 100 Å				
Analytical Valco type columns				
4.6 mm		719454	719455	719542
NUCLEOGEL® RP 100-8 particle size 8 μm, pore size 100 Å				
Analytical Valco type columns				
4.6 mm		719456	719520	719542
NUCLEOGEL® RP 300-5 particle size 5 μm, pore size 300 Å				
Analytical Valco type columns				
4.6 mm	719459			719542
NUCLEOGEL® RP 300-8 particle size 8 μm, pore size 300 Å				
Analytical Valco type columns				
4.6 mm	719460			719542

* Valco type guard columns measure 5 x 3 mm and require Guard column holder B, REF 719539, see page 250.
Columns in packs of 1, guard columns in packs of 2.

NUCLEOSIL® Carbohydrate separation of mono- and disaccharides · USP L8

Technical data

· Matrix: NUCLEOSIL® silica with amino modification, particle size 10 µm

Recommended application

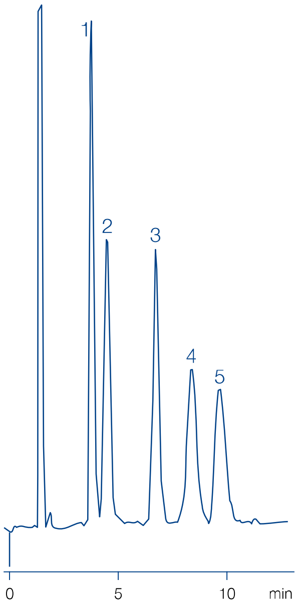
· RP separation of mono- and disaccharides

Separation of sugars

MN Appl. No. 102480

Column: 250 x 4 mm NUCLEOSIL® Carbohydrate
Eluent: acetonitrile – water (79:21, v/v)
Flow rate: 2 mL/min
Temperature: 25 °C
Detection: RI
Injection: 10 µL

Peaks:
1. Fructose
2. Glucose
3. Saccharose
4. Maltose
5. Lactose



Ordering information

Eluent in column acetonitrile – water (79:21, v/v)

ID	Length → 250 mm	EC guard columns*
NUCLEOSIL® Carbohydrate		
Analytical EC columns		
4 mm	720905.40	721170.30

* EC 4/3 guard columns for EC columns with 4 mm ID require the Column Protection System guard column holder (REF 718966, see page 251). Columns and guard columns in packs of 1.

NUCLEOGEL® SUGAR 810 separation of sugars · USP L17 (H-Form) · USP L19 (Ca form)

Technical data

- Sulfonated polystyrene - divinylbenzene resins in different ionic forms; due to a different selectivity pattern compared to NUCLEOGEL® SUGAR columns, the range of application is considerably enlarged
- Separation mechanism: ion exclusion, ion exchange, size exclusion, ligand exchange, NP and RP chromatography

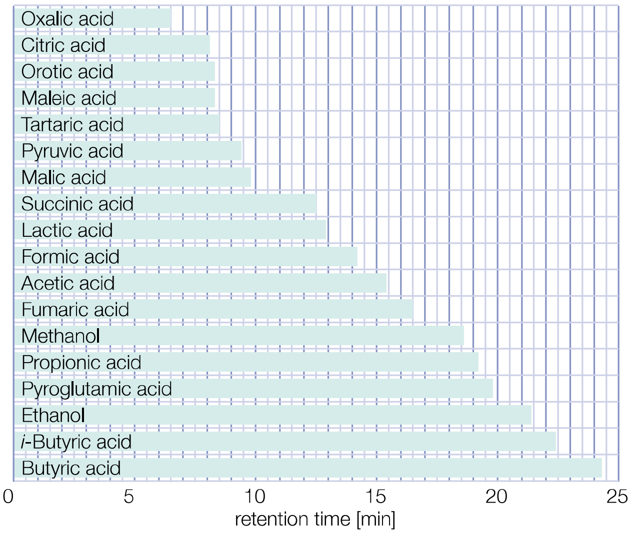
Recommended application

- H^+ form:
  Separation of sugars, sugar alcohols and organic acids; eluent in column 5 mmol/L H_2SO_4
- Ca^{2+} form:
  Separation of mono-, di- and oligosaccharides; eluent in column water

