Chromatography

MACHEREY-NAGEL

NUCLEODUR® and NUCLEOSHELL®

Modern HPLC phases

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. In 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 to describe the high performance method developed from the column liquid chromatography that came about in the 1930s. At the beginning of the 21st century HPLC was complemented by 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 isolation of biopolymers.

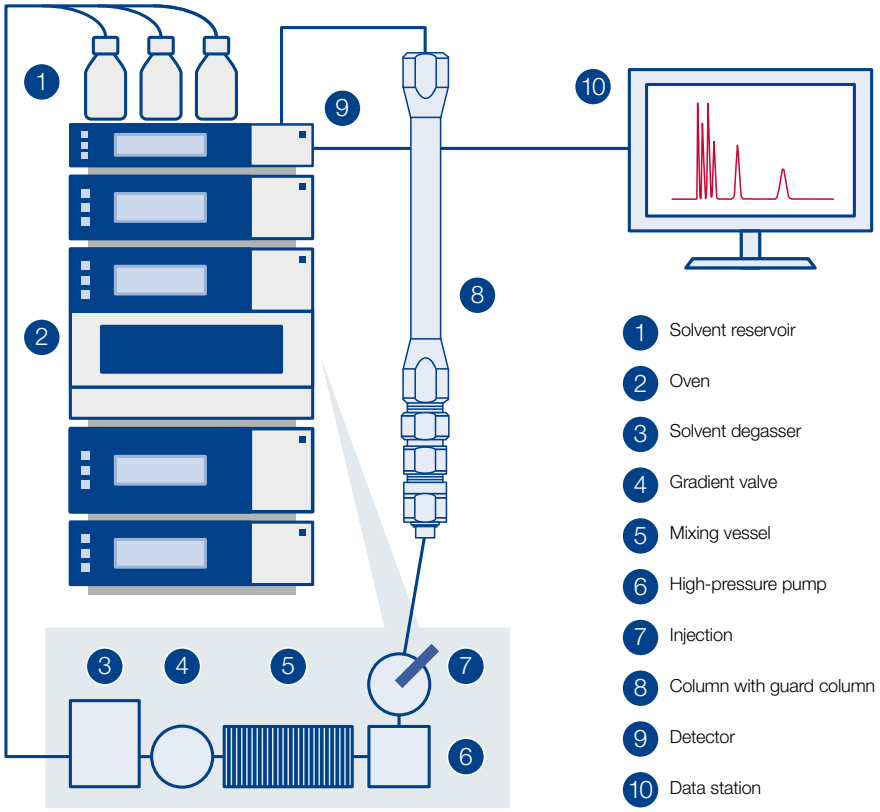
Basic principle

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 guard and 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.



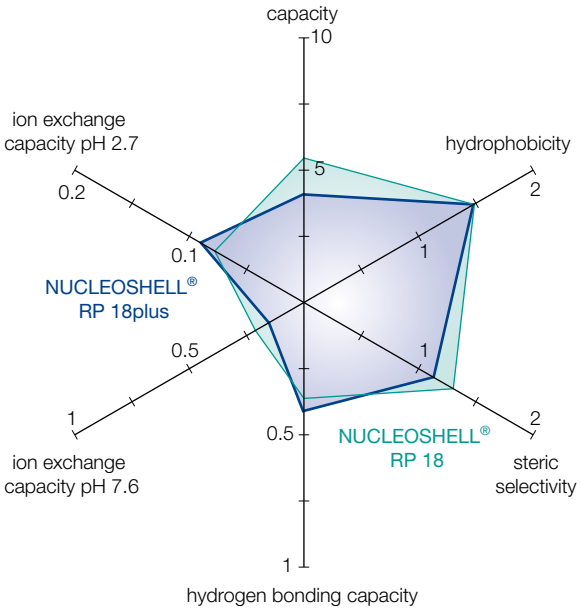
Separation mechanism

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 [1].

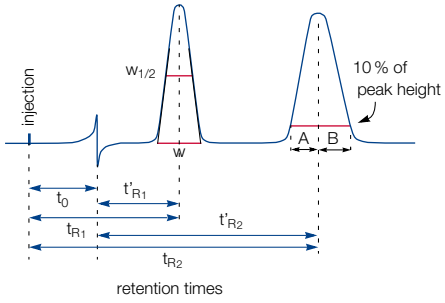


Parameter of the Tanaka diagram:
Capacity = k' (pentylbenzene)
Hydrophobicity = α (pentylbenzene, butylbenzene) Steric selectivity = α (triphenyl, *o*-terphenyl)
Hydrogen bonding capacity (capacity of silanol) = α (caffeine, phenol) Ion exchange capacity at pH 2.7 = α (benzylamine, phenol)
Ion exchange 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 legend	
Peak width:	
$w_{½}$	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'.

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

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 $w_{½}$ 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.

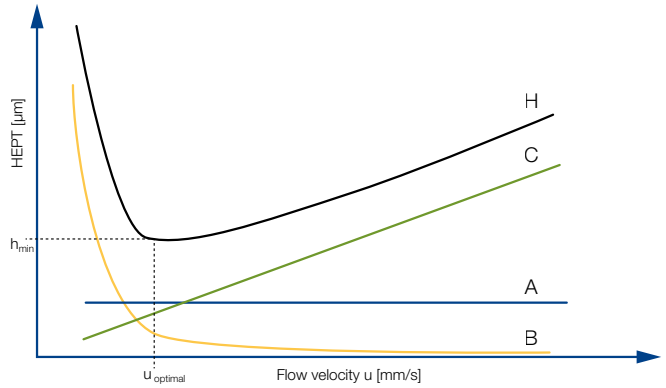
$$N = 5.54 \cdot \left(\frac{t_{R1}}{w_{1/2}} \right)^2$$

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

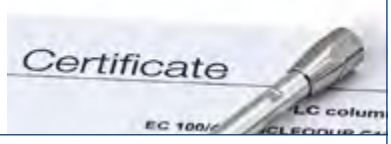
The A term, also called eddy-diffusion, is a function of the particle size, the B term a function of the diffusion coefficient of the substance in the mobile phase and the C term the retardation of a substance by the interface between stationary and mobile phase. At the point of intersection of h_{min} 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 modern high-purity silica phases NUCLEODUR® and Core-Shell material NUCLEOSHELL® as well as the respective HPLC- and UHPLC-columns can be found on the following pages.

Strict quality specifications
Outstanding reliability



Highest production standard

- Our facilities are ISO 9001 certified
- Perfect reproducibility from batch-to-batch and within each lot
- Individually tested columns, supplied with test chromatogram and conditions

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® C18 ec	38
		NUCLEODUR® C18 Gravity	18
		NUCLEODUR® C18 Gravity-SB	22
		NUCLEODUR® C18 HTec	45
		NUCLEODUR® C18 Isis	24
		NUCLEODUR® C18 Pyramid	26
		NUCLEODUR® PolarTec	28
		NUCLEODUR® Sphinx RP	36
		NUCLEOSHELL® RP 18	70
		NUCLEOSHELL® RP 18plus	72
		NUCLEOSHELL Bluebird RP 18	75
USP L3	porous silica particles, 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® SiOH	54
USP L7	octyl silane chemically bonded to totally porous silica particles,1.8 to 10 µm diameter	NUCLEODUR® C8 ec	38
		NUCLEODUR® C8 Gravity	18
USP L8	an essentially monomolecular layer of aminopropyl silane chemically bonded to totally porous silica gel support, 1.5 to 10 µm diameter	NUCLEODUR® NH2 / NH2-RP	52
USP L10	nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® CN / CN-RP	50
USP L11	phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® Phenyl-Hexyl	30
		NUCLEODUR® π^2	34
		NUCLEODUR® Sphinx RP	36
		NUCLEOSHELL® Phenyl-Hexyl	78
		NUCLEOSHELL Biphenyl	81
USP L26	butyl silane chemically bonded to totally porous silica particles, 5 to 10 µm diameter	NUCLEODUR® C4 ec	38
USP L43	pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm diameter	NUCLEODUR® PFP	32
		NUCLEOSHELL® PFP	84
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	28
USP L118	aqueous polymerized C_{18} groups on silica particles, 1.2 to 5 µm in diameter	NUCLEODUR® C18 PAH	56

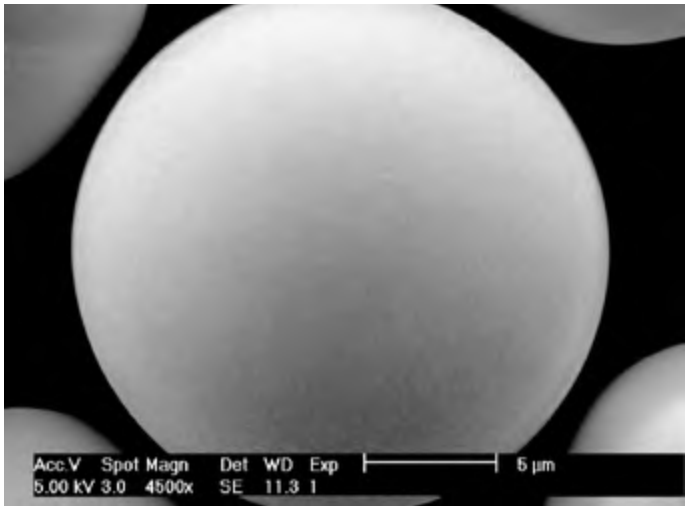
NUCLEODUR® high purity silica for HPLC

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 on the following page.

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 Å	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 two decades 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 see page 14.

CHROMABOND® QuEChERS
Mixes for sample preparation

“Quick Easy Cheap Effective Rugged Safe”

- High throughput due to easy handling
- Solvent and time-saving procedure
- High reproducibility and recovery rates
- Broad range of applications (e.g. pesticides from food)

1.8 µm particles for increased separation efficiency

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 × 4.6 mm NUCLEODUR® C18 Gravity

· 3 µm: N ≥ 100 000 plates/m (h-value ≤ 10)

· 1.8 µm: N ≥ 166 667 plates/m (h-value ≤ 6)

Increasing the plate number by ~ 67 % offers the possibility of using shorter columns with equal plate number, therefore 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 $N \propto 1/d_P$, d_P = particle diameter

Key features

- 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 specially fractionated to limit the increase in back pressure.

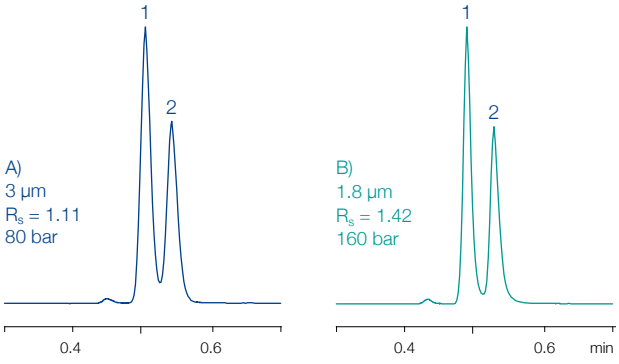
Availability

- The following NUCLEODUR® phases are available in 1.8 µm: C18 Gravity, C8 Gravity, C18 Gravity-SB, C18 Isis, C18 Pyramid, PolarTec, Phenyl-Hexyl, PFP, Sphinx RP, C18 HTec and HILIC

Resolution as function of particle size

Säule: 50 x 4 mm NUCLEODUR® C18 Gravity
A) 3 µm, B) 1.8 µm
Eluent: acetonitrile – water (80:20, v/v)
Flussrate: 2 mL/min
Detektion: 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 (non-dimensional), LC = column length, η = viscosity, u = linear velocity, d_P = particle diameter

The high sphericity of the NUCLEODUR® particles and a 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 × 4.6 mm

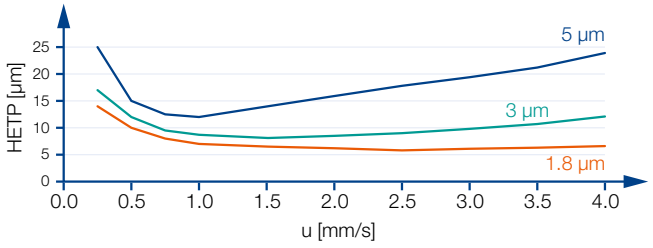
	NUCLEODUR® C18 Gravity	Competitor
3 µm	70 bar	–
1.8 µm	130 bar	170 bar

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

Van Deemter curves

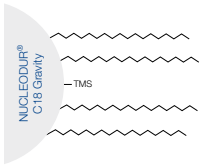
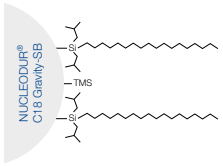
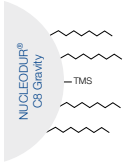
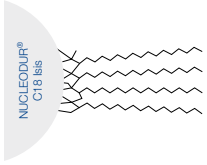
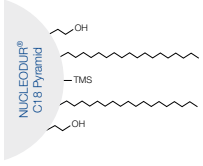
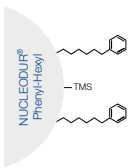
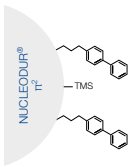
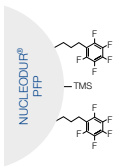


Column 50 x 4.6 mm, acetonitrile – water (50:50, v/v), analyte toluene

Technical requirements

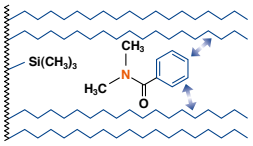
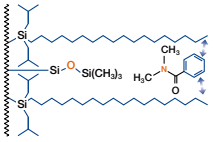
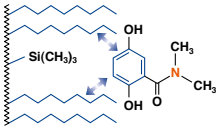
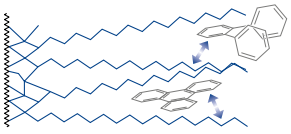
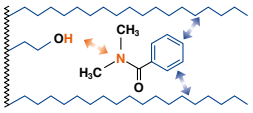
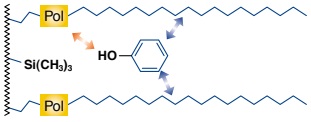
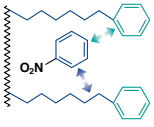
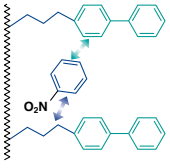
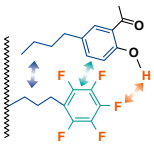
To gain best results with 1.8 µm particles certain technical demands must be met including pumps for flow rates of 2–3 mL with pressures of 250–1000 bar, minimized dead volume, and fast data recording.

NUCLEODUR® phase overview

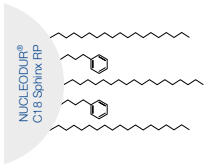
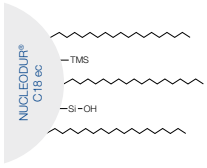
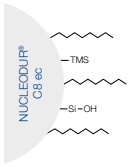
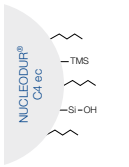
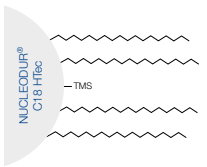
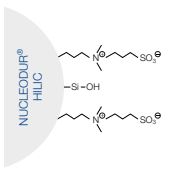
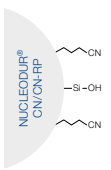
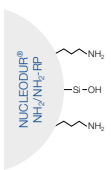
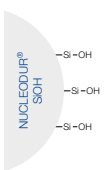
Phase	Specification	Page	Characteristic*	Stability	Structure
C18 Gravity	octadecyl, high density coating, multi-endcapping 18 % C USP L1	18	A ●●●●● B ◖ C ●●◖	pH 1–11, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C18 Gravity-SB	octadecyl (monomeric), extensive endcapping 13 % C USP L1	22	A ●●●● B ●●◖ C -	pH 1–9, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C8 Gravity	octyl, high density coating, multi-endcapping 11 % C USP L7	18	A ●●◖ B ● C ●◖	pH 1–11, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C18 Isis	octadecyl phase with specially crosslinked surface modification, endcapping 20 % C USP L1	24	A ●●●●◖ B ●● C ●●●●●	pH 1–10, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C18 Pyramid	octadecyl with polar endcapping 14 % C USP L1	26	A ●●●● B ●●◖ C ●●	stable in 100 % aqueous eluent, pH 1–9, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
PolarTec	octadecyl with embedded polar group, endcapping 17 % C USP L1 and L60	28	A ●●●● B ●●◖ C ●●●●	stable in 100 % aqueous eluent, pH 1–9, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
Phenyl-Hexyl	phenylhexyl, multi-endcapping 10 % C USP L11	30	A ●● B ●●● C ●	pH 1–10, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
π^2	biphenylpropyl, multi-endcapping 17 % C USP L11	34	A ●●◖ B ●●●● C ●●●	pH 3–10	NUCLEODUR® (Si-O$_2$)$_n$ 
PFP	pentafluorophenylpropyl, multi-endcapping 8 % C USP L43	32	A ●● B ●●●● C ●●●●	pH 1–9, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity
** phases which provide a similar selectivity based on chemical and physical properties

NUCLEODUR® phase overview

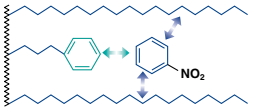
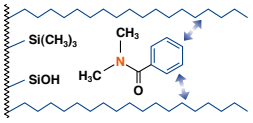
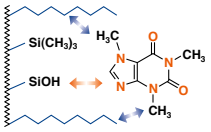
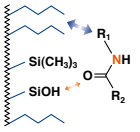
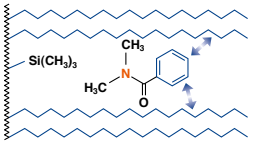
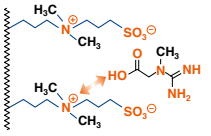
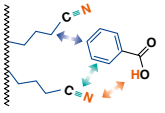
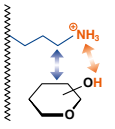
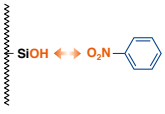
Application	Similar phases**	Interactions · retention mechanism	
in general compounds with ionizable functional groups such as basic pharmaceuticals and pesticides	NUCLEOSIL® C18 HD Xterra® RP18 / MS C18; Luna® C18(2), Gemini®, Synergi® Max RP; Zorbax® Extend-C18; Inertsil® ODS III; Purospher® STAR RP-18; 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® C8 HD Xterra® RP8 / MS C_8; Luna® C_8; Zorbax® Eclipse XDB-C_8	hydrophobic (van der Waals interactions)	
high steric selectivity, thus suited for separation of positional and structural isomers, planar / nonplanar molecules	NUCLEOSIL® C18 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® C18 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	
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	

NUCLEODUR® phase overview

Phase	Specification	Page	Characteristic*	Stability	Structure
Sphinx RP	bifunctional, balanced ratio of propylphenyl and octadecyl, endcapping 15 % C USP L1 and L11	36	A ●●● B ●●◖ C ●	pH 1–10, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C18 ec	octadecyl, medium density, endcapping, available in 110 Å and 300 Å pore size 17.5 % / 4 % C USP L1	38	A ●●●● B ● C ●●●◖	pH 1–9	NUCLEODUR® (Si-O$_2$)$_n$ 
C8 ec	octyl, medium density, endcapping 10.5 % C USP L7	38	A ●● B ●◖ C ●●◖	pH 1–9	NUCLEODUR® (Si-O$_2$)$_n$ 
C4 ec	butyl, medium density, endcapping, 300 Å pore size 2.5 % C USP L26	38	A ● B ●● C ●◖	pH 1–9	NUCLEODUR® (Si-O$_2$)$_n$ 
C18 HTec	octadecyl, high density coating, high capacity, multi-endcapping 18 % C USP L1	45	A ●●●●● B ◖ C ●●◖	pH 1–11, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
HILIC	zwitterionic ammonium-sulfonic acid phase, no endcapping 7 % C	48	A ● B ●●●●● C -	pH 2–8.5	NUCLEODUR® (Si-O$_2$)$_n$ 
CN / CN-RP	cyano (nitrile) for NP and RP separations, endcapping 7 % C USP L10	50	A ● B ●●●● C -	pH 1–8, stable towards highly aqueous mobile phases	NUCLEODUR® (Si-O$_2$)$_n$ 
NH2 / NH2-RP	aminopropyl for NP and RP separations, no endcapping 2.5 % C USP L8	52	A ● B ●●●● C -	pH 2–8, stable towards highly aqueous mobile phases	NUCLEODUR® (Si-O$_2$)$_n$ 
SiOH	unmodified high purity silica, no endcapping USP L3	54	A - B - C -	pH 2–8	NUCLEODUR® (Si-O$_2$)$_n$ 

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity
** phases which provide a similar selectivity based on chemical and physical properties

NUCLEODUR® phase overview

Application	Similar phases**	Interactions · retention mechanism	
compounds with aromatic and multiple bond systems	no similar phases	π-π and hydrophobic	
robust C_{18} phase for routine analyses	NUCLEOSIL® C18 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® C8 ec / C8 Spherisorb® C_8; Symmetry® C_8; Hypersil® MOS; Kromasil® C_8; LiChrospher® RP-8	hydrophobic (van der Waals interactions) some residual silanol interactions	
biological macromolecules like proteins or peptides	Jupiter® C_4; ACE® C_4	hydrophobic (van der Waals interactions) some residual silanol interactions	
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)	
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	
sugars, sugar alcohols and other hydroxy compounds, DNA bases, polar compounds in general	NUCLEOSIL® NH2 / NH2-RP	polar / ionic and hydrophobic	
polar compounds in general	NUCLEOSIL® SiOH	polar / ionic	

Base deactivation

NUCLEODUR® C18 Gravity and NUCLEODUR® C8 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 40.

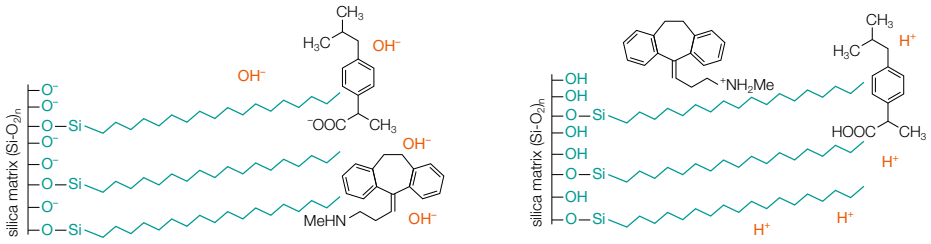
Enhanced pH stability

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® C18 and C_8 Gravity allow for use at an expanded pH range from pH 1 to 11.

Benefits of enhanced pH stability

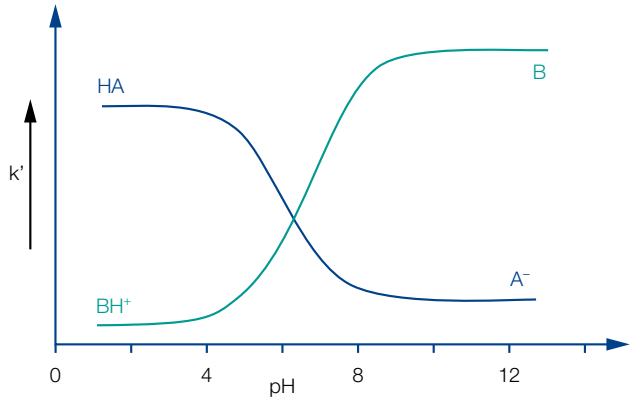
An expanded pH range is often required in method development. Many nitrogen containing compounds like basic drugs are protonated at acidic or neutral pH and exhibit poor retention on a standard C_{18} phase. The retention behavior can be improved by working at a higher pH, where the analyte is no longer 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

Key features

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

Technical data

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

Recommended applications

- USP listing L1
- Overall sophisticated analytical separations
- Compound classes separated include pharmaceuticals, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants

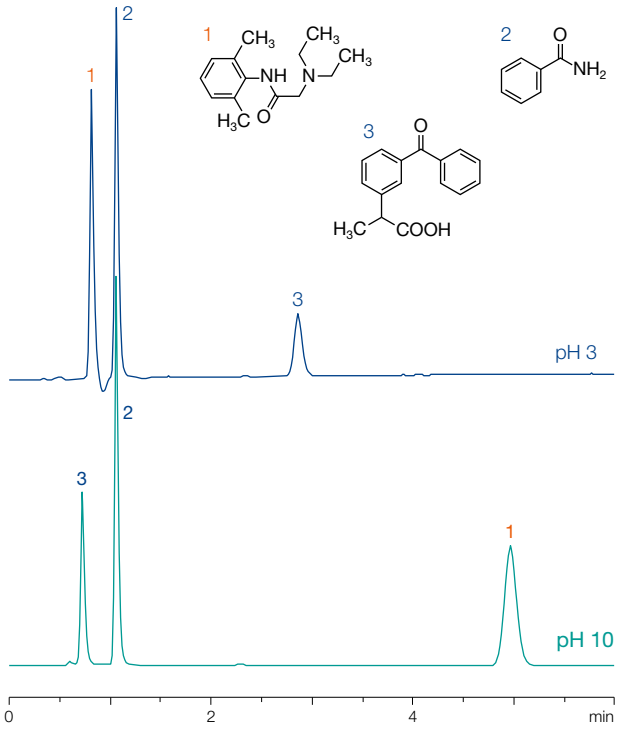
between analyte and C_{18} chains, while the formally neutral ketoprofen is eluted after about 3 min. However, at pH 10 a reversal of the elution order, with a visibly longer retention time for the basic lidocaine, is observed.

Influence of the pH value on selectivity

MN Appl. No. 120860

Column:	125 x 4 mm NUCLEODUR® C18 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



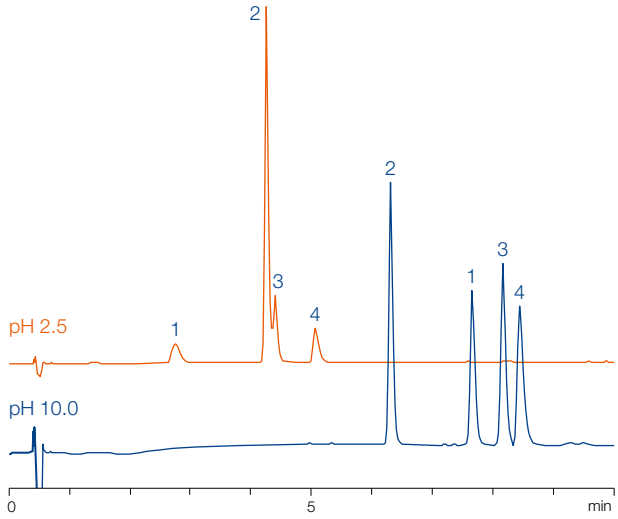
As mentioned above, pH stability of the stationary phase can be helpful for improving selectivity in method development. The following figure shows the separation of 4 basic drugs under acidic and basic conditions. At pH 2.5 the protonated analytes exhibit poor retention (early elution) and in addition 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® C18 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



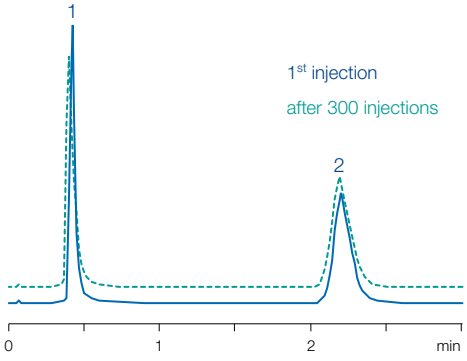
The following chromatogram demonstrates the stability of NUCLEODUR® C18 Gravity under alkaline conditions. The ultra-pure Gravity with its unique high density surface bonding technology withstands strong alkaline mobile phase conditions.

Stability of NUCLEODUR® C18 Gravity at pH 11

MN Appl. No. 120850

Column:	50 x 4.6 mm NUCLEODUR® C18 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.

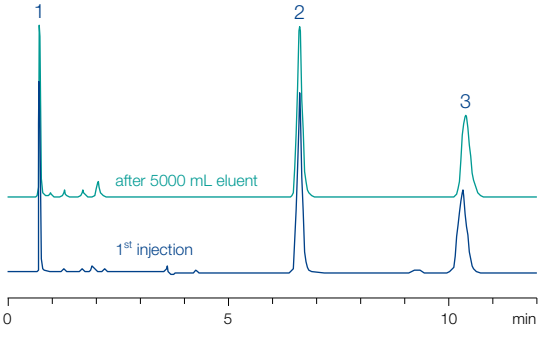
The following chromatograms show the excellent column stability of NUCLEODUR® C18 Gravity in acidic conditions. Retention times of all three compounds in the column performance test remain consistent and virtually unchanged, even after the column is run with 5000 mL eluent. Due to the extremely stable surface modification, no cleavage of the Si-O-Si bonding occurs, column deterioration is therefore successfully prevented.

Stability of NUCLEODUR® C18 Gravity at pH 1.5

MN Appl. No. 120840

Column:	125 x 4 mm NUCLEODUR® C18 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

NUCLEODUR® C18 Gravity

Analytical EC columns NUCLEODUR® C18 Gravity (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760101.46	761903.30
250	4	5	760101.40	761903.30
250	3	5	760101.30	761903.30
150	4.6	5	760103.46	761903.30
250	3	3	760082.30	761902.30
150	2	3	760083.20	761902.20
125	4.6	3	760081.46	761902.30
50	4.6	3	760080.46	761902.30
100	4.6	1.8	760076.46	761901.30
50	2	1.8	760079.20	761901.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

Ordering information

NUCLEODUR® C8 Gravity

Analytical EC columns NUCLEODUR® C8 Gravity (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760753.46	761907.30
250	4	5	760753.40	761907.30
150	4.6	5	760752.46	761907.30
150	4	5	760752.40	761907.30
125	4.6	5	760751.46	761907.30
125	4	5	760751.40	761907.30
250	4.6	3	760659.46	761906.30
50	3	3	760653.30	761906.30
150	2	1.8	760759.20	761905.20
50	3	1.8	760755.30	761905.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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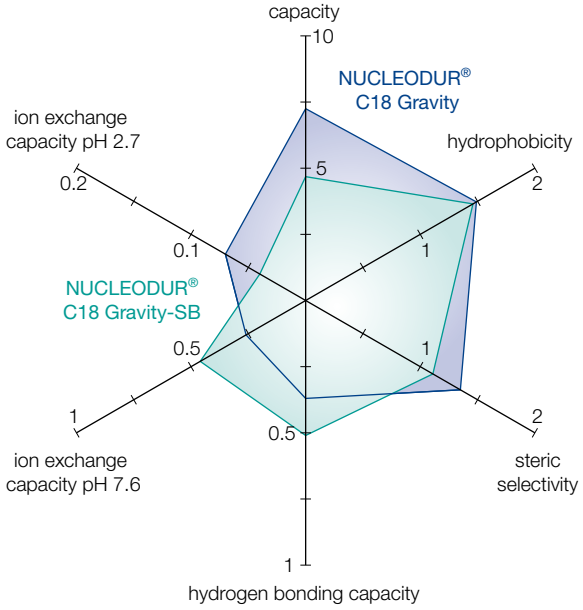
NUCLEODUR® C18 Gravity-SB

Hydrophobic phase with polar selectivity

NUCLEODUR® C18 Gravity-SB excels with a relatively high hydrophobicity – similar to C18 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 NUCLEODUR® C18 Gravity-SB shows similar hydrophobicity than C18 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® C18 Gravity-SB is versatile applicable, especially for polar analytes like nucleobases or pesticides the column shows good separation efficiency.



Key features

- 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 phase; extensively endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 13 %; pH stability 1–9

Recommended applications

- USP listing L1
- Overall sophisticated analytical separations, especially for polar compounds, e.g., antibiotics, water-soluble vitamins, organic acids

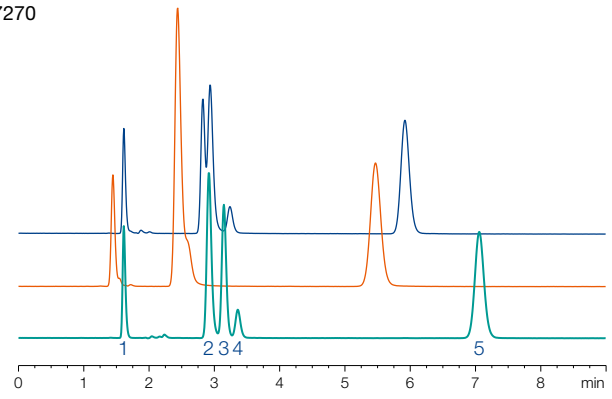
Selectivity comparison of nucleobases

MN Appl. No. 127270

Columns:	EC 150/4.6 mm
	NUCLEODUR® C18 Gravity-SB, 5 µm
	NUCLEODUR® C18 Gravity, 5 µm
	NUCLEODUR® C18 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.

NUCLEODUR® C18 Gravity-SB

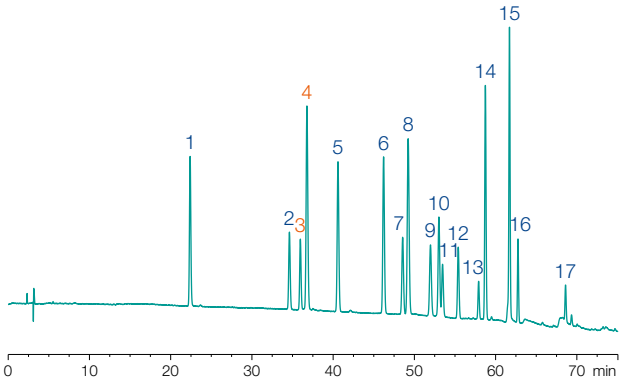
Pesticide mix (Ehrenstorfer, 17 components)

MN Appl. No. 127330

Column:	EC 250/4.6 NUCLEODUR® C18 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
2. Metoxuron
3. Hexazinone
4. Simazine
5. Cyanazine
6. Methabenzthiazuron
7. Chlortoluron
8. Atrazine
9. Monolinuron
10. Isoproturon
11. Diuron
12. Metobromuron
13. Metazachlor
14. Sebuthylazin
15. Terbuthylazine
16. Linuron
17. Metolachlor



Good separation of the critical pair hexazinone/simazine

Ordering information

NUCLEODUR® C18 Gravity-SB				
Analytical EC columns NUCLEODUR® C18 Gravity-SB (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760619.46	761992.30
150	4.6	5	760618.46	761992.30
150	3	5	760618.30	761992.30
125	4	5	760617.40	761992.30
250	3	3	760609.30	761991.30
150	4.6	3	760608.46	761991.30
100	2	3	760606.20	761991.20
150	2	1.8	760598.20	761990.20
100	2	1.8	760596.20	761990.20
50	2	1.8	760593.20	761990.20

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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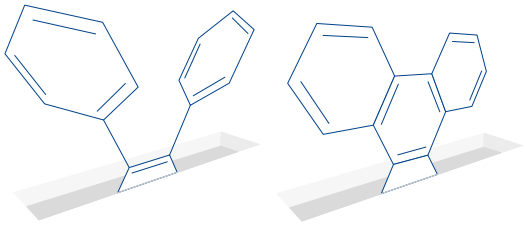
NUCLEODUR® C18 Isis

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® C18 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 [2] 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 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 (left structure) is retained longer than *o*-terphenyl (right structure).



Key features

- Phase with exceptional steric selectivity
- Outstanding surface deactivation
- Suitable for LC/MS

Technical data

- C_{18} phase with special polymeric, cross-linked surface modification; endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 20 %; pH stability 1–10

Recommended applications

- USP listing L1
- Steroids, (*o*,*p*,*m*-)substituted aromatics, fat-soluble vitamins

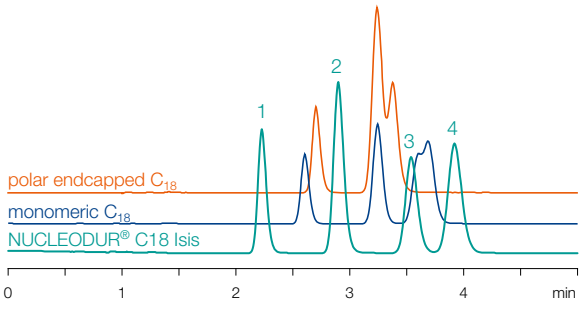
Steric selectivity

The following chromatograms reveal the improved resolution for positional isomers in a test mixture of aromatic compounds on NUCLEODUR® C18 Isis (green) in direct comparison with monomerically coated (blue) and polar endcapped (orange) C_{18} columns.

Steric selectivity of NUCLEODUR® C18 Isis

Columns:	125 x 4 mm NUCLEODUR® C18 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 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 shown on the next page the α value is considerable larger on NUCLEODUR® C18 Isis compared to a conventional C_{18} column.

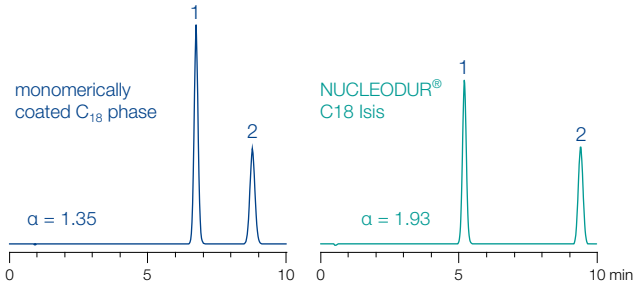
The surface bonding technology also provides improved stability features for the NUCLEODUR® C18 Isis phase.

NUCLEODUR® C18 Isis

Steric selectivity of NUCLEODUR® C18 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



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 No. 121210 at ChromaAppDB.mn-net.com).

Ordering information

NUCLEODUR® C18 Isis

Analytical EC columns NUCLEODUR® C18 Isis (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760414.46	761912.30
250	3	5	760414.30	761912.30
125	4	5	760412.40	761912.30
50	3	5	760410.30	761912.30
250	4.6	3	760404.46	761911.30
150	4	3	760403.40	761911.30
100	4.6	3	760401.46	761911.30
100	4	3	760401.40	761911.30
100	3	1.8	760407.30	761910.30
50	4.6	1.8	760405.46	761910.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® C18 Pyramid

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 [3].

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.

Key features

- 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 C_{18} phase; polar endcapped
- 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 applications

- USP listing L1
- Analgesics, penicillin antibiotics, nucleic acid bases, water-soluble vitamins, complexing agents, organic acids

Stability features

NUCLEODUR® C18 Pyramid is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. The lower figure shows the retention behavior of tartaric, acetic and maleic acid under purely aqueous conditions on NUCLEODUR® C18 Pyramid in comparison with a conventionally bonded C_{18} phase.

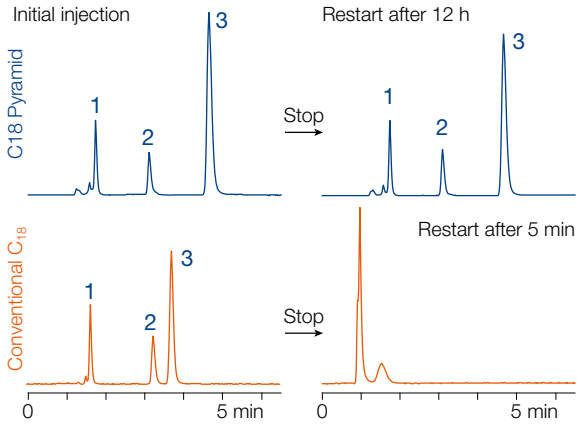
It can be shown that the retention times for NUCLEODUR® C18 Pyramid remain nearly unchanged between initial injection and restart after the flow has been stopped for 12 h, whilst the performance of the conventional RP column already totally collapsed after 5 min.

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



NUCLEODUR® C18 Pyramid

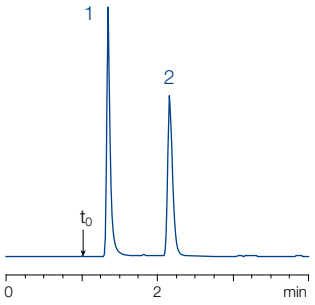
Retention characteristics

Separation of very polar compounds

MN Appl. No. 119170

Column: 125 x 4 mm NUCLEODUR® C18 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



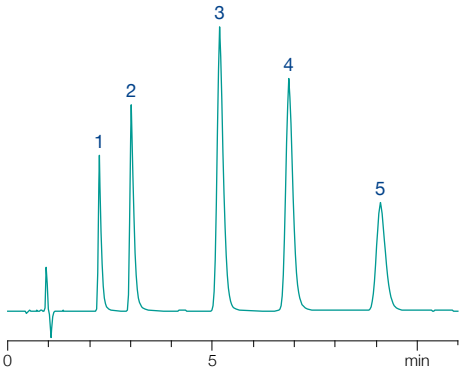
The polar surface exhibits retention characteristics different from conventional C_{18} phases. Application 119170 shows improved retention behavior of very polar short chain organic acids, which are insufficiently retained on RP columns with predominantly hydrophobic surface properties. In addition to the exceptional polar selectivity NUCLEODUR® C18 Pyramid also provides adequate hydrophobic retention (application No. 119190 at ChromaAppDB.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 in application 119200.