Organic acids and alcohols

MN Appl. No. 113870

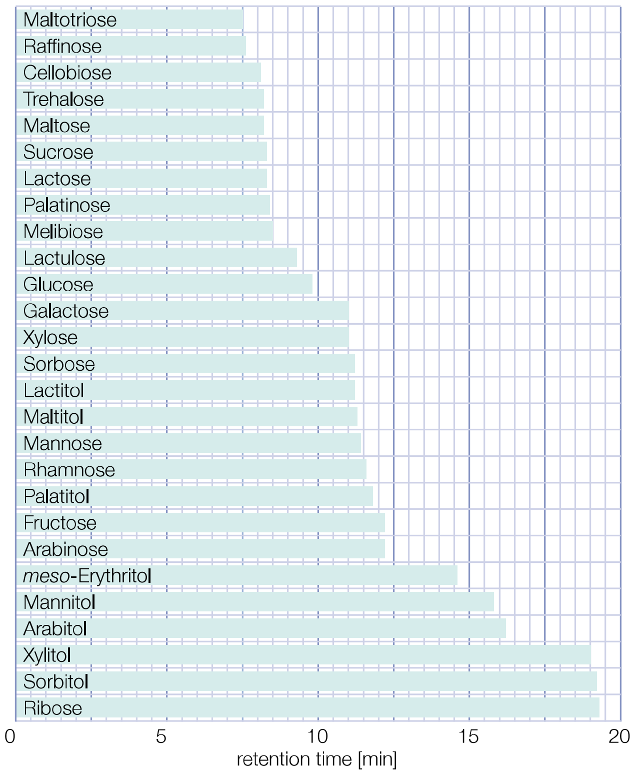
Column:	300 x 7.8 mm NUCLEOGEL® SUGAR 810 H
Eluent:	5 mmol/L H_2SO_4
Flow rate:	0.6 mL/min
Temperature:	35 °C
Detection:	RI
Injection:	5 μL



Sugars and sugar alcohols

MN Appl. No. 114160

Column:	300 x 7.8 mm NUCLEOGEL® SUGAR 810 Ca
Eluent:	water
Flow rate:	0.6 mL/min
Temperature:	85 °C
Detection:	RI



Ordering information

	ID	Length → 300 mm	Guard columns*
NUCLEOGEL® SUGAR 810 H eluent in column 5 mmol/L H_2SO_4			
Analytical Valco type columns			
	7.8 mm	719574	719575
NUCLEOGEL® SUGAR 810 Ca eluent in column water			
Analytical Valco type columns			
	7.8 mm	719570	719571

* NUCLEOGEL® SUGAR 810 guard columns measure 30 x 4 mm and require the CC column holder 30 mm (REF 721823)
Columns in packs of 1, guard columns in packs of 2.

NUCLEOGEL® ION 300 OA / SUGAR

separation of sugars · USP L17 (H form) · USP L19 (Ca form) · USP L34 (Pb form) · USP L58 (Na form)

Technical data

- Sulfonated spherical PS/DVB resins in different ionic forms; mean particle size 10 µm, pore size 100 Å
- Separation mechanism includes steric exclusion, ligand exchange and partition effects, ligand exchange being the predominant force, since the hydrated metal ions form strong interactions with the hydroxyl groups of the sample molecules. The intensity of these interactions decreases in the sequence Pb > Ca > Na
- Recommended operating temperatures: 60–95 °C; maximum pressure 70 bar

Recommended application

NUCLEOGEL® ION 300 OA:
H^+ form for separation of sugars, alcohols and organic acids

NUCLEOGEL® SUGAR:
Ca^{2+} form: separation of mono- and oligosaccharides, sugar alcohols