Tricyclic antidepressants

MN Appl. No. 119200

Column: 125 x 4 mm NUCLEODUR® C18 Pyramid, 5 µm
Eluent: methanol – 20 mM $NH_4H_2PO_4$, pH 6.95 (70:30, v/v)
Temperature: 40 °C
Injection volume: 5 µL
Detection: UV, 254 nm
Injection volume: 2 µL

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



Ordering information

NUCLEODUR® C18 Pyramid				
Analytical EC columns NUCLEODUR® C18 Pyramid (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760202.46	761917.30
250	4	5	760202.40	761917.30
150	4.6	5	760203.46	761917.30
125	4	5	760201.40	761917.30
150	4.6	3	760261.46	761916.30
125	3	3	760260.30	761916.30
100	4.6	3	760264.46	761916.30
50	2	3	760263.20	761916.20
100	2	1.8	760273.20	761915.20
50	2	1.8	760272.20	761915.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® PolarTec

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.

Key features

- RP phase with embedded polar group
- Excellent base deactivation
- Pronounced steric selectivity
- Suitable for LC/MS and 100 % aqueous eluents

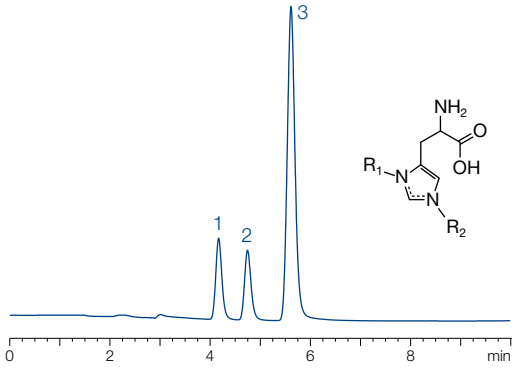
Separation of histidines

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 top-notch of embedded polar group phases on the market. The pronounced steric selectivity 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.

Technical data

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

Recommended applications

- USP listing L1 and L60
- Exceptional selectivity for phenols and nitrogen containing compounds, polar compounds like basic pharmaceuticals, organic acids, pesticides, amino acids, water-soluble vitamins, etc.

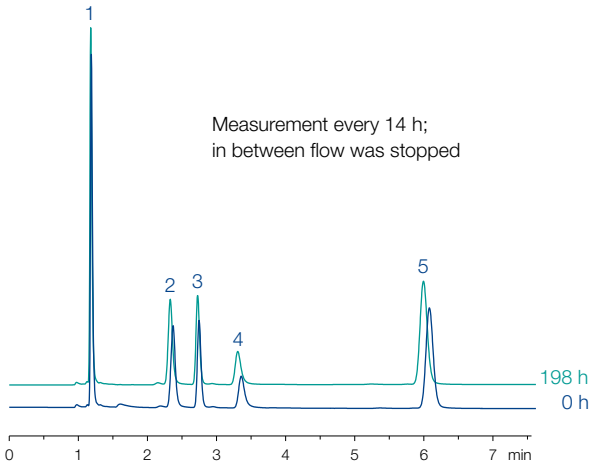
Stability of NUCLEODUR® PolarTec

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



NUCLEODUR® PolarTec

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

NUCLEODUR® PolarTec				
Analytical EC columns NUCLEODUR® PolarTec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760489.46	761982.30
250	4	5	760489.40	761982.30
150	4.6	5	760488.46	761982.30
150	4	5	760488.40	761982.30
250	4.6	3	760479.46	761981.30
150	4.6	3	760478.46	761981.30
150	3	3	760478.30	761981.30
150	2	1.8	760468.20	761980.20
100	4.6	1.8	760466.46	761980.30
50	2	1.8	760463.20	761980.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® Phenyl-Hexyl

Alternative selectivity to C_{18} phases

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. Therefor 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 with good resolution.

Key features

- Suitable for polar / aromatic compounds
- 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 phenylhexyl modification; multi-endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 10 %; pH stability 1–10

Recommended applications

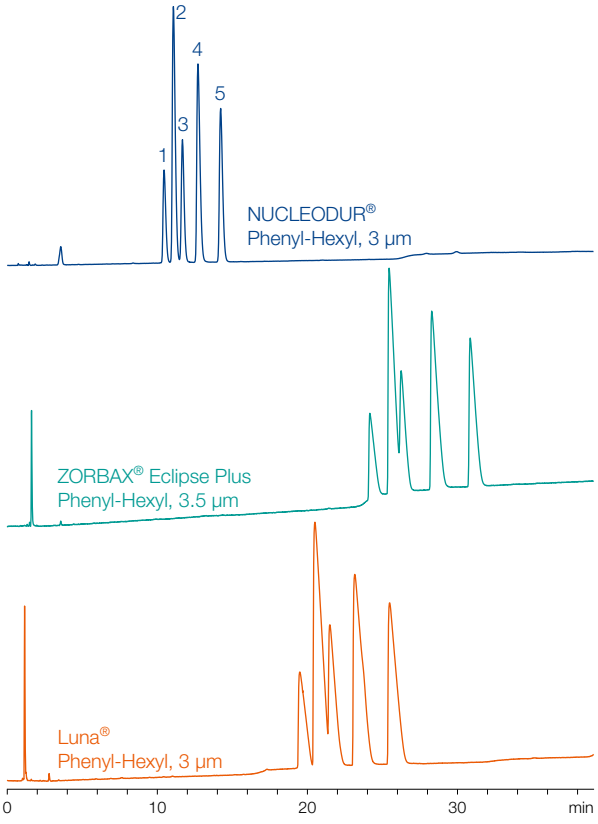
- USP listing L11
- Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics

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



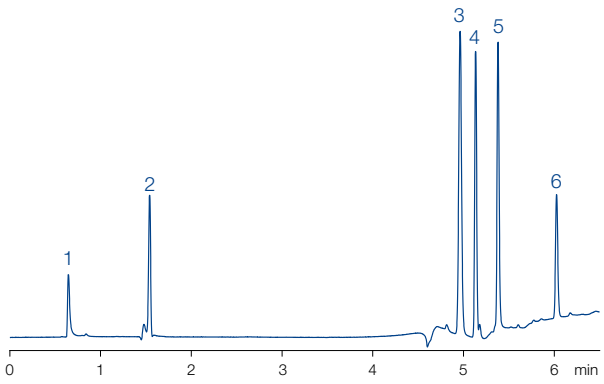
NUCLEODUR® Phenyl-Hexyl

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



Ordering information

NUCLEODUR® Phenyl-Hexyl

Analytical EC columns NUCLEODUR® Phenyl-Hexyl (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760589.46	761987.30
250	4	5	760589.40	761987.30
250	3	5	760589.30	761987.30
150	4.6	5	760588.46	761987.30
150	4.6	3	760578.46	761986.30
150	2	3	760578.20	761986.20
100	4.6	3	760576.46	761986.30
100	2	3	760576.20	761986.20
100	3	1.8	760566.30	761985.30
50	4.6	1.8	760563.46	761985.30

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® PFP

Orthogonality in selectivity

Fluorinated stationary phases have been of increasing interest in HPLC. Most common representative of fluorinated silica phases is the pentafluorophenyl modification (PFP or F5). 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.

Key features

- Hydrophobic pentafluorophenyl 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

- Pentafluorophenylpropyl phase; multi-endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 8 %; pH stability 1–9

Recommended applications

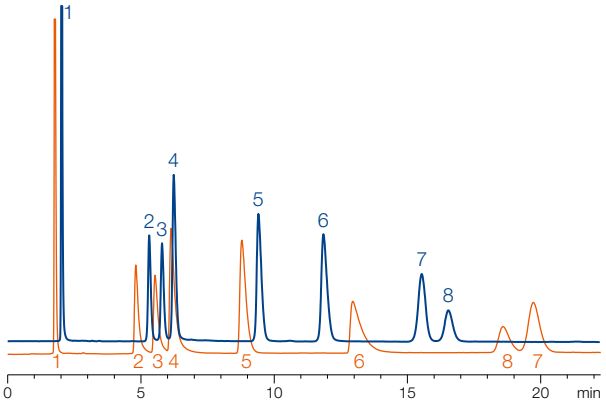
- USP listing L43
- Aromatic and unsaturated compounds, phenols, halogen compounds, isomers, polar compounds like pharmaceuticals, antibiotics; strong retention of basic compounds

Separation of antihistamines

MN Appl. No. 124861

Columns: 250 x 3 mm NUCLEODUR® PFP, 5 µm
250 x 3 mm NUCLEODUR® C18 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



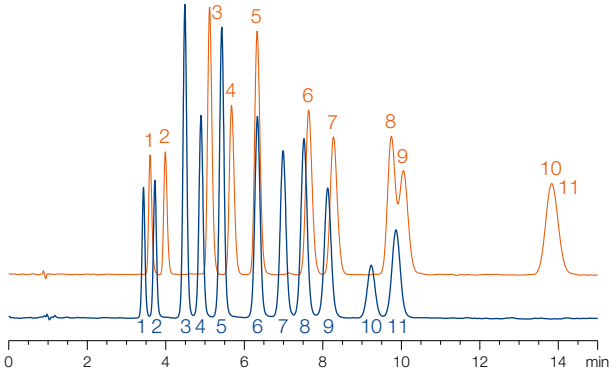
Separation of phenol isomers

MN Appl. No. 124531

Column: 125 x 4 mm NUCLEODUR® PFP, 5 µm
125 x 4 mm NUCLEODUR® C18 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

NUCLEODUR® PFP				
Analytical EC columns NUCLEODUR® PFP (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760459.46	761977.30
250	4	5	760459.40	761977.30
150	4.6	5	760458.46	761977.30
125	3	5	760457.30	761977.30
125	4	3	760447.40	761976.30
125	3	3	760447.30	761976.30
100	3	3	760446.30	761976.30
100	2	1.8	760436.20	761975.20
50	4.6	1.8	760433.46	761975.30
50	2	1.8	760433.20	761975.20

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® π²

Highest aromatic and orthogonal selectivity

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 to C_{18} modified phases. Thereby displaying 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, which can be seen e.g., in application 127910.

Key features

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

Technical data

- Biphenylpropyl phase; multi-endcapped
- Pore size 110 Å; particle size 3 µm and 5 µm; carbon content 17 %; pH stability 3–10

Recommended applications

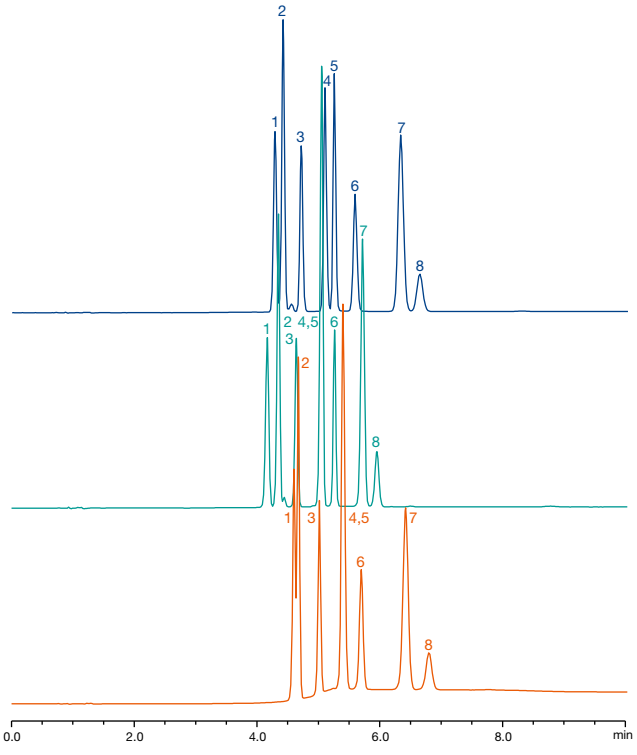
- USP listing L11
- Overall sophisticated analytical separations, especially aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics, steroids

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



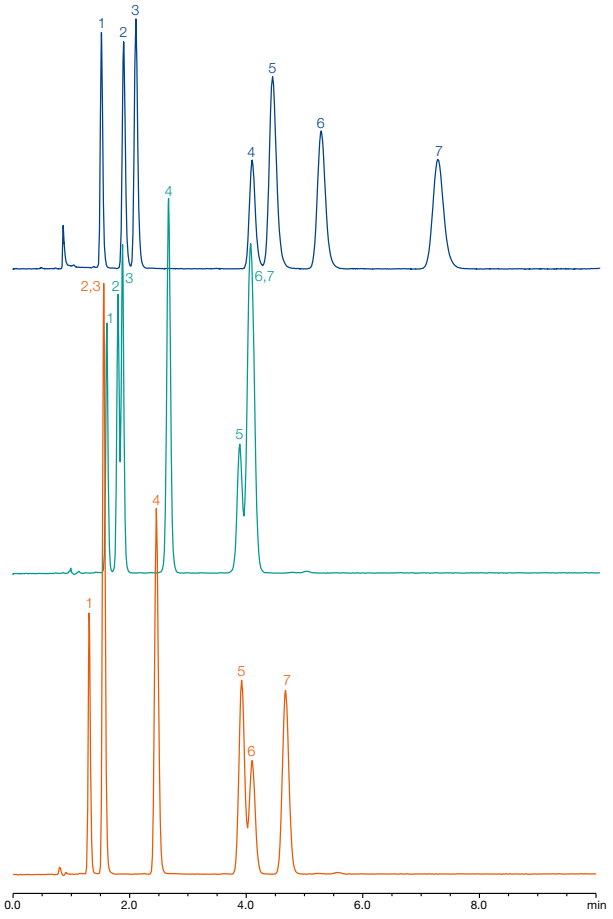
NUCLEODUR® π²

Steroids

MN Appl. No. 127910

Columns:	125 x 4 mm each NUCLEODUR® π², 5 µm NUCLEODUR® Phenyl-Hexyl, 5 µm NUCLEODUR® C18 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

NUCLEODUR® π²				
Analytical EC columns NUCLEODUR® π² (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760625.46	761810.30
250	4	5	760625.40	761810.30
250	3	5	760625.30	761810.30
250	2	5	760625.20	761810.20
150	2	5	760624.20	761810.20
250	4.6	3	760639.46	761811.30
150	4.6	3	760638.46	761811.30
150	4	3	760638.40	761811.30
125	2	3	760637.20	761811.20
100	3	3	760636.30	761811.30

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® Sphinx RP

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.

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® C8/C18 Gravity and the polar endcapped NUCLEODUR® C18 Pyramid.

Key features

- Bifunctional RP phase with distinct selectivity based on well-balanced 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 bifunctional phase; endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 15 %; pH stability 1–10

Recommended applications

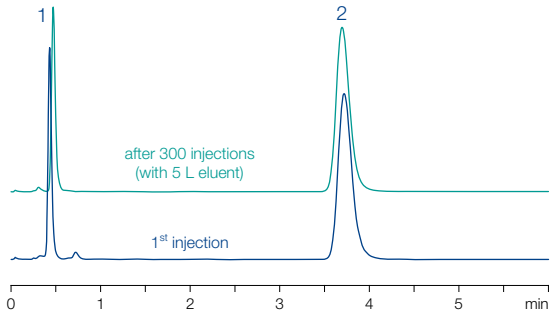
- USP listing L1 and L11
- Quinolone antibiotics, sulfonamides, xanthines, substituted aromatics

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



NUCLEODUR® Sphinx RP

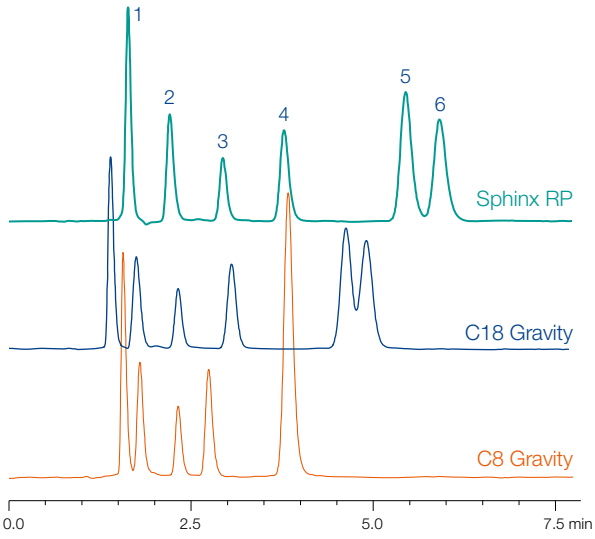
Separation of flavonoids on three different NUCLEODUR® phases

MN Appl. No. 119830

Columns:	150 x 4.6 mm
	NUCLEODUR® Sphinx RP, 5 µm
	NUCLEODUR® C18 Gravity, 5 µm
	NUCLEODUR® C8 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

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

NUCLEODUR® Sphinx RP

Analytical EC columns NUCLEODUR® Sphinx RP (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760803.46	761922.30
150	4.6	5	760802.46	761922.30
125	4	5	760801.40	761922.30
250	4.6	3	760808.46	761921.30
250	3	3	760808.30	761921.30
150	3	3	760805.30	761921.30
100	2	3	760812.20	761921.20
100	2	1.8	760823.20	761920.20
50	4	1.8	760822.40	761920.30
50	3	1.8	760822.30	761920.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® C18 ec for daily routine analysis

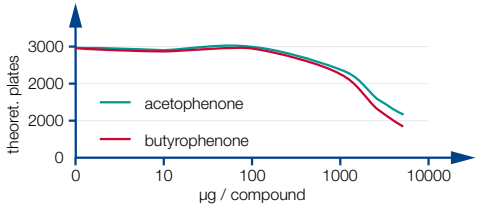
The efficiency of a separation is controlled by particle size and selectivity of the stationary phase. The exceptional surface coverage of monomeric bonded alkylsilanes, combined with an exhaustive endcapping, results in a surface with lowest silanol activity. This allows the tailing-free elution of polar compounds such as basic drugs. NUCLEODUR® C18 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® C18 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 C18 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 C18 ec
Eluent:	acetonitrile – H_2O 80:20 (v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 280 – 370 nm



Key features

- Nonpolar phases for routine analysis
- 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) modification with pore size of 110 Å for a wide range of applications
- Octadecyl (C_{18}) and butyl (C_4) modification with pore size of 300 Å for the separation of biomolecules
- High batch-to-batch reproducibility

Technical data

- Medium density octadecyl, octyl and butyl phase; endcapped
- 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
- Pore size 300 Å; particle size 5 µm, carbon content 4 % for C_{18}, 2.5 % for C_4; pH stability 1–9

Recommended applications

- USP listing L1 (C_{18}) · L7 (C_8) · L26 (C_4)
- 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

Chemical stability

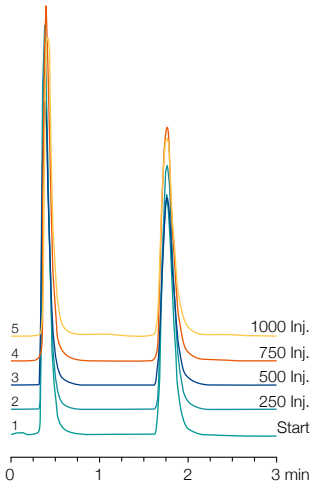
The utmost purity of the base silica and the exceptional silane bonding chemistry minimize the risk of dissolution, or hydrolysis at pH extremes.

The chromatograms show the retention behavior at pH values of 1.5 and 10.0 for NUCLEODUR® 100-5 C18 ec.

pH stability of NUCLEODUR® C18 ec

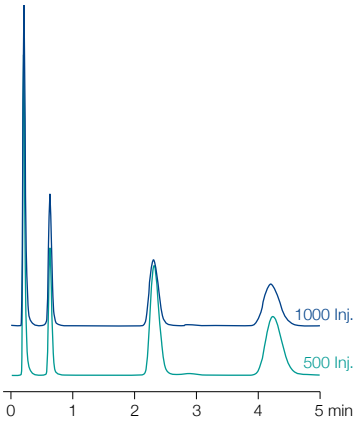
Separation of theophylline and caffeine at pH 10

Column:	30 x 3 mm NUCLEODUR® 100-5 C18 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 C18 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



NUCLEODUR® octyl phases

In addition to NUCLEODUR® C18 phases MACHEREY-NAGEL offers octyl modified NUCLEODUR® C8 Gravity and NUCLEODUR® C8 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® C8 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 nonpolar 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® C8 ec and NUCLEODUR® C8 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® C8 ec and C18 ec. The separation of phenols below 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.

Good to know

- 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.

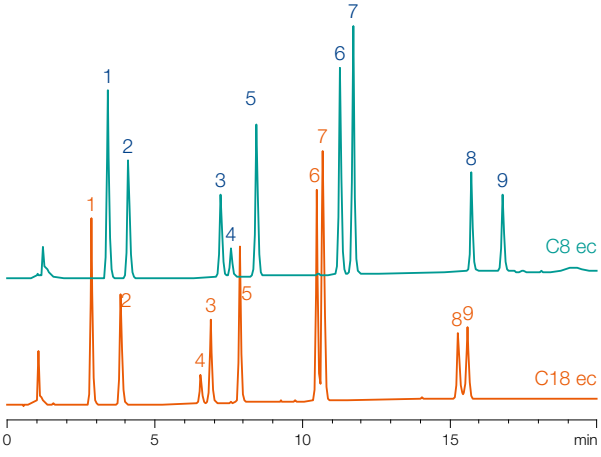
Separation of phenols

MN Appl. Nos. 12089 / 120891

Columns:	250 x 4 mm NUCLEODUR® 100-5 C8 ec / C18 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:

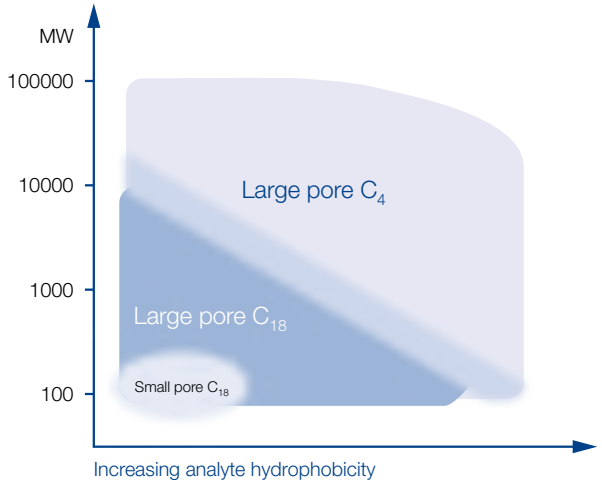
1. Resorcinol	4. Phenol	7. Veratrol
2. Pyrocatechol	5. 2-Methoxyphenol	8. Biphenyl-2-ol
3. 4-Methoxyphenol	6. 2-Ethoxyphenol	9. Phenetole

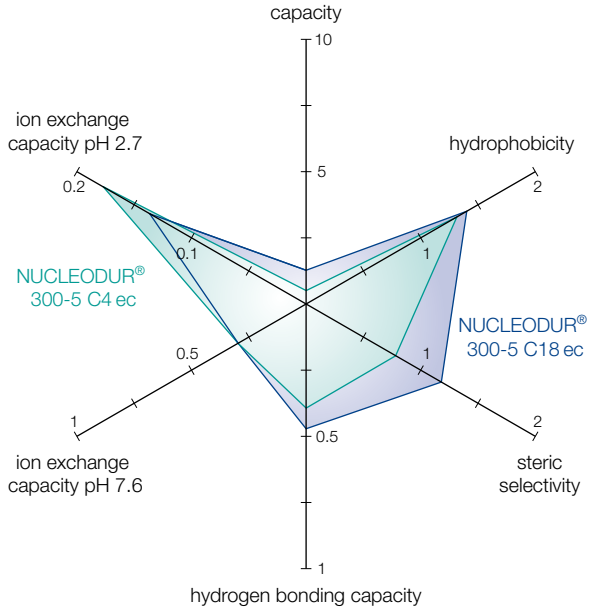


NUCLEODUR® phases for biochromatography

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 seen on the following pages.

Column selection by analyte characteristics





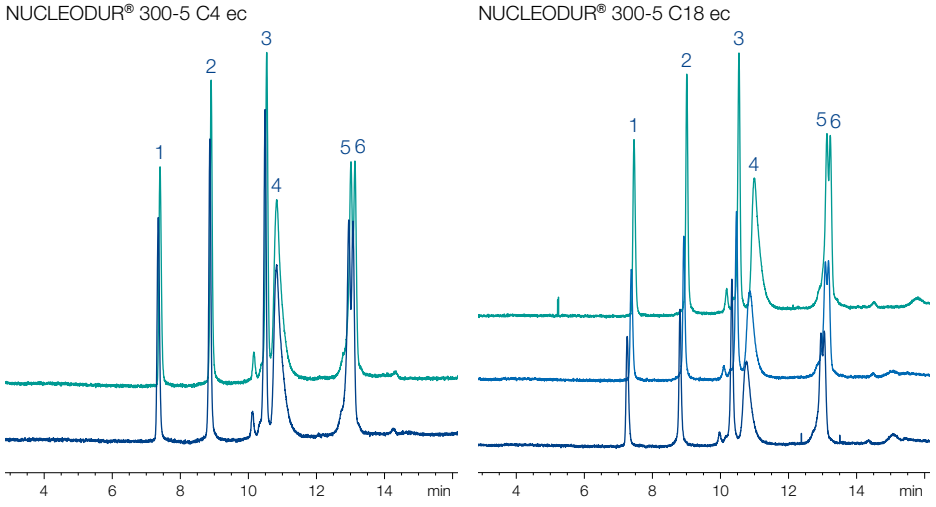
Tanaka plots of NUCLEODUR® wide pore phases

Batch-to-batch reproducibility of NUCLEODUR® 300-5 C4 ec and NUCLEODUR® 300-5 C18 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

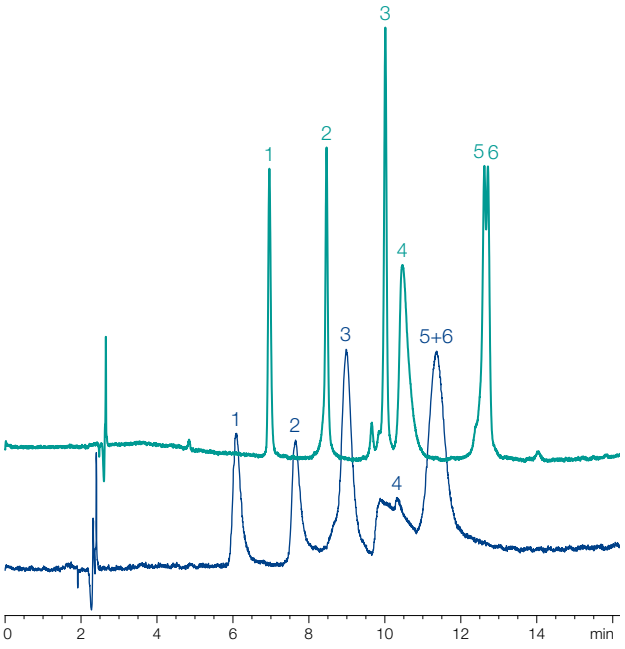


Comparison of narrow and wide pore NUCLEODUR® for the separation of proteins

MN Appl. No. 126590

Columns:	250 x 4,6 mm NUCLEODUR® 300-5 C18 ec
	250 x 4,6 mm NUCLEODUR® C18 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

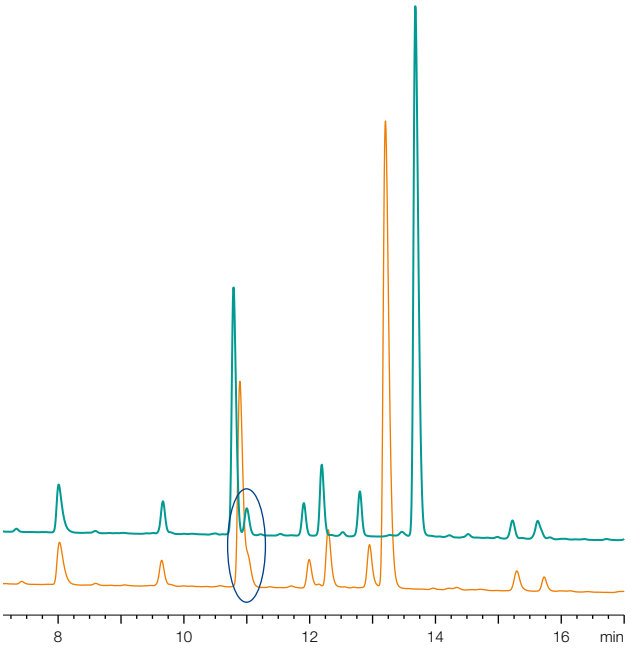


Sharper peaks of larger molecules on wide pore material.

Tryptic digest of cytochrome C

MN Appl. No. 126600

Columns:	250 x 4.6 mm NUCLEODUR® 300-5 C18 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® 300-5 C18 ec.

Ordering information

NUCLEODUR® C18 ec				
Analytical EC columns NUCLEODUR® C18 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760002.46	761932.30
250	4	5	760002.40	761932.30
150	4.6	5	760008.46	761932.30
125	4.6	5	760001.46	761932.30
125	4	5	760001.40	761932.30
125	3	5	760001.30	761932.30
125	2	5	760001.20	761932.20
250	4.6	3	760052.46	761931.30
250	4	3	760052.40	761931.30
150	4.6	3	760053.46	761931.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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Ordering information

NUCLEODUR® C18 ec				
Preparative VP columns NUCLEODUR® C18 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	21	10	762010.210	762090.160
250	10	10	762010.100	762090.80
250	21	5	762022.210	762090.160
250	10	5	762022.100	762090.80
50	10	5	762003.100	762090.80

* For more information of guard columns for preparative VP columns please see page 91.

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Ordering information

NUCLEODUR® 300-5 C18 ec				
Analytical EC columns NUCLEODUR® 300-5 C18 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760186.46	761988.30
250	4	5	760186.40	761988.30
150	4.6	5	760185.46	761988.30
150	2	5	760185.20	761988.20
100	4.6	5	760183.46	761988.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

Ordering information

NUCLEODUR® C8 ec				
Analytical EC columns NUCLEODUR® C8 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760703.46	761937.30
250	4	5	760703.40	761937.30
150	4.6	5	760702.46	761937.30
125	4	5	760701.40	761937.30
50	4.6	5	760700.46	761937.30
100	3	5	760704.30	761937.30
250	4	3	760062.40	761936.30
150	4.6	3	760061.46	761936.30
125	4.6	3	760060.46	761936.30
125	2	3	760060.20	761936.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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Ordering information

NUCLEODUR® C8 ec				
Preparative VP columns NUCLEODUR® C8 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	21	5	762062.210	762092.160
250	10	5	762062.100	762092.80
125	10	5	762061.100	762092.80

* For more information of guard columns for preparative VP columns please see page 91.

For more products and information
Or visit *www.mn-net.com*

Ordering information

NUCLEODUR® 300-5 C4 ec				
Analytical EC columns NUCLEODUR® 300-5 C4 ec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760196.46	761989.30
150	4.6	5	760195.46	761989.30
100	4.6	5	760193.46	761989.30
100	4	5	760193.40	761989.30
100	2	5	760193.20	761989.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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Or visit *www.mn-net.com*

NUCLEODUR® C18 HTec

The preparative octadecyl phase

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® C18 HTec scores in low bleed characteristics and is therefore highly suitable for LC/MS.

Key features

- Base-deactivated preparative octadecyl phase
- Reliable and durable standard RP phase for up-scaling to preparative scale
- High loading capacity and excellent stability
- Suitable for LC/MS

Technical data

- High density octadecyl (C_{18}) phase; multi-endcapped
- 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

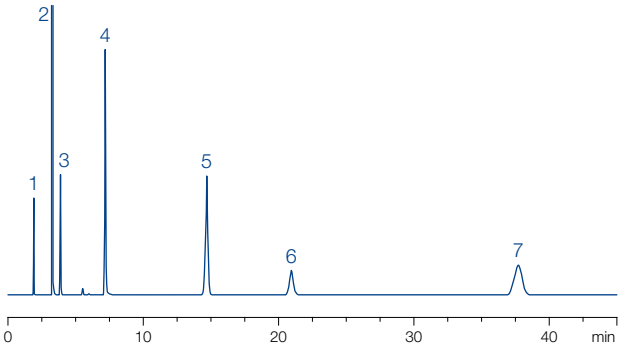
Engelhardt test

MN Appl. No. 123580

Column:	250 x 4 mm NUCLEODUR® C18 HTec, 5 µm
Eluent:	methanol – water (49:51, w/w)
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® C18 HTec offers outstanding mechanical rigidity and thus the perfect choice for self-packing of prep-columns, too. The special surface modification and endcapping procedure results in high chemical stability even at extreme chromatographic conditions like high flow rates, high temperature or critical solvents (DMSO). Furthermore, NUCLEODUR® C18 HTec columns show a remarkably long lifetime in acidic (pH 1) as well as basic (pH 10) mobile phases.

Recommended applications

- USP listing L1
- Sophisticated analytical and preparative separations of basic, neutral and acidic pharmaceuticals, derivatized amino acids, pesticides, fat-soluble vitamins, aldehydes, ketones and phenolic compounds

pH stability test

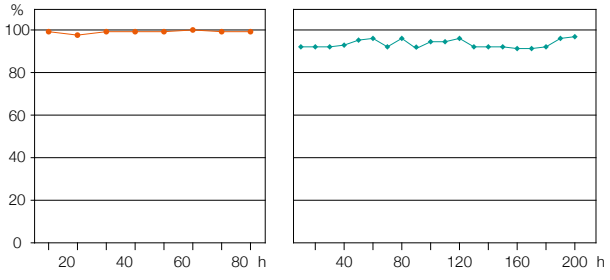
Column:	150 x 4 mm NUCLEODUR® C18 HTec, 5 µm
Flow rate:	1 mL/min
Detection:	UV, 254 nm
Injection:	5 µL

• 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



NUCLEODUR® C18 HTec

Up-scaling

Due to highest quality standards in silica production and phase chemistry combined with optimized packing technology, NUCLEODUR® C18 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).

Good to know

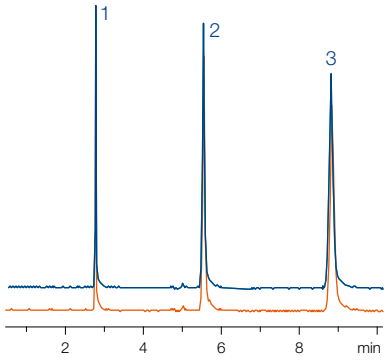
- Due to innovative surface coating procedures NUCLEODUR® C18 HTec offers excellent analytical separation properties and is the first choice for up-scaling to preparative column dimensions.

Up-scaling with NUCLEODUR® C18 HTec

MN Appl. No. 123780

Columns:	EC 250 x 4,6 mm NUCLEODUR® C18 HTec, 5 µm
	VP 250 x 21 mm NUCLEODUR® C18 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

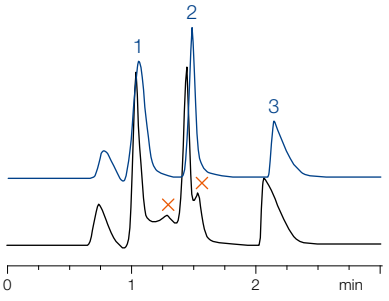
A vital criterion for efficiency in preparative HPLC is the capacity of the separation medium. NUCLEODUR® C18 HTec is characterized by a notably high loading capacity under both basic and acidic conditions, while competitor columns show overload effects even at lower loadings (×).

Loading capacity under acidic conditions

MN Appl. No. 123890

Columns:	VP 100 x 21 mm NUCLEODUR® C18 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)



NUCLEODUR® C18 HTec

Ordering information

NUCLEODUR® C18 HTec				
Analytical EC columns NUCLEODUR® C18 HTec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760316.46	761927.30
250	4	5	760316.40	761927.30
150	4.6	5	760315.46	761927.30
125	4	5	760314.40	761927.30
250	4.6	3	760326.46	761926.30
150	4.6	3	760325.46	761926.30
150	2	3	760325.20	761926.20
125	3	3	760324.30	761926.30
150	2	1.8	760308.20	761925.20
100	2	1.8	760306.20	761925.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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Or visit *www.mn-net.com*

Ordering information

NUCLEODUR® C18 HTec				
Preparative VP columns NUCLEODUR® C18 HTec (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	50	10	762576.500	762592.500
250	21	10	762576.210	762591.160
250	10	10	762576.100	762591.80
150	32	10	762575.320	762592.320
250	21	7	762566.210	762591.160
250	10	7	762566.100	762591.80
250	50	5	762556.500	762592.500
250	40	5	762556.400	762592.320
250	32	5	762556.320	762592.320
250	21	5	762556.210	762591.160
250	16	5	762556.160	762591.160
250	10	5	762556.100	762591.80
250	8	5	762556.80	762591.80
150	32	5	762555.320	762592.320
100	21	5	762553.210	762591.160

* For more information of guard columns for preparative VP columns please see page 91.

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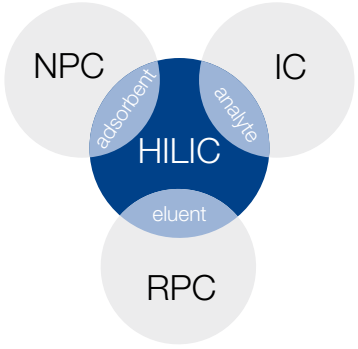
NUCLEODUR® HILIC

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 [4].

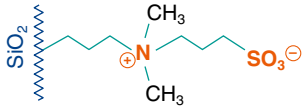
HILIC combines the characteristics of the 3 major methods in liquid chromatography reversed phase (RPC), normal phase (NPC) and ion chromatography (IC):

· 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 modifiers 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 ammonium-sulfonic acid ligands results in total charge equalization and in an overall neutrally 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.

Stability features

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

Key features

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

Technical data

- Zwitterionic ammonium-sulfonic acid phase; not endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 7 %; pH stability 2–8.5

Recommended applications

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

NUCLEODUR® HILIC

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

Good to know

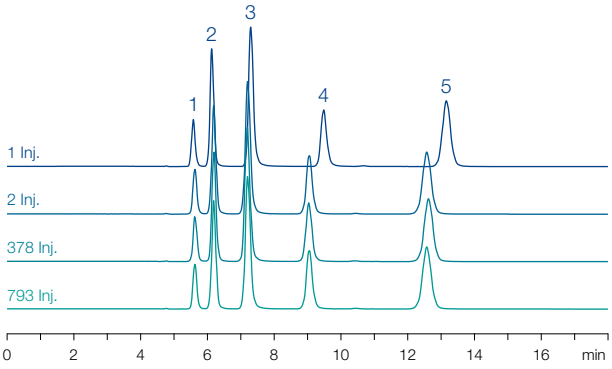
NUCLEODUR® HILIC is a patented phase modification (pat. number DE102009006007 (B4))

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



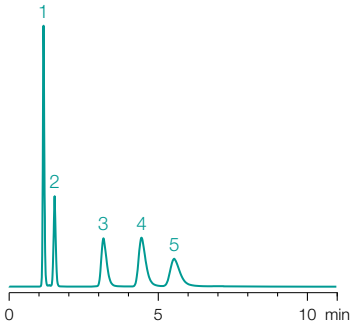
Overall NUCLEODUR® HILIC provides excellent chromatographic features and is hereby the perfect choice for separation of polar or charged compounds which can be shown in application 122920.

Separation of adenosine and phosphates

MN Appl. No. 122920

Column: 125 x 4 mm NUCLEODUR® HILIC, 5 µm
Eluent: acetonitrile – 100 mM ammonium acetate, pH 5.3 (70:30, v/v)
Flow rate: 1.3 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

Peaks:
1. Adenosine
2. cAMP
3. AMP
4. ADP
5. ATP



Ordering information

NUCLEODUR® HILIC

Analytical EC columns NUCLEODUR® HILIC (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760550.46	761962.30
150	2	5	760553.20	761962.20
250	4.6	3	760530.46	761961.30
250	3	3	760530.30	761961.30
150	4.6	3	760533.46	761961.30
125	4.6	3	760531.46	761961.30
125	2	3	760531.20	761961.20
100	3	3	760534.30	761961.30
100	2	1.8	760526.20	761960.20
50	2	1.8	760523.20	761960.20

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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Alternative bonded-phase functionality

In reversed phase HPLC it is fairly common to start with C_{18} or C_8 columns when developing new methods. However, superior polarity and selectivity properties often required for more sophisticated separations, are not always sufficiently provided by classical RP phases. These classical RP phases 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 clearly recognizable and different retention behavior compared to purely alkyl-functionalized surface modifications (see application 119340).