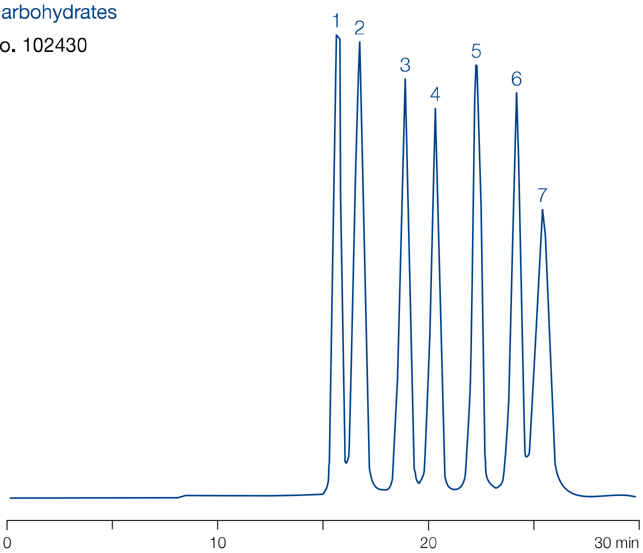
Pb^{2+} form: separation of mono- and disaccharides from food and biological samples

Na^+ form: separation of oligosaccharides from starch hydrolysates and food

Separation of carbohydrates
MN Appl. No. 102430

Column:	300 x 7.8 mm NUCLEOGEL® SUGAR Pb
Eluent:	deionized water
Flow rate:	0.4 mL/min
Temperature:	80 °C
Detection:	RI

Peaks:
1. Sucrose
2. Maltose
3. Glucose
4. Xylose
5. Galactose
6. Arabinose
7. Mannose



Ordering information

ID		Length → 300 mm	Guard columns*
NUCLEOGEL® ION 300 OA eluent in column 5 mmol/L H_2SO_4 5 mmol/L H_2SO_4			
Analytical Valco type columns			
	7.8 mm	719501	719537
NUCLEOGEL® SUGAR Ca eluent in column water + 0.02 % azide			
Analytical Valco type columns			
	6.5 mm	719531	719535
NUCLEOGEL® SUGAR Pb eluent in column water + 0.02 % azide			
Analytical Valco type columns			
	7.8 mm	719530	719534
NUCLEOGEL® SUGAR Na eluent in column water + 0.02 % azide			
Analytical Valco type columns			
	7.8 mm	719532	719536

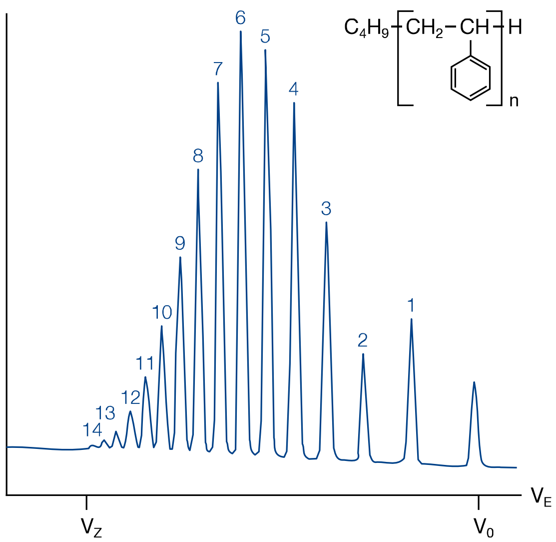
* Valco Type guard columns measure 21 x 4 mm and require the guard column holder C, REF 719538, see page 250.
Columns in packs of 1, guard columns in packs of 2.

NUCLEOGEL® GPC for GPC of water-insoluble substances

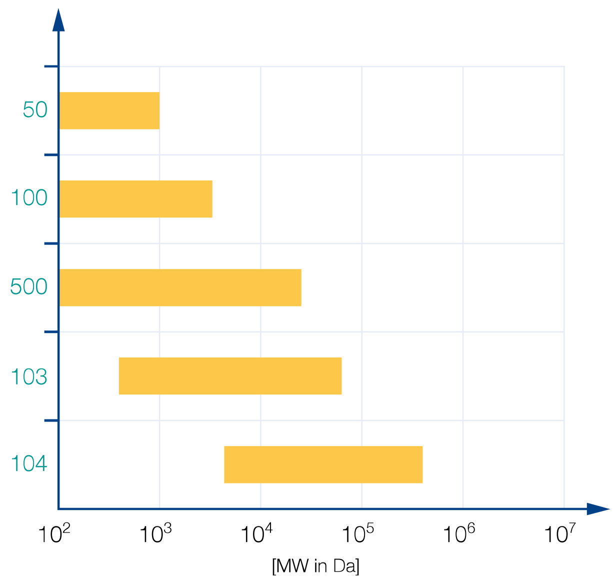
Technical data

- Highly crosslinked macroporous, spherical polystyrene – divinylbenzene polymer matrix with good mechanical stability