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 [5]. 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) [6].

Short-chain bonded phases sometimes reveal shortcomings in stability towards hydrolysis at low pH [7]. 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).

Key features

- Multi-mode phase modification (RP and NP) widens scope of selectivity
- High retention capacity especially for very polar and unsaturated compounds
- Stable against hydrolysis at low pH
- Different retention characteristics in comparison to C_8 and C_{18} phases

Technical data

- Cyanopropyl high purity phase; specially endcapped
- Pore size 110 Å; particle sizes 3 µm and 5 µm; carbon content 7 %; pH stability 1–8

Recommended applications

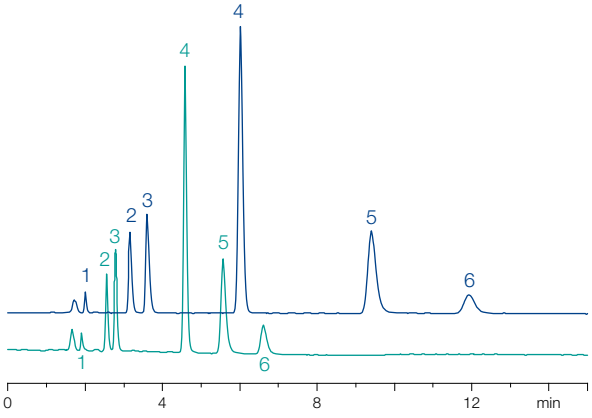
- USP listing L10
- Tricyclic antidepressants, steroids, organic acids

Separation of cold medicine ingredients on two different NUCLEODUR® phases

MN Appl. No. 119340

Columns:	250 x 4 mm NUCLEODUR® 100-5 C18 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

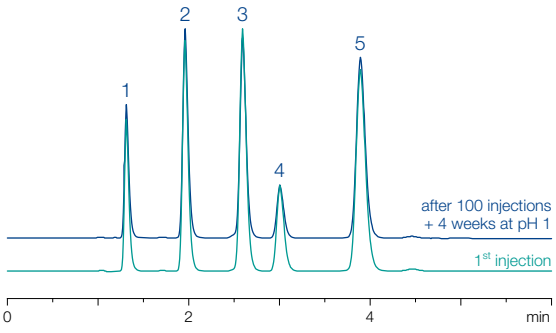


Stability of NUCLEODUR® CN-RP at pH 1

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 some of the basic drugs analyzed in applications 119271 and 119272.

Separation of steroids in normal phase and reversed phase mode

MN Appl. Nos. 119271 / 119272

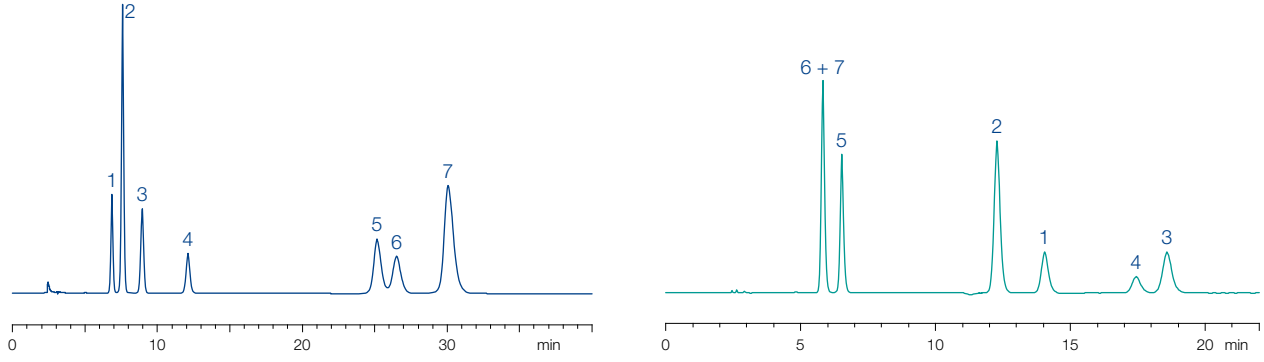
Normal phase mode

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 in normal phase mode

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



Ordering information

NUCLEODUR® CN-RP / CN				
Analytical EC columns NUCLEODUR® CN-RP (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760152.46	761944.30
250	4	5	760152.40	761944.30
150	4.6	5	760154.46	761944.30
125	4.6	5	760153.46	761944.30
150	4.6	3	760156.46	761941.30
150	4	3	760156.40	761941.30
50	2	3	760159.20	761941.20
Analytical EC columns NUCLEODUR® CN (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760150.46	761943.30
250	4	5	760150.40	761943.30
150	4.6	5	760149.46	761943.30

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEODUR® NH2 / NH2-RP

Amino modified HPLC phase

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. They both feature an important advantage – that they can be run in the RP and NP mode. RP mode using aqueous-organic eluent mixtures and NP mode with hexane as a possible mobile phase.

NUCLEODUR® NH2, belongs to the so-called multi-mode 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.

The main field of application of NUCLEODUR® NH2 is the separation of simple and complex sugars, sugar alcohols, and other hydroxy compounds under RP conditions as well as hydrocarbons under NP conditions.

Key features

- Multi-mode phase modification (for RP, NP and IC)
- Stable against hydrolysis at low pH and stable in 100 % aqueous eluents
- Widens scope of analytical HPLC into the polar range
- Suitable for LC/MS

Technical data

- Aminopropyl high purity phase; not endcapped
- Pore size 110 Å; particle sizes 3 µm, 5 µm and 7 µm; carbon content 2.5 %; pH stability 2–8

Recommended applications

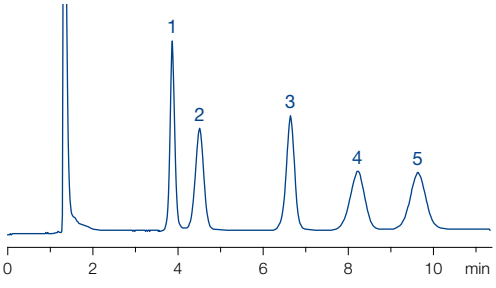
- USP listing L8
- Polar compounds under RP conditions (sugars, DNA bases), hydrocarbons under NP conditions

Reversed phase separation of sugars

MN Appl. No. 122160

Column: 250 x 4 mm NUCLEODUR® 100-5 NH2-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

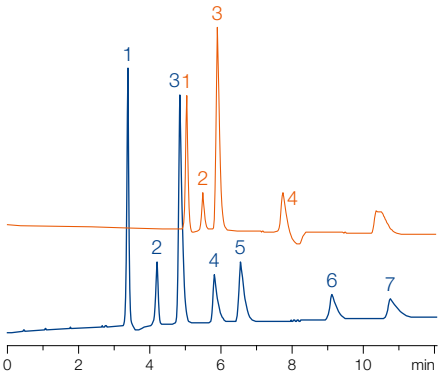


Normal phase separation of middle distillates in accordance with DIN EN 12916

MN Appl. No. 122180

Columns: A) 250 x 4 mm NUCLEODUR® 100-5 NH2
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



NUCLEODUR® NH2 / NH2-RP

Due to the special method of surface modification NUCLEODUR® NH2 features a pronounced stability at higher as well as 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.

The following example shows enhanced pH stability of NUCLEODUR® NH2 and outstanding suitability for the separation of total herbicides (AMPA, glyphosate, glufonisate, …) - see application No. 122190 in our online database at ChromaAppDB.mn-net.com.

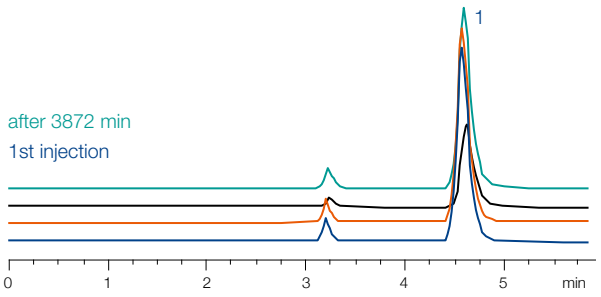
Good to know

- Normal phase chromatography (NP) with hexane, dichloromethane or 2-propanol as mobile phase for polar compounds
- 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

Hydrolytical resistance of NUCLEODUR® NH2-RP

Column: 250 x 4 mm NUCLEODUR® 100-5 NH2-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)



Based on spherical NUCLEODUR® silica 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® NH2 enables reliable analyses especially for routine work.

Ordering information

NUCLEODUR® NH2-RP / NH2

Analytical EC columns NUCLEODUR® NH2-RP (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760732.46	761953.30
250	4	5	760732.40	761953.30
250	2	5	760732.20	761953.20
125	4	5	760730.40	761953.30
250	4.6	3	760739.46	761951.30
150	4.6	3	760742.46	761951.30
100	2	3	760740.20	761951.20

Analytical EC columns NUCLEODUR® NH2 (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760722.46	761952.30
250	4	5	760722.40	761952.30
125	4.6	5	760720.46	761952.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

NUCLEODUR® SiOH

Unmodified ultra-pure spherical silica gel

Unmodified silica gels were the first substrates used for liquid chromatography. Our ultra-pure spherical silica gel NUCLEODUR® SiOH can be used under normal phase as well as HILIC conditions. With no phase modification and no endcapping, the bare silica phase is suited for applications with polar to midpolar compounds. Due to this, very polar silica surface with silanol and siloxane groups a non-polar mobile phase is necessary for a perfect chromatographic performance. NUCLEODUR® SiOH can be used for analytical and preparative HPLC applications.

Key features

- Unmodified silica for NP chromatography
- 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 phase; not endcapped
- 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 10)

Recommended applications

- USP listing L3
- Polar and midpolar compounds under normal phase and HILIC conditions

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Ordering information

NUCLEODUR® SiOH

Analytical EC columns NUCLEODUR® SiOH (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760007.46	761967.30
250	4	5	760007.40	761967.30
150	4.6	5	760012.46	761967.30
250	4.6	3	760173.46	761966.30
150	4.6	3	760172.46	761966.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

CHROMABOND® SPE products
For a wide variety of applications

High-performance products for sample preparation

- Comprehensive range of RP and normal phases as well as ion exchangers
- Polymer and silica based phases
- Phases for special applications like food or environmental analysis

NUCLEODUR® C18 PAH

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, and tabacco; hence it can found globally. Today most PAHs accrue from anthropogenic processes – but also natural origins such as forest fire. In the past the production of coke and gas from black coal had a considerable impact on environmental pollution. Waste products (e.g., tar) from coking or gas plants are often the 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. Therefore control of the PAH content in 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.

Key features

- Special octadecyl phase for PAH analysis
- Base material high purity NUCLEODUR® silica

Technical data

- Special octadecyl phase with polymerically coated base material; endcapped
- Pore size 110 Å, particle sizes 1.8 µm and 3 µm

Recommended applications

- USP listing L1
- 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

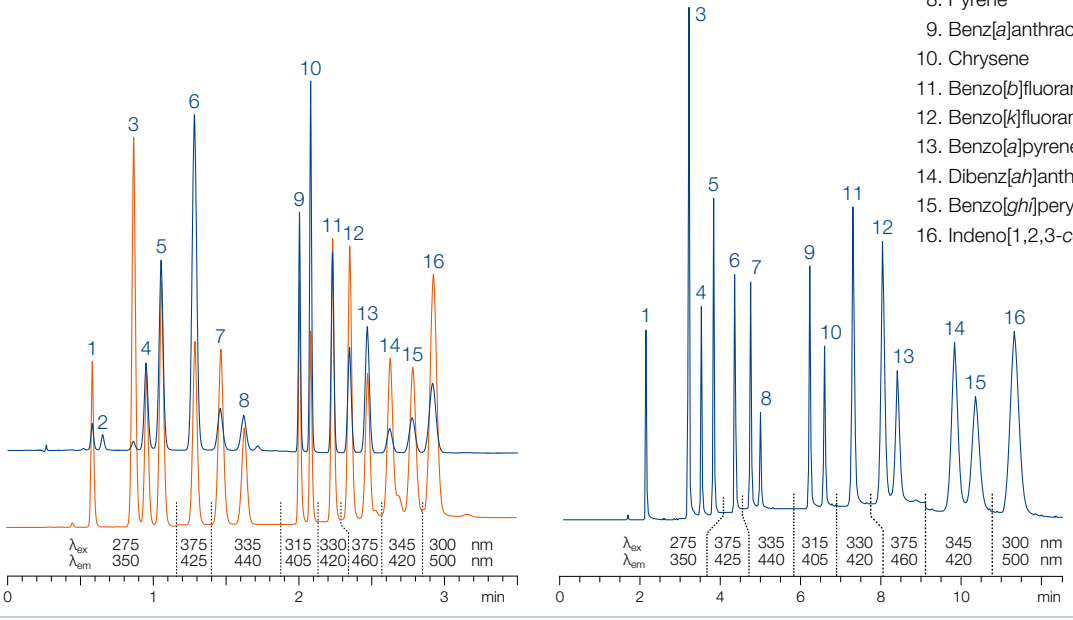
Eluent: A) water B) methanol 65–97 % B in 6 min, 97 % B for 5 min, 97–65 % B in 0.5 min

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).

NUCLEODUR® C18 PAH

Separation of 18 PAHs on NUCLEODUR® C18 PAH

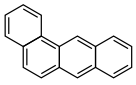
MN Appl. No. 123840

Column:	125 x 4 mm NUCLEODUR® C18 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)

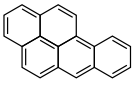
Peaks:

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

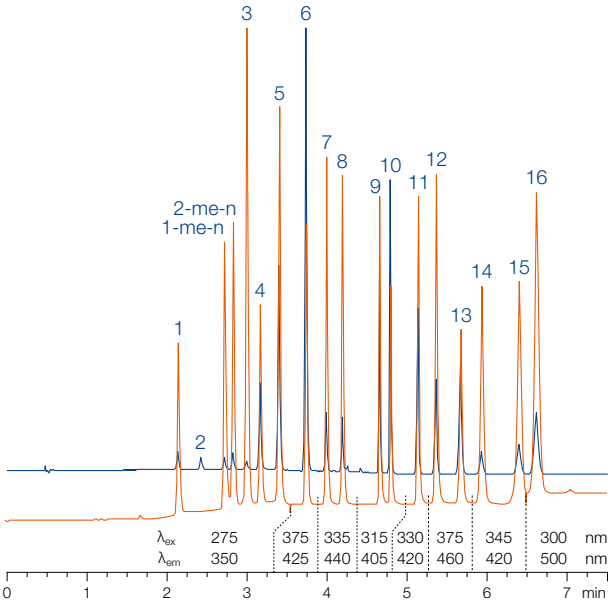
1-me-n: 1-methylnaphthalene
2-me-n: 2-methylnaphthalene



Benzo[a]anthracen



Benzo[a]pyren



NUCLEODUR® C18 PAH

HPLC columns for PAH analysis

For PAH analyses we have developed a specially modified C_{18} phase based on NUCLEODUR® which allows efficient gradient separation of 16 PAHs according to EPA regulations. 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 the eluent. For rapid analysis NUCLEODUR® C18 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® C18 PAH.

Ordering information

NUCLEODUR® C18 PAH				
Analytical EC columns NUCLEODUR® C18 PAH (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4	3	760786.40	761971.30
250	3	3	760786.30	761971.30
150	3	3	760785.30	761971.30
125	4	3	760784.40	761971.30
125	3	3	760784.30	761971.30
100	3	3	760783.30	761971.30
100	4	1.8	760773.40	761970.30
100	3	1.8	760773.30	761970.30
100	2	1.8	760773.20	761970.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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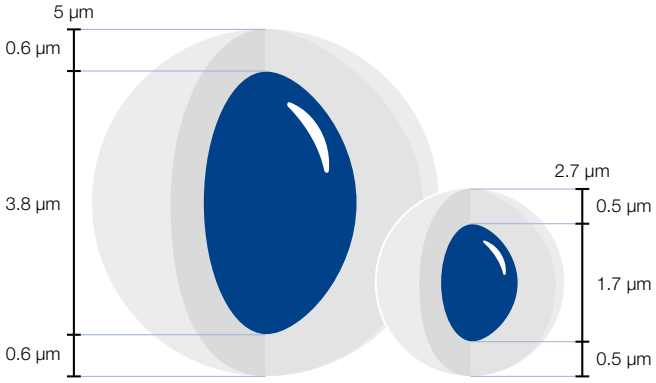
OPTIMA® GC columns
For sophisticated separations

OPTIMA® 1301 MS

- USP G43 medium polar cross-linked silarylene phase
- Excellent deactivation with very low MS bleeding
- Especially suitable for environmental analysis (e.g. PAHs, PCBs and pesticides)

NUCLEOSHELL® core-shell silica for HPLC

Core-shell technology



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 savings. However they generate a high back pressure of the mobile phase during column runs which requires specifically designed equipment.

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.

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

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

Key features

- 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) m^2/g lower back pressure enables use on conventional LC systems
- Pressure stability up to 600 bar

NUCLEOSHELL® core-shell silica for HPLC

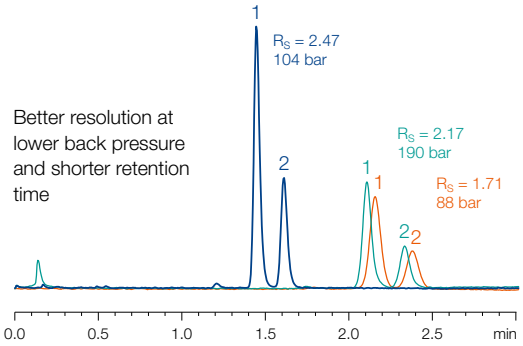
Resolution R_S as function of particle size

MN Appl. No. 125270

Columns: 50 x 4 mm
NUCLEOSHELL® RP 18, 2.7 µm
NUCLEODUR® C18 Gravity, 3 µm
NUCLEODUR® C18 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

Better resolution at lower back pressure and shorter retention time

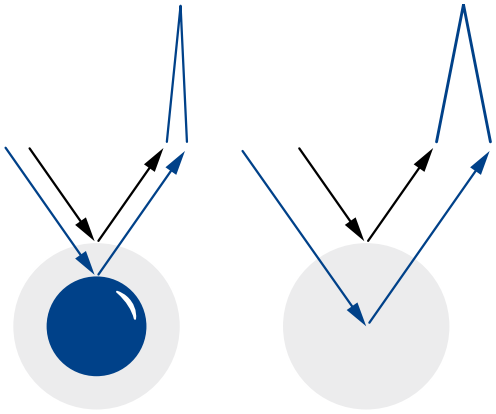


Theoretical column efficiency (optimal conditions)

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



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.

Benefits

- 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)

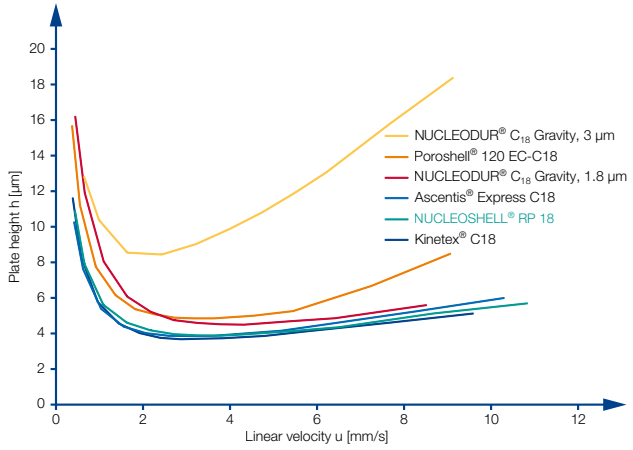
$$H = A + \frac{B}{u} + C \cdot u$$

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



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.

Good to know

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.

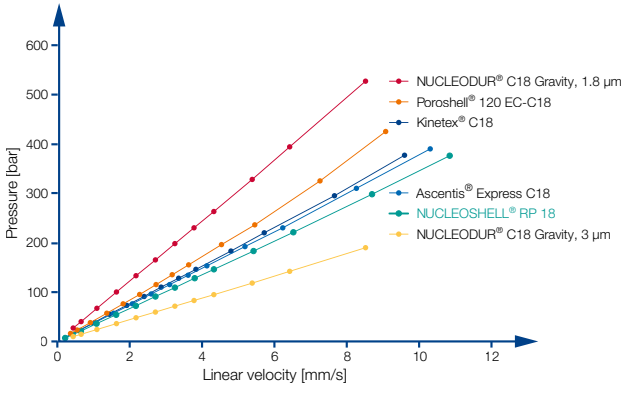
$$\Delta_P = \frac{\Phi \cdot L_C \cdot \eta \cdot u}{d_P^2}$$

Δ_P = pressure drop, Φ = flow resistance (non-dimensional), 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



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.

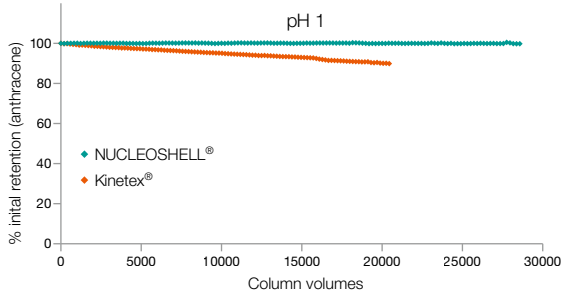
The following figure shows a column stability test of NUCLEOSHELL® RP 18 at mobile phase levels pH 1 and pH 10 compared with three competing phases.

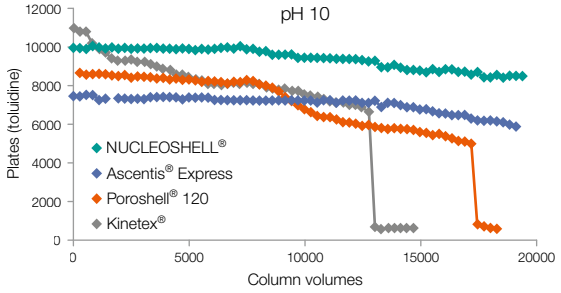
Stability under acidic and basic conditions

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
Eluent:	acetonitrile – 1 % TFA in water, pH 1 (50:50, v/v)
Flow rate:	1.3 mL/min
Temperature:	80 °C
Detection:	UV, 254 nm
Analyt:	anthracene

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:	20 mmol/L Na borate – 10 mmol/L NaOH – methanol, pH 10 (21:49:30, v/v/v)
Flow rate:	1.5 mL/min
Temperature:	40 °C
Detection:	UV, 220 nm
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:

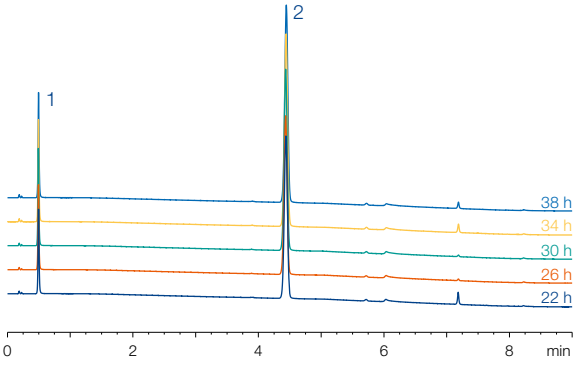
Column:	50 x 2 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent:	A) 10 mmol/L ammonium formate – methanol (9:1, v/v) + 120 µL formic acid, ~ pH 4 B) 10 mmol/L ammonium formate – methanol (1:9, v/v) + 120 µL formic acid, ~ pH 4 0–100 % B in 7 min
Flow rate:	0.5 mL/min,
Temperature:	100 °C
Detection:	UV, 220 nm

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



NUCLEOSHELL® core-shell silica for HPLC

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

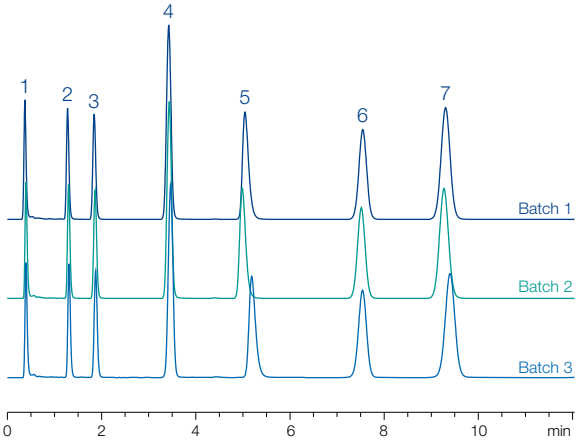
Batch-to-batch reproducibility

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



CHROMAFIL® syringe filters

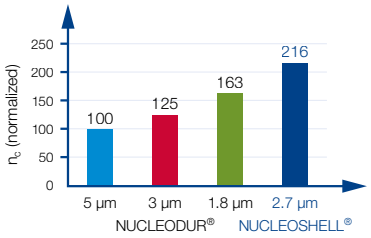
Protecting your columns from solid contamination

Ideal for filtration of GC and (U)HPLC sample solutions

- Diverse membrane types and filter sizes
- Lowest content of extractable substances
- Luer lock inlet, Luer outlet

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.

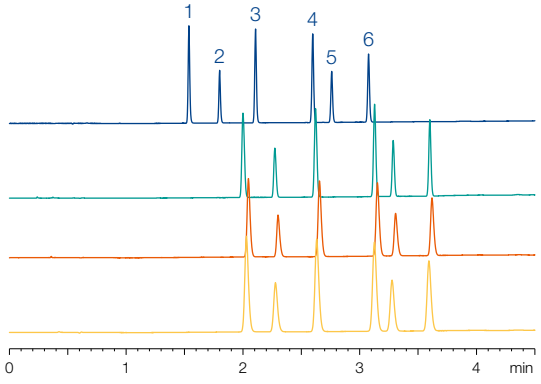
Peak capacity

MN Appl. No. 125540

Columns: 100 x 4.6 mm each
NUCLEOSHELL® RP 18, 2.7 µm
NUCLEODUR® C18 Gravity, 1.8 µm
NUCLEODUR® C18 Gravity, 3 µm
NUCLEODUR® C18 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

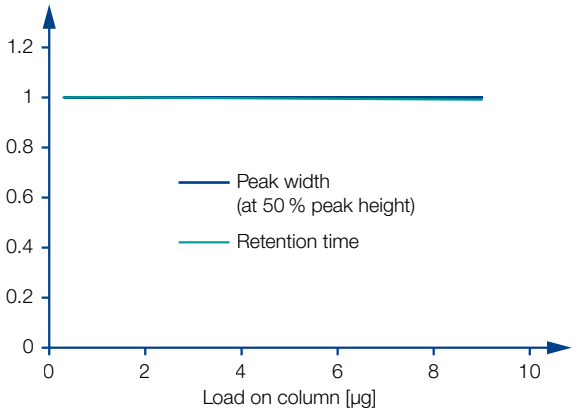


	Max. pressure [bar]	Resolution (4, 5)
NUCLEOSHELL®, 2.7 µm	255	5.45
NUCLEODUR®, 1.8 µm	450	4.14
NUCLEODUR®, 3 µm	214	2.97
NUCLEODUR®, 5 µm	142	2.30

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 even though core-shell particles are suspected of showing a slightly lower loading capacity compared to fully porous silica materials.

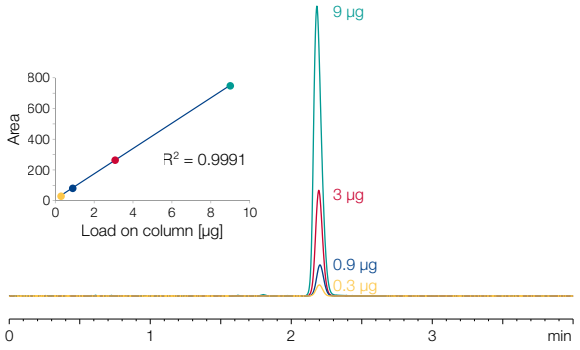
Normalized column parameters



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 a particular 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 C18, 5 µm

Eluent: methanol – water + 0.1 % formic acid (35:65, v/v)

Flow rate: 1.3 mL/min

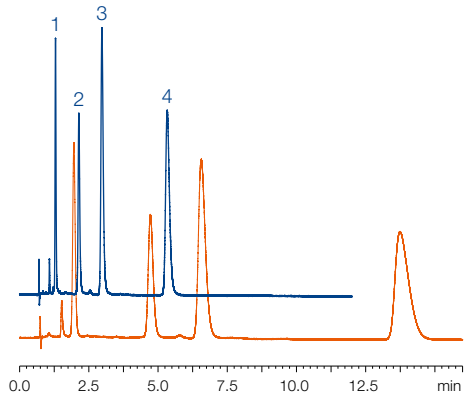
Pressure: 182 bar, 219 bar

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



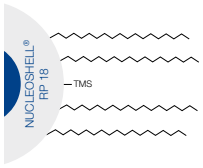
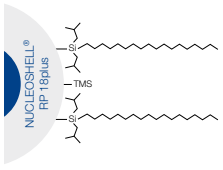
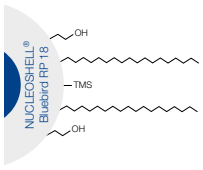
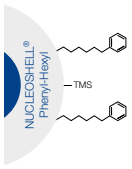
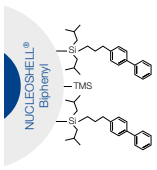
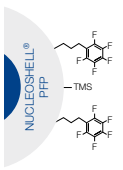
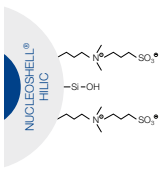
Column protection system

Increasing the lifetime of your HPLC columns

Ideal protection for your analytical main column

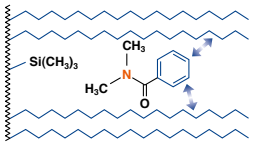
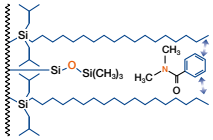
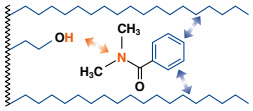
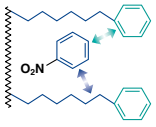
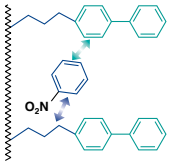
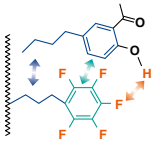
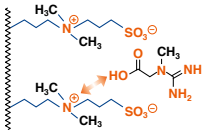
- Universal screw-on guard column holder system
- Suitable for all analytical HPLC columns with 1/16" fittings
- Special ferrules for UHPLC: pressure stability up to 1300 bar (18850 psi)

NUCLEOSHELL® phase overview

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	70	A ●●●●● B ◖ C ●●◗	pH 1–11, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 RP 18plus	octadecyl (monomeric), multi-endcapping 5.7 % C (2.7 µm particles) 4.4 % C (5 µm particles) USP L1	72	A ●●●● B ●●◗ C -	pH 2–9, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 Bluebird RP 18	octadecyl, hydrophilic endcapping 5 % C (2.7 µm particles) USP L1	75	A ●●●● B ●●◗ C ●●	stable in 100 % aqueous eluent, pH 1–8, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 Phenyl-Hexyl	phenylhexyl, multi-endcapping 4.5 % C (2.7 µm particles) USP L11	78	A ●● B ●●● C ●	pH 1–10, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 Biphenyl	biphenylpropyl, multi-endcapping 5.2 % C (2.7 µm particles) USP L11	81	A ●●● B ●●● C ●●●	stable in 100 % aqueous eluent, pH 1.5–8.5, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 PFP	pentafluorophenyl, multi-endcapping ~ 3 % C (2.7 µm particles) USP L43	84	A ●● B ●●●● C ●●●●	pH 1–9, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 
 HILIC	zwitterionic ammonium-sulfonic acid, no endcapping 1.3 % C (2.7 µm particles)	86	A ● B ●●●●● C -	pH 2–8.5, suitable for LC/MS	NUCLEOSHELL® (Si-O$_2$)$_n$ 

* A = ● hydrophobic selectivity, B = ● polar / ionic selectivity, C = ● steric selectivity
** phases which provide a similar selectivity based on chemical and physical properties

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; HALO® C18; Shim-pack Velox® 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; Shim-pack Velox® SP-18	hydrophobic (van der Waals interactions)	
overall sophisticated analytical separations, especially for very polar compounds, e.g., pesticides, sweeteners, nitrosamines, water-soluble vitamins, organic acids, pharmaceuticals	Kinetex® Polar C18	hydrophobic and polar (H bonds)	
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; HALO® Phenyl-Hexyl	π-π and hydrophobic	
aromatic and unsaturated compounds, mycotoxins, phthalates, hormones, polar compounds like pharmaceuticals, antibiotics, pesticides	Kinetex® Biphenyl, Raptor® Biphenyl, HALO® Biphenyl; Shim-pack Velox® Biphenyl	π-π and hydrophobic	
aromatic and unsaturated compounds, phenols, halogenated hydrocarbons, isomers, polar compounds like pharmaceuticals, antibiotics	Kinetex® PFP; Ascentis® Express F5; Accucore® PFP; Shim-pack Velox® PFP; HALO® PFP; Raptor® 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	

NUCLEOSHELL® RP 18

High density, base-deactivated core-shell silica

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.

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

Key features

- Nonpolar high density phase
- Suitable for LC/MS and HPLC at pH extremes (pH 1–11)
- Superior base deactivation, ideal for method development

Technical data

- Octadecyl phase; multi-endcapped
- Pore size 90 Å, particle sizes 2.7 and 5 µm, carbon content 7.8 % for 2.7 µm, 6.1 % for 5 µm; pH stability 1–11

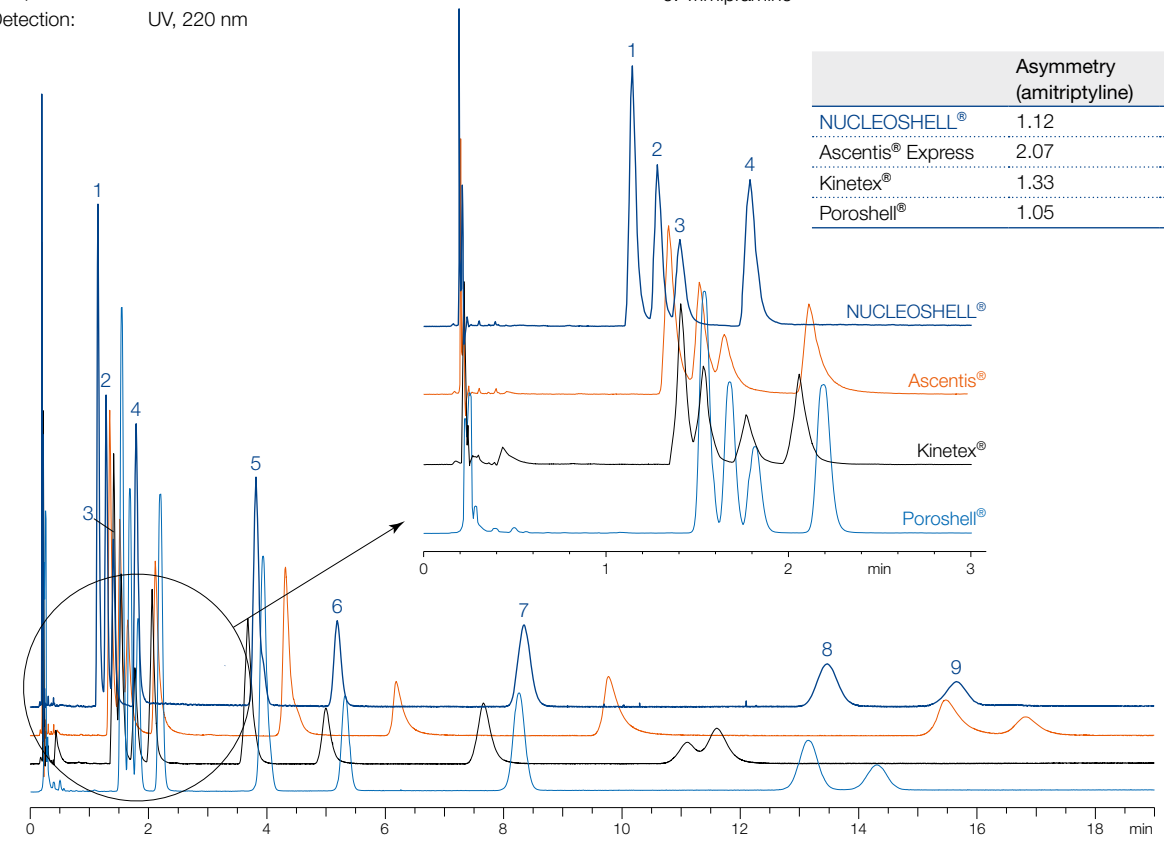
Tricyclic antidepressants · comparison of selectivity and resolution

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



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.

Recommended applications

- USP listing L1
- Overall sophisticated analytical separations, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants

NUCLEOSHELL® RP 18

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

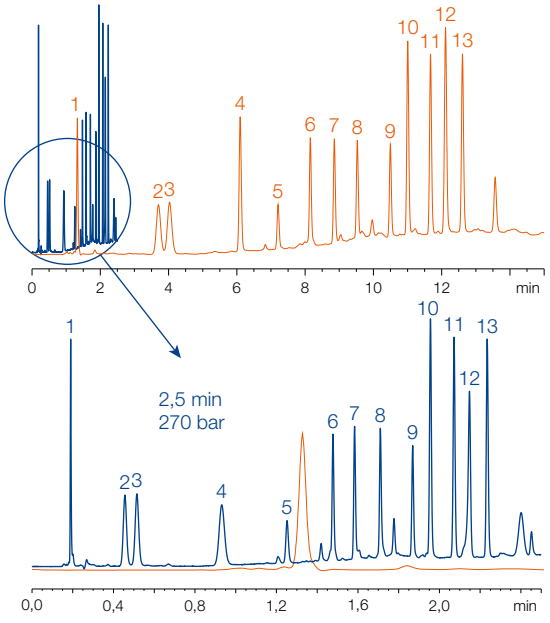
13 β-lactam antibiotics in less than 3 min

MN Appl. No. 124940

Columns:	50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm 150 x 4 mm NUCLEODUR® C18 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

NUCLEOSHELL® RP 18

Analytical EC columns NUCLEOSHELL® RP 18 (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763136.46	763138.30
150	4	2.7	763136.40	763138.30
150	3	2.7	763136.30	763138.30
150	2	2.7	763136.20	763138.20
100	4.6	2.7	763134.46	763138.30
100	4	2.7	763134.40	763138.30
100	3	2.7	763134.30	763138.30
100	2	2.7	763134.20	763138.20
50	3	2.7	763132.30	763138.30
50	2	2.7	763132.20	763138.20
250	4.6	5	763157.46	763158.30
250	4	5	763157.40	763158.30
250	3	5	763157.30	763158.30
150	4.6	5	763156.46	763158.30
150	4	5	763156.40	763158.30
150	3	5	763156.30	763158.30
100	4.6	5	763154.46	763158.30
100	3	5	763154.30	763158.30
100	2	5	763154.20	763158.20
50	4.6	5	763152.46	763158.30

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

NUCLEOSHELL® RP 18plus

Hydrophobic phase with polar selectivity

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.