Chromatogram of styrene oligomers



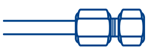
Working ranges for polystyrene

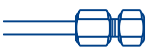


Ordering information

Eluent in column toluene

Phase	Exclusion limit [kDalton]	Application	Column 300 x 7.7 mm
5 µm particle size			
Analytical Valco type columns			
NUCLEOGEL GPC 50	2	low molecular weight organics	719402
NUCLEOGEL GPC 100	4	oligomers, oils	719403
NUCLEOGEL GPC 500	25	low molecular weight polymers	719404
NUCLEOGEL GPC 103	60	low molecular weight polymers	719405
NUCLEOGEL GPC 104	500	polymers up to 500 kDa	719406
		guard columns 50 x 7.7 mm	719409
10 µm particle size			
Analytical Valco type columns			
NUCLEOGEL GPC 50	2	low molecular weight organics	719410
NUCLEOGEL GPC 100	4	oligomers, oils	719411
NUCLEOGEL GPC 500	25	low molecular weight polymers	719412
NUCLEOGEL GPC 103	60	low molecular weight polymers	719413
NUCLEOGEL GPC 104	500	polymers up to 500 kDa	719414
		guard columns 50 x 7.7 mm	719418





Columns and guard columns in packs of 1.

EC standard columns for analytical HPLC / UHPLC

- Analytical column system manufactured from stainless steel M8 outer threads on both ends combination of sealing element and very fine-meshed stainless steel screen, PTFE ring and fitting adaptor column heads SW 12, with inner threads M8 x 0.75 and UNF 10-32 (= 1/16" connection)
- EC column hardware guarantees pressure stability of 1200 bar - hereby EC columns are suitable for UHPLC applications (ultra fast HPLC) and all modern HPLC systems.
- As screw-on guard column system we recommend the Column Protection System used with EC guard column cartridges with 4 mm length.
- EC guard columns supplied with NUCLEODUR®, NUCLEOSIL® spherical silicas and NUCLEOSHELL® spherical core shell silica particles

Available standard dimensions of EC columns

ID	Length → 20 mm	30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	200 mm	250 mm	300 mm
2 mm	+	+	+	+	+	+	+	+	+	+
3 mm	+	+	+	+	+	+	+	+	+	+
4 mm	+	+	+	+	+	+	+	+	+	+
4.6 mm	+	+	+	+	+	+	+	+	+	+

Please ask for availability of certain phases.

Note: NUCLEODUR® and NUCLEOSHELL® column head must not be removed!

Guard columns for EC columns

EC column with ID	EC guard column*
2 mm	4/2
3 mm	4/3
3 mm	4/3
3 mm	4/3

Packs of 3 cartridges

* Information about the Column Protection System on page 251.

For preparative applications MN offers the so-called VarioPrep® hardware system, which is described from page 252 on.

Valco type columns

- Analytical column system manufactured from stainless steel
- Available inner diameters: 4.6 mm ID (1/4" OD) and 7.7 mm (3/8" OD)
- Mainly used for NUCLEOGEN® and NUCLEOGEL® (see page 226)

Ordering information

Description	Pack of	REF
Accessories for Valco type columns		
Guard column holder B for VA columns 5 x 3 mm	1	719539
Guard column holder C for VA guard columns 21 x 4 mm	1	719538

Column Protection System

Innovative and universal guard column holder system

- Suitable for all analytical HPLC columns with 1/16” fittings
- Cartridges filled with special NUCLEODUR®, NUCLEOSIL® and NUCLEOSHELL® HPLC adsorbents
- Ideal protection for your analytical main column → significant increase in column lifetime
- Minimized dead volume → suitable also for ultra-fast HPLC
- Special ferrules → pressure stability up to 1300 bar (18 850 psi)
- Visual contamination check → in-time changing of the guard column
- Suitable guard columns with 4 mm length, 2 mm ID (for main columns with 2 mm ID); 3 mm ID (for main columns with 3, 4 and 4.6 mm), respectively
- UNIVERSAL RP guard columns suitable for all HPLC columns under RP conditions