Key features

- C_{18} phase with polar selectivity
- Hydrophobic C_{18} phase with distinct polar selectivity ideal for method development and suitable for LC/MS
- Excellent performance under highly aqueous conditions

Technical data

- Monomeric octadecyl phase; 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

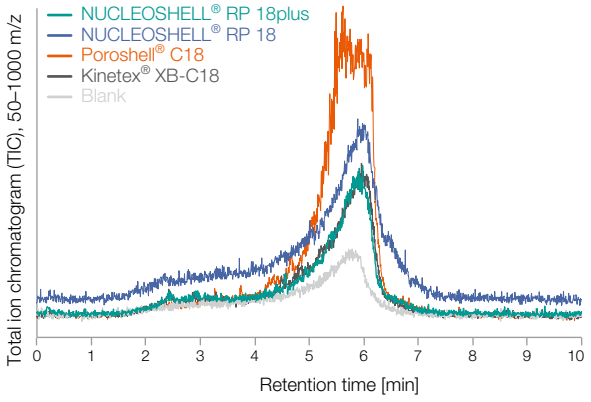
Recommended applications

- USP listing L1
- Overall sophisticated analytical separations, especially for polar compounds, e.g., pharmaceuticals like antibiotics, water-soluble vitamins, organic acids

Bleeding characterisitics

MN Appl. No. 126640

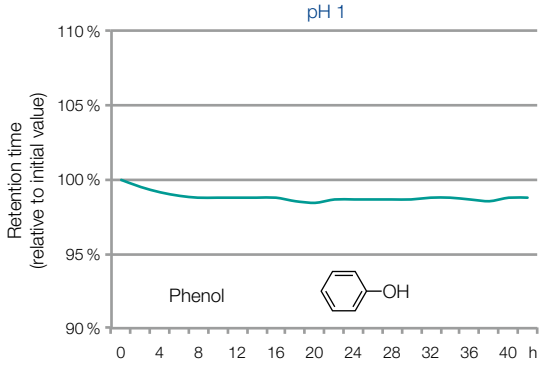
Column:	50 x 2 mm NUCLEOSHELL® RP 18plus, 2.7 µm
Eluent:	A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile 95 % A → 5 % A in 4.5 min (0.5 min) → 95 % A in 0.5 min (4.5 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	MS

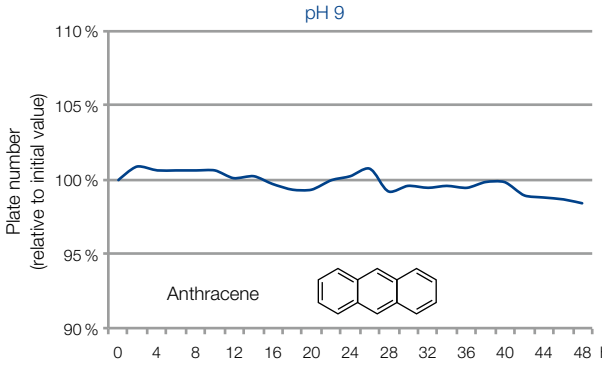


pH stability of NUCLEOSHELL® RP 18plus

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





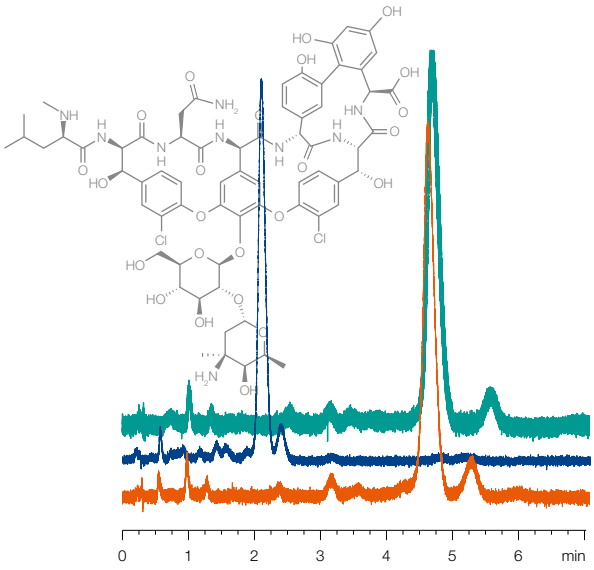
NUCLEOSHELL® RP 18plus

A comparison of retention of the glycopeptide antibiotic vancomycin on several octadecyl modified core-shell phases also 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



NUCLEOSHELL® RP 18plus

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

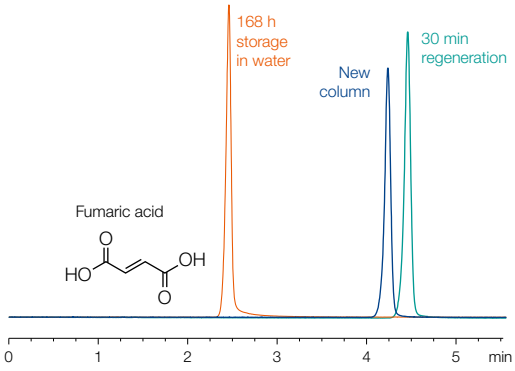
Good to know

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.

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

NUCLEOSHELL® RP18 plus

Analytical EC columns NUCLEOSHELL® RP18 plus (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763236.46	763238.30
150	4	2.7	763236.40	763238.30
150	2	2.7	763236.20	763238.20
100	4.6	2.7	763234.46	763238.30
100	4	2.7	763234.40	763238.30
100	3	2.7	763234.30	763238.30
100	2	2.7	763234.20	763238.20
50	3	2.7	763232.30	763238.30
50	2	2.7	763232.20	763238.20
30	2	2.7	763231.20	763238.20
250	4.6	5	763257.46	763258.30
250	4	5	763257.40	763258.30
250	3	5	763257.30	763258.30
150	4.6	5	763256.46	763258.30
150	4	5	763256.40	763258.30
150	2	5	763256.20	763258.20
100	4.6	5	763254.46	763258.30
100	3	5	763254.30	763258.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

NUCLEOSHELL® Bluebird RP 18

Core-Shell technology suitable for highly aqueous mobile phases

NUCLEOSHELL® Bluebird RP 18 is an octadecyl modified superficially porous silica. Due to an excellent base deactivation and a special hydrophilic endcapping procedure, NUCLEOSHELL® Bluebird RP 18 is extremely durable in 100 % aqueous mobile phase.

A robust bonding chemistry leads to low bleeding characteristics and therefor an excellent suitability for LC/MS applications.

The polar surface chemistry of NUCLEOSHELL® Bluebird RP 18 leads to retention characteristics distinctly different from conventional C_{18} phases. Sulfa drugs and various polar drug analytes can be very well separated as shown in the following applications (MN application numbers 128340 and 128390).

Key features

- Special core-shell phase with hydrophilic endcapping
- Stable in 100 % aqueous mobile phase
- Distinct polar selectivity features
- Excellent base deactivation; suitable for LC/MS due to low bleeding characteristics

Technical data

- Octadecyl phase; polar endcapped
- Pore size 90 Å; particle size 2.7 µm. carbon content 5 %; pH stability 1–8

Recommended applications

- USP listing L1
- Pesticides, pharmaceuticals, water-soluble vitamins, sweeteners, nitrosamines, organic acids, very polar analytes

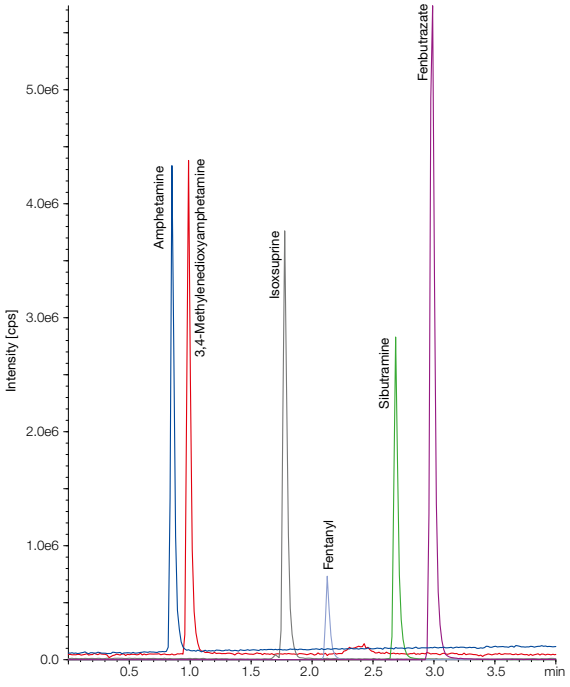
Drug analytes

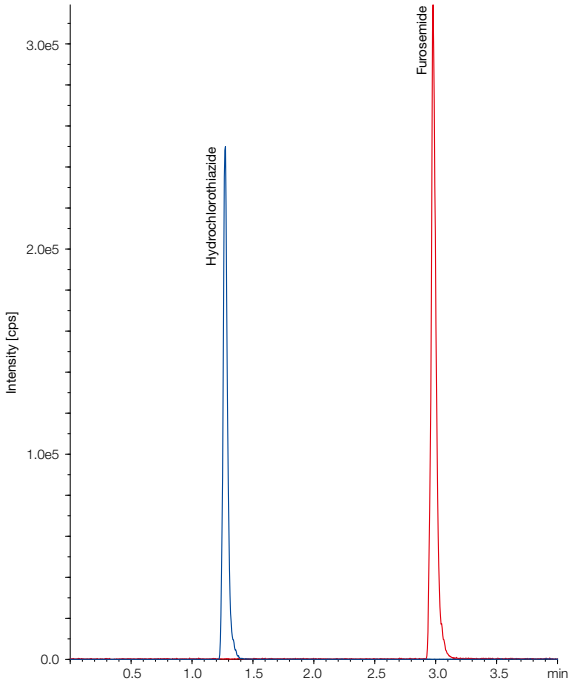
MN Appl. No. 128340

Column:	50 x 4.6 mm NUCLEOSHELL® Bluebird RP 18, 2.7 µm
Eluent:	A) 0.1 % formic acid in water
	B) 0.1 % formic acid in methanol
Gradient:	in 4.5 min from 5 % to 90 % B, hold for 0.5 min, in 0.5 min to 5 % B, hold 0 % B for 4.5 min
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	MS, SMRM
Injection:	5 µL
Concentration:	50 ng/mL for each analyte

MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Amphetamine	0.85	136.0	91.1	108.9
3,4-Methylenedi-oxyamphetamine	0.99	180.0	163.1	105.0
Isoxsuprine	1.78	303.0	285.1	77.1
Fentanyl	2.13	337.0	304.9	105.1
Sibutramine	2.69	280.0	125.0	139.1
Fenbutrazate	2.99	368.2	191.1	91.1
Analyte	**RT [min]**	**$[M-H]^-$**	**Q_1 (Quantifier)**	**Q_2 (Qualifier)**
Hydrochlorothiazide	1.27	295.9	268.7	98.9
Furosemide	2.98	329.0	283.2	255.2



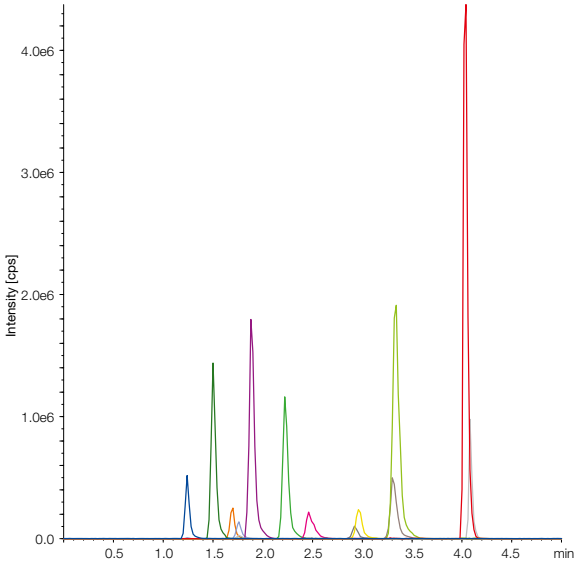


Sulfa drugs

MN Appl. No. 128390

Column: 50 x 4.6 mm NUCLEOSHELL® Bluebird RP 18, 2.7 µm
Eluent: A) 0.1 % formic acid in water
B) 0.1 % formic acid in methanol
Gradient: in 4.0 min from 5 % to 20 % B, in 1.0 min to 80 % B, hold 80 % B for 0.5 min, in 0.1 min to 5 % B, hold 5 % B for 4.4 min
Flow rate: 1.3 mL/min
Temperature: 50 °C
Detection: MS, MRM
Injection: 5 µL
Concentration: 100 ng/mL for each analyte
MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Sulfacetamide	1.24	215.2	156.2	92.1
Sulfadiazine	1.50	251.2	156.1	92.1
Sulfapyridine	1.69	250.2	156.1	92.0
Sulfatiazole	1.75	256.2	156.2	92.1
Sulfamerazine	1.89	265.1	156.1	92.1
Sulfadimidine	2.22	279.2	185.9	65.0
Sulfamethoxypyridazine	2.46	281.2	156.1	92.2
Sulfamonomethoxine	2.92	281.2	156.1	92.2
Sulfachlorpyridazine	2.96	285.2	156.1	92.1
Sulfamethoxazole	3.31	254.2	156.1	92.1
Sulfadoxine	3.72	311.1	156.1	92.1
Sulfadimethoxine	4.03	311.1	156.1	92.1
Sulfaquinoxaline	4.08	301.2	156.1	92.1



The reliable phase modification process leads to a high batch-to-batch reproducibility, where different batches show very consistent performance results. This can be shown in application 128610 with analytes of different polarities, which also demonstrate the hydrophobic properties of this C_{18} phase.

Batch-to-batch reproducibility

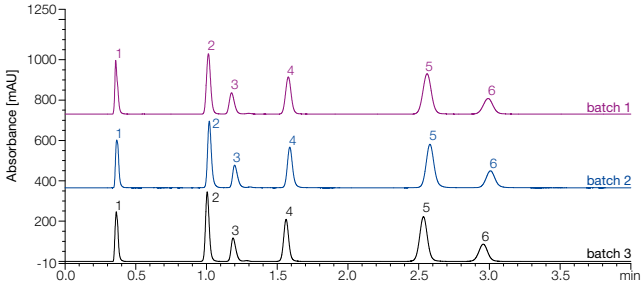
MN Appl. No. 128610

Column: 50 x 4 mm NUCLEOSHELL® Bluebird RP 18, 2.7 µm
Eluent: 25 mM ammonium dihydrogen phosphate solution – methanol (35:65, v/v), pH = 7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV, 254 nm
Injection: 5 µL
Concentration:

Uracil	45 µg/mL
Ethyl benzoate	181 µg/mL
Lidocaine	1134 µg/mL
Naphthalene	1134 µg/mL
Biphenyl	45 µg/mL
Acenaphthene	227 µg/mL

The mixture was diluted to 4 mL with water

Peaks:
1. Uracil
2. Ethyl benzoate
3. Lidocaine
4. Naphthalene
5. Biphenyl
6. Acenaphthene



NUCLEOSHELL® Bluebird RP 18

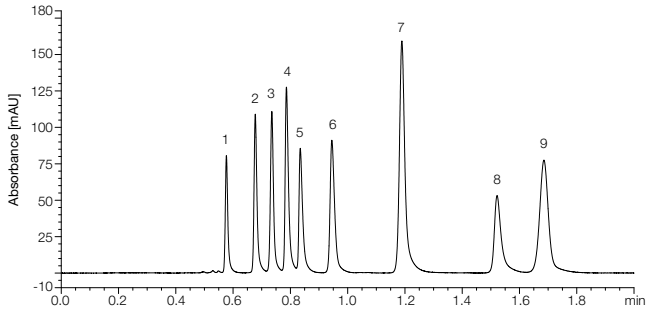
In addition even very polar organic acids can be analyzed while retaining an excellent performance on NUCLEOSHELL® Bluebird RP 18 using 100 % aqueous mobile phase.

Organic acids

MN Appl. No. 128330

Column:	150 x 4 mm NUCLEOSHELL® Bluebird RP 18, 2.7 µm
Eluent:	50 mM potassium dihydrogen phosphate solution, pH = 2.5
Flow rate:	2.0 mL/min
Temperature:	40 °C
Detection:	UV, 210 nm
Injection:	3 µL
Concentration (in water)	
Tartaric acid	135 µg/mL
Malic acid	2162 µg/mL
Shikimic acid	27 µg/mL
Lactic acid	2703 µg/mL
Acetic acid	2703 µg/mL
Citric acid	1081 µg/mL
Fumaric acid	41 µg/mL
Acrylic acid	676 µg/mL
Arbutin	216 µg/mL

Peaks:
1. Tartaric acid
2. Malic acid
3. Shikimic acid
4. Lactic acid
5. Acetic acid
6. Citric acid
7. Fumaric acid
8. Acrylic acid
9. Arbutin



Ordering information

NUCLEOSHELL® Bluebird RP 18

Analytical EC columns NUCLEOSHELL® Bluebird RP 18 (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763436.46	763438.30
150	4	2.7	763436.40	763438.30
150	3	2.7	763436.30	763438.30
150	2	2.7	763436.20	763438.20
100	4.6	2.7	763434.46	763438.30
100	4	2.7	763434.40	763438.30
100	3	2.7	763434.30	763438.30
100	2	2.7	763434.20	763438.20
50	4.6	2.7	763432.46	763438.30
50	3	2.7	763432.30	763438.30

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEOSHELL® Phenyl-Hexyl

Alternative selectivity to C_{18} phases

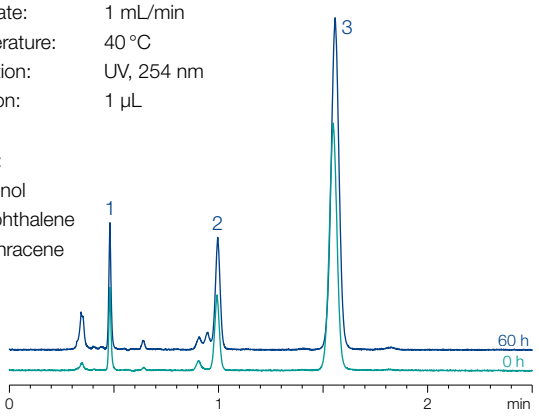
Phenylhexyl modified phases offer an excellent separation efficiency especially for aromatic and unsaturated compounds with electron-withdrawing groups. The combination of hydrophobic and π-π interactions results in an alternative and interesting selectivity profile compared to C_{18} or C_8 modifications. 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 a pH stability from 1 to 10.

Stability of NUCLEOSHELL® Phenyl-Hexyl at pH 10

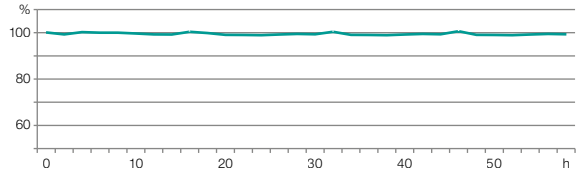
MN Appl. No. 126420

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



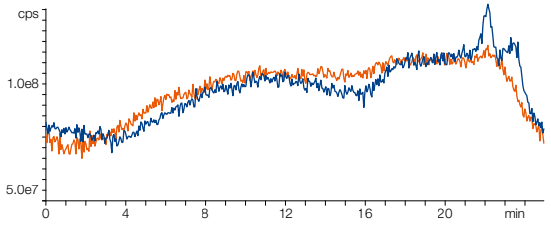
Relative plate numbers



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 phenylhexyl phases, which underlines the excellent base deactivation.

Key features

- Suitable for polar / aromatic compounds
- 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

Technical data

- Phenylhexyl phase; multi-endcapped
- Pore size 90 Å, particle size 2.7 µm; carbon content 4.5 %; pH stability 1–10

Recommended applications

- USP listing L11
- Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics

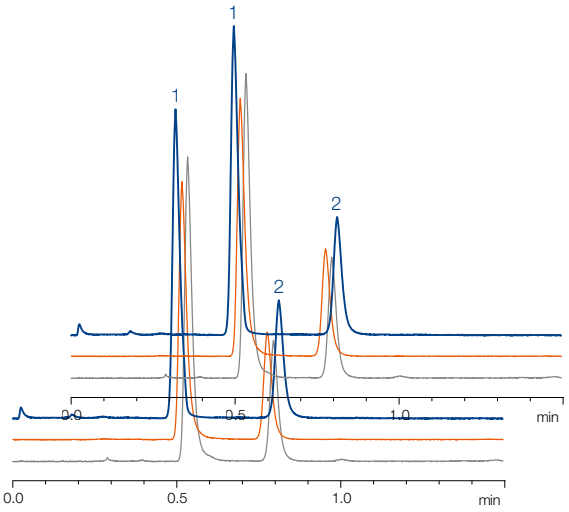
NUCLEOSHELL® Phenyl-Hexyl

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



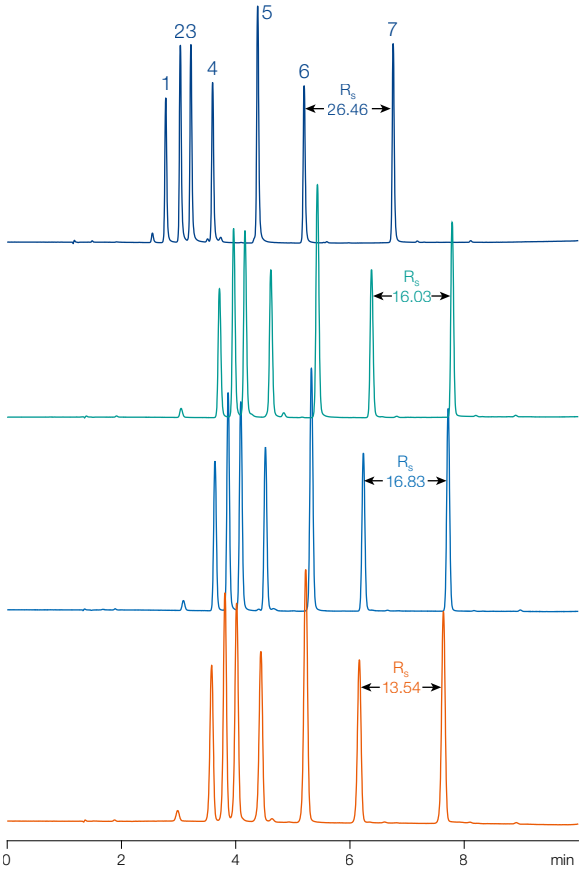
Comparing the separation of sulfonamides on NUCLEODUR® Phenyl-Hexyl with different particle sizes

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. The core-shell silica exhibits identical selectivity, narrower peaks and slightly shorter retention under the same conditions.