Content of the Column Protection System

Description	Pack of	REF
Guard column holder	1	718966
Capillaries (0.12 mm ID)	2	
Ferrules	3	
Wrenches	2	
Manual	1	

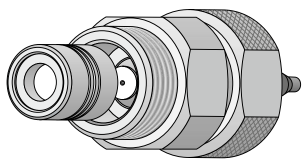
Ordering information

Description	Pack of	REF
Replacement parts for the Column Protection System		
Special ferrules made of PEEK	5	718967
Replacement connector including O-ring	1	718968
Stainless steel capillaries 0.12 mm ID, nuts and metal ferrules	3	718969
Stainless steel capillaries 0.18 mm ID (for higher flow rates), nuts and metal ferrules	3	718971
Wrench (size 12 and 14 mm)	1	718970
EC 4/2 UNIVERSAL RP guard column (for main columns with 2 mm ID)	3	728777.20
EC 4/2 UNIVERSAL RP guard column (for main columns with 2 mm ID), value pack	9	728778.20
EC 4/3 UNIVERSAL RP guard column (for main columns with 3, 4 and 4.6 mm ID)	3	728777.30
EC 4/3 UNIVERSAL RP guard column (for main columns with 3, 4 and 4.6 mm ID), value pack	9	728778.30

Visual contamination check

The cartridge is fitted with a special filter membrane:

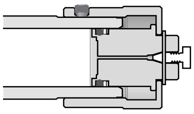
- If this silver membrane is contaminated (bright or dark discoloration), it is advisable to replace the cartridge.
- If the contaminants are colorless, replace the cartridge if the pressure rises or the chromatographic performance decreases.



VarioPrep (VP) columns for preparative HPLC

- Column system for preparative HPLC, manufactured from stainless steel with two adjustable end fittings, suitable for frequent use of back-flushing techniques
- Allows compensation of a dead volume, which could occur at the column inlet after some time of operation, without need for opening the column
- Can be packed with all NUCLEODUR® and NUCLEOSIL® spherical silicas

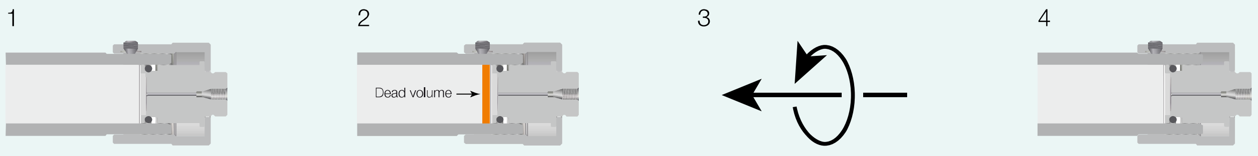
Available standard dimensions of VarioPrep columns with axially adjustable end fittings



End fitting design	ID	Length → 10* mm	15* mm	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	500 mm
	8	+		+		+	+	+	+	
	10			+		+	+	+	+	
	16	+		+		+	+	+	+	
	21			+	+	+	+	+	+	
	32		+			+		+	+	
	40			+		+	+	+	+	+
	50		+			+		+	+	
	80								+	+

* 10 x 8, 10 x 16, 15 x 32 and 15 x 50 mm ID columns are used as guard columns and require the respective holders, see page 253.

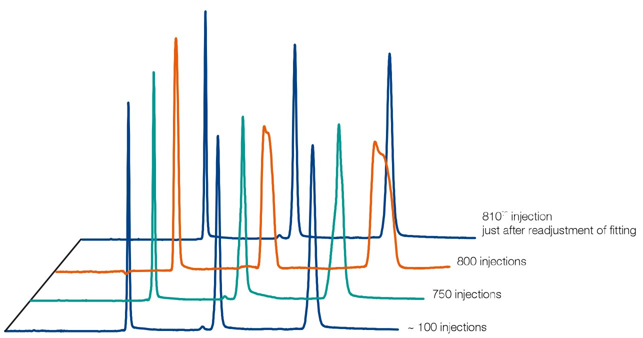
The VarioPrep principle



Readjustment of fitting

VarioPrep columns are produced with highest packing quality and bed density (1). Due to intensive chemical and/or mechanical exposure of the column adsorbent, shrinking of the column bed can occur (2; orange gap). in this even unlikely case readjustment of the VarioPrep column fitting (3; turning the nut at the column inlet clockwise) will eliminate the emerged dead volume (4). The performance of the VarioPrep column is completely reconstituted and column lifetime is significantly extended.

Column reconstitution



Reconstitution of VarioPrep column performance

- Slight peak broadening and deformation after 800 injections under strongly demanding conditions (pH 11; 50 °C; sample in DMSO)
- Readjustment of the column fitting restores column performance and prolongs column lifetime noticeably.

The improved guard column system for (semi-) preparative HPLC

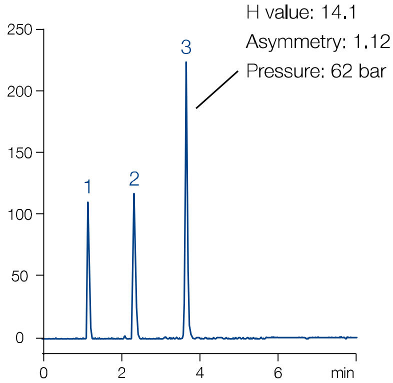
① VP 15/32 for 32 and 40 mm ID columns
② VP 10/16 for 16 and 21 mm ID columns
③ VP 10/8 for 8 and 10 mm ID columns
④ VP 15/50 for ≥ 50 mm ID columns

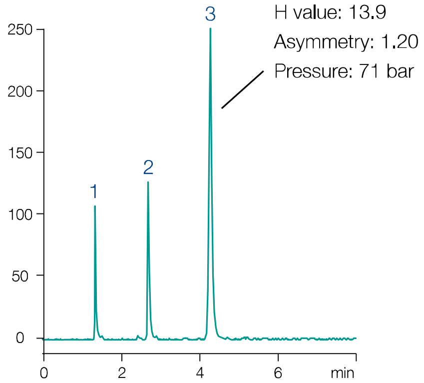
- Easy handling and cartridge exchange
- Robust hardware
- Free rotary plunger fittings – low O-ring abrasion
- Cost-efficient cartridges
- Minimally invasive / no disturbance of the separation efficiency of main column
- Low back pressure
- Designed for pressures up to 400 bar

Column performance without and with guard column

Columns: 125 x 16 mm NUCLEODUR® C_{18} HTec, 5 µm
125 x 16 mm NUCLEODUR® C_{18} HTec, 5 µm + 10 x 16 mm NUCLEODUR® C_{18} HTec guard column
Eluent: acetonitrile – water (80:20, v/v)
Flow rate: 16 mL/min
Temperature: 22 °C

Peaks:
1. Phenol
2. Naphthalene
3. Anthracene





Using VarioPrep guard columns provides ideal protection of your main column – symmetry, pressure and retention stay almost constant.

Technical data

· 1/16“ thread · free rotary plunger fittings – low O-ring abrasion · stainless steel

Guard cartridge	Holder REF	Holder ID	Recommended for column ID	Preferred capillary ID	Typical flow rate
VP 10/8	718251	8 mm	8 and 10 mm ID	0.17 and 0.25 mm	1–12 mL/min
VP 10/16	718256	16 mm	16 and 21 mm ID	0.17, 0.25 and 0.5 mm	2–32 mL/min
VP 15/32	718253	32 mm	32 and 40 mm ID	0.25, 0.5 and 1.0 mm	5–150 mL/min
VP 15/50	718255	50 mm	≥ 50 mm ID	0.5 and 1.0 mm	20–250 mL/min

Ordering information

Guard column holders for VarioPrep columns

	VP Guard columns for VarioPrep columns with ID →				Pack of	Replacement O-ring	Holder	
	8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	guard columns	(pack of 2)	ID	REF
VP	10/8				2	718975	8 mm	718251
VP		10/16			2	718976	16 mm	718256
VP			15/32		1	718977	32 mm	718253
VP				15/50	1	718978	50 mm	718255

For REF numbers of individual VP guard column cartridges see respective NUCLEODUR® and NUCLEOSIL® phases.