NUCLEOSHELL® Phenyl-Hexyl

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

Ordering information

NUCLEOSHELL® Phenyl-Hexyl				
Analytical EC columns NUCLEOSHELL® Phenyl-Hexyl (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763736.46	763738.30
150	4	2.7	763736.40	763738.30
150	3	2.7	763736.30	763738.30
150	2	2.7	763736.20	763738.20
100	4.6	2.7	763734.46	763738.30
100	4	2.7	763734.40	763738.30
100	3	2.7	763734.30	763738.30
100	2	2.7	763734.20	763738.20
50	4.6	2.7	763732.46	763738.30
50	2	2.7	763732.20	763738.20

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEOSHELL® Biphenyl

Core-Shell technology suitable for highly aqueous mobile phases

NUCLEOSHELL® Biphenyl is a biphenyl modified superficially porous silica.

The special phase modification of NUCLEOSHELL® Biphenyl with iso-butyl sidechains leads to low bleeding characteristics even at very acidic pH values compared to competitor columns (as shown in application 128780). Due to these iso-butyl sidechains and multi-endcapping procedures no phase collapse occurs and stability in 100 % aqueous mobile phase is ensured. Additionally NUCLEOSHELL® Biphenyl shows an excellent suitability for LC/MS applications.

A reliable phase modification process guarantees a high batch-to-batch reproducibility. This can be shown in application 128760 with different analytes. The separation of these compounds with various polarities demonstrates the hydrophobic as well as polar properties of this biphenyl phase.

Key features

- Enhanced retention for aromatic and unsaturated substances due to a separation principle based on 2 retention mechanisms: π-π interactions and hydrophobic interactions
- Stable in 100 % aqueous mobile phase systems
- Suitable for LC/MS due to low bleeding characteristics

Technical data

- Biphenylpropyl phase; multi-endcapped
- Pore size 90 Å; particle size 2.7 µm. carbon content 5.2 %; pH stability 1.5–8.5

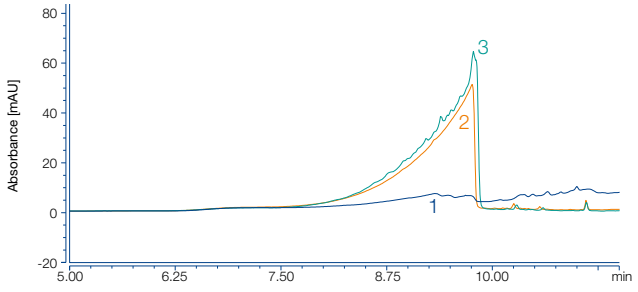
Recommended applications

- USP listing L11
- Pesticides, pharmaceuticals, mycotoxins, phthalates, hormones, DNPH aldehydes, aromatic and unsaturated compounds

Stability in acidic medium (gradient method)

MN Appl. No. 128780

Column:	100 x 3 mm NUCLEOSHELL® Biphenyl, 2.7µm
Eluent:	A) 1 % H_3PO_4 (pH = 1.2) B) acetonitrile
Gradient:	equilibration 10 min 10 % B, hold 10 % B for 5 min, from 10 % to 90 % B in 5 min, hold 90 % B for 3 min, in 1.0 min to 10 % B
Flow rate:	0.56 mL/min
Temperature:	40 °C
Detection:	UV, 254 nm
1.	NUCLEOSHELL® Biphenyl, 2.7 µm
2.	Kinetex® Biphenyl, 2.6 µm
3.	Raptor® Biphenyl, 2.7 µm



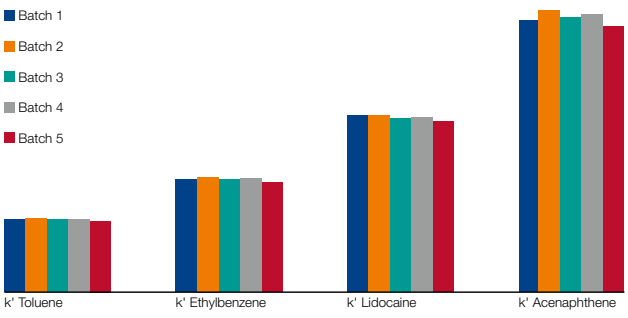
Batch-to-batch reproducibility

MN Appl. No. 128760

Column:	50 x 4 mm NUCLEOSHELL® Biphenyl, 2.7 µm
Eluent:	25 mM potassium dihydrogen phosphate solution – methanol (70:30, v/v), pH = 7.0
Flow rate:	1.0 mL/min
Run time:	10 min
Temperature:	30 °C
Detection:	UV, 254 nm
Injection:	1 µL

Concentration (in methanol)

Uracil	40 µg/mL (void volume marker)
Toluene	1250 µg/mL
Ethylbenzene	1250 µg/mL
Lidocaine	500 µg/mL
Acenaphthene	230 µg/mL



NUCLEOSHELL® Biphenyl

Phthalates

MN Appl. No. 128830

Columns: 100 x 3 NUCLEOSHELL® Biphenyl, 2.7 µm
100 x 3 NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
100 x 3 NUCLEOSHELL® PFP, 2.7 µm

Eluent: A) water
B) 0.1 % water in acetonitrile

Gradient: hold 50 % B for 1.5 min, in 6.0 min to 95 % B, hold 95 % B for 3.5 min, in 2.0 min to 50 % B, hold 50 % B for 4.5 min

Flow rate: 1.0 mL/min

Temperature: 30 °C

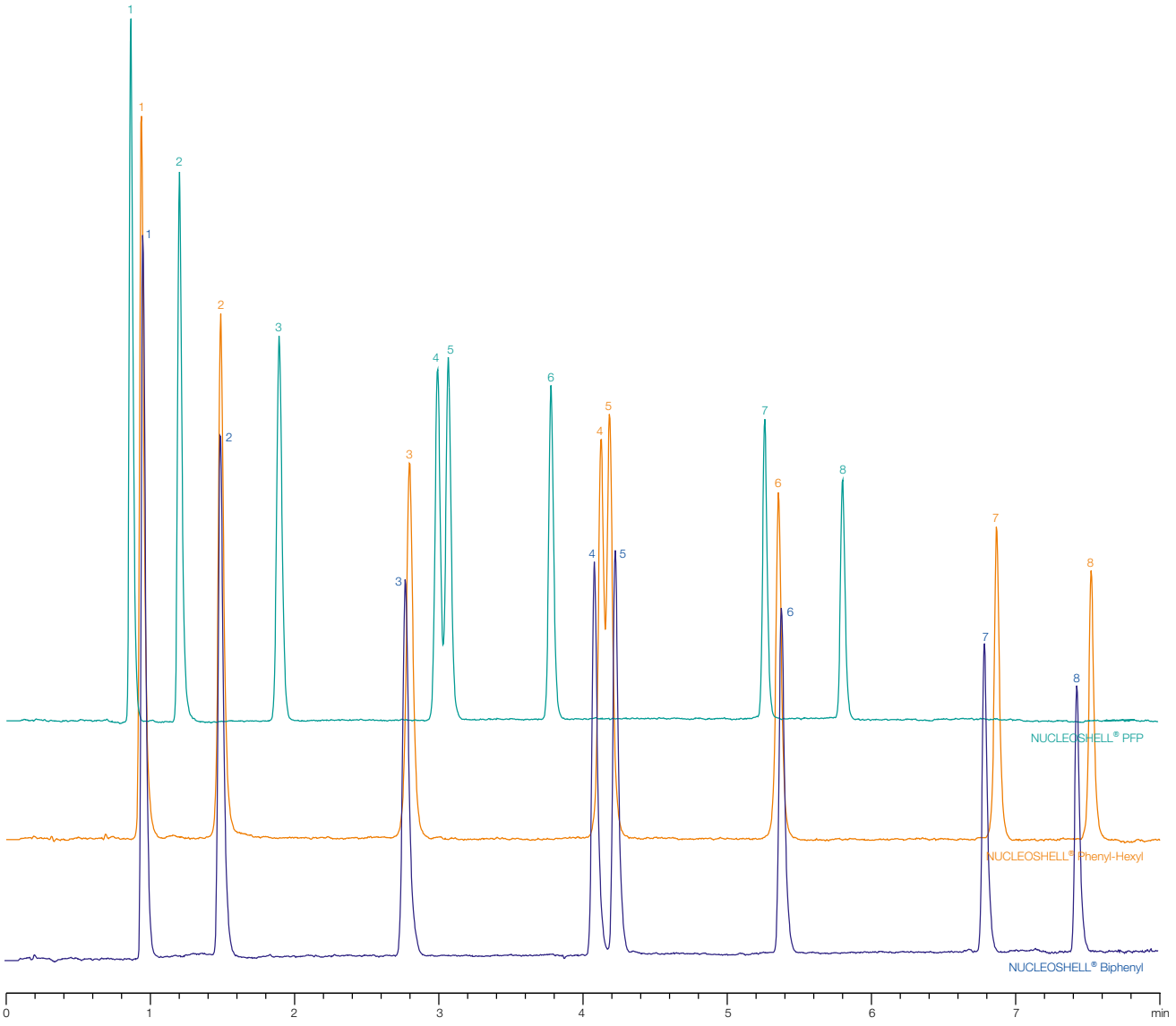
Detection: UV, 228 nm

Injection: 5 µL

Concentration: 10.0 ng/mL for each analyte in water – acetonitrile (1:1, v/v)

Retention times

	Analyte	Biphenyl RT [min]	Phenyl-Hexyl RT [min]	PFP RT [min]
1	Dimethyl phthalate	0.96	0.94	0.86
2	Diethyl phthalate	1.50	1.49	1.20
3	Dipropyl phthalate	2.87	2.80	1.89
4	Dibutyl phthalate	4.09	4.13	2.99
5	Benzyl butyl phthalate	4.24	4.19	3.07
6	Dicyclohexyl phthalate	5.39	5.36	3.78
7	Diheptyl phthalate	6.80	6.87	5.26
8	Dioctyl phthalate	7.44	7.53	5.80



Compared to other aryl HPLC modifications NUCLEOSHELL® Biphenyl shows more pronounced π-π interactions. In application 128830 NUCLEOSHELL® Biphenyl is able to separate the critical analyte pair dibutyl phthalate and benzyl butyl phthalate whereas other aryl phases cannot achieve a baseline separation.

NUCLEOSHELL® Biphenyl

Ordering information

NUCLEOSHELL® Biphenyl				
Analytical EC columns NUCLEOSHELL® Biphenyl (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763636.46	763638.30
150	4	2.7	763636.40	763638.30
150	3	2.7	763636.30	763638.30
150	2	2.7	763636.20	763638.20
100	4.6	2.7	763634.46	763638.30
100	4	2.7	763634.40	763638.30
100	3	2.7	763634.30	763638.30
100	2	2.7	763634.20	763638.20
50	3	2.7	763632.30	763638.30
50	2	2.7	763632.20	763638.20

*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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MN Application database
with a new design

The MN chromatography application database

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- ChromaAppDB.mn-net.com

NUCLEOSHELL® PFP

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 modiication (PFP or F5). 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.

Key features

- 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)
- Suitable for LC/MS

Technical data

- Phase with pentafluorophenylpropyl phase; multi-endcapped
- Pore size 90 Å, particle size 2.7 µm; carbon content ~ 3 %; pH stability 1–9;

Recommended applications

- USP listing L43
- Aromatic and unsaturated compounds, phenols, halogen compounds, isomers, polar compounds like pharmaceuticals, antibiotics; strong retention of basic compounds

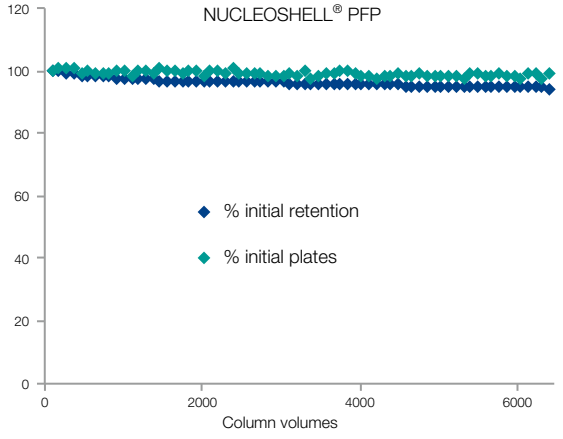
Good to know

- 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.

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



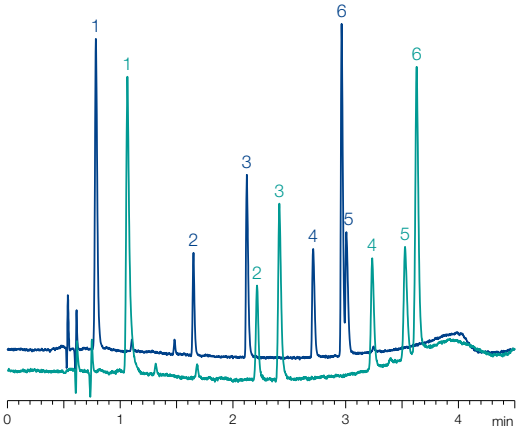
β-Blockers · orthogonal selectivity of NUCLEOSHELL® PFP

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



NUCLEOSHELL® PFP

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

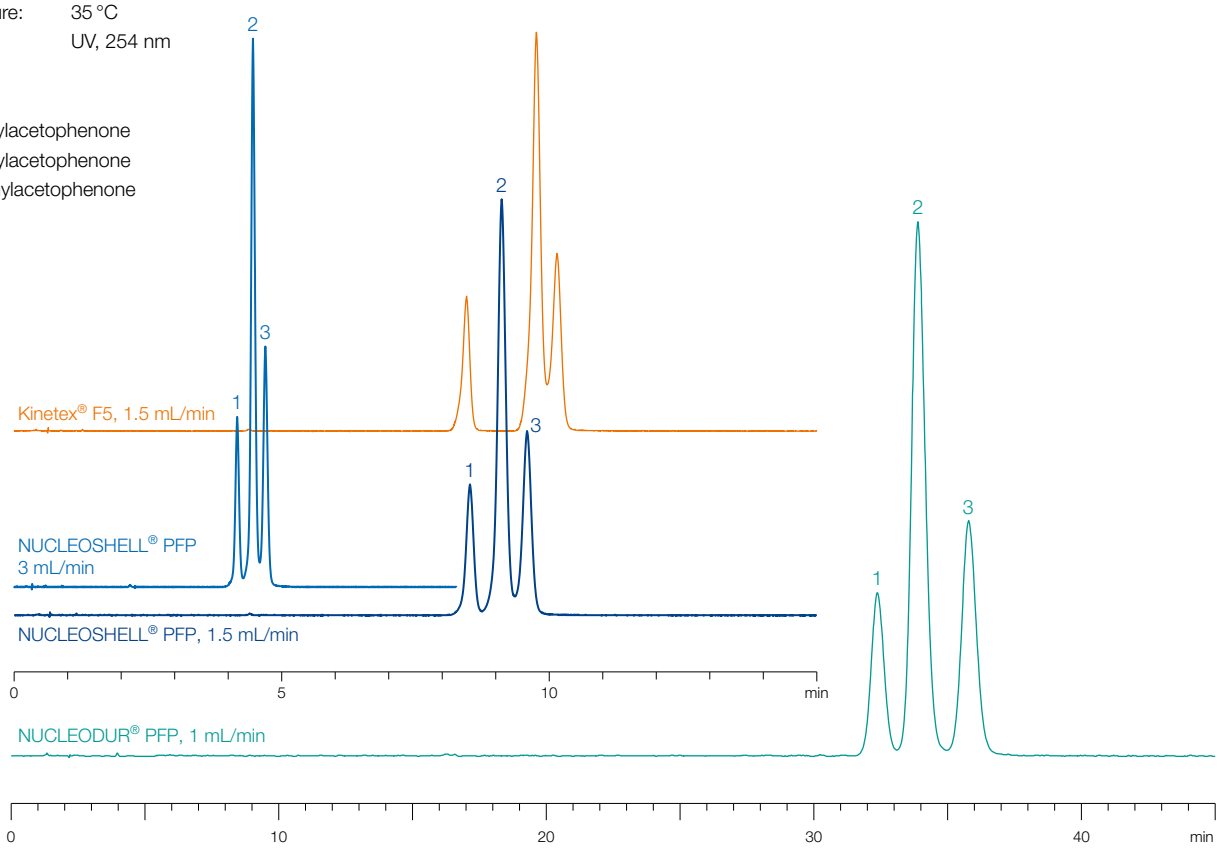
Eluent: Methanol – water (35:65, v/v)

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



Ordering information

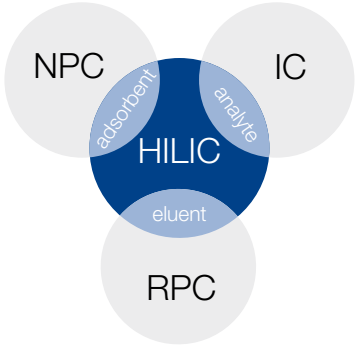
NUCLEOSHELL® PFP				
Analytical EC columns NUCLEOSHELL® PFP (pack of 1)				
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763536.46	763538.30
150	4	2.7	763536.40	763538.30
150	3	2.7	763536.30	763538.30
150	2	2.7	763536.20	763538.20
100	4.6	2.7	763534.46	763538.30
100	4	2.7	763534.40	763538.30
100	3	2.7	763534.30	763538.30
100	2	2.7	763534.20	763538.20
50	3	2.7	763532.30	763538.30
50	2	2.7	763532.20	763538.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

NUCLEOSHELL® HILIC

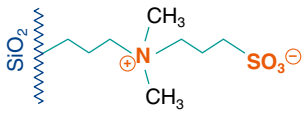
Hydrophilic interaction chromatography



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 will not 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. 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.

Key features

- Ideal for reproducible and stable chromatography of highly polar analytes
- Very short column equilibration times
- Suitable for LC/MS

Technical data

- Zwitterionic ammonium-sulfonic acid phase; not endcapped
- Pore size 90 Å, particle size 2.7 µm; carbon content 1.3 %; pH stability 2–8.5

Recommended applications

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

Good to know

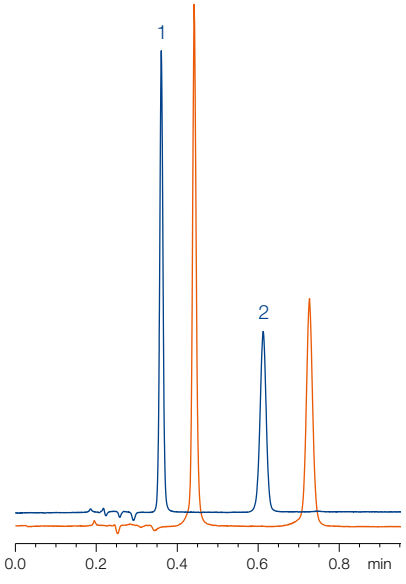
NUCLEODUR® HILIC is a patented phase modification (pat. number DE102009006007 (B4))

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



NUCLEOSHELL® HILIC

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 %.

Good to know

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

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)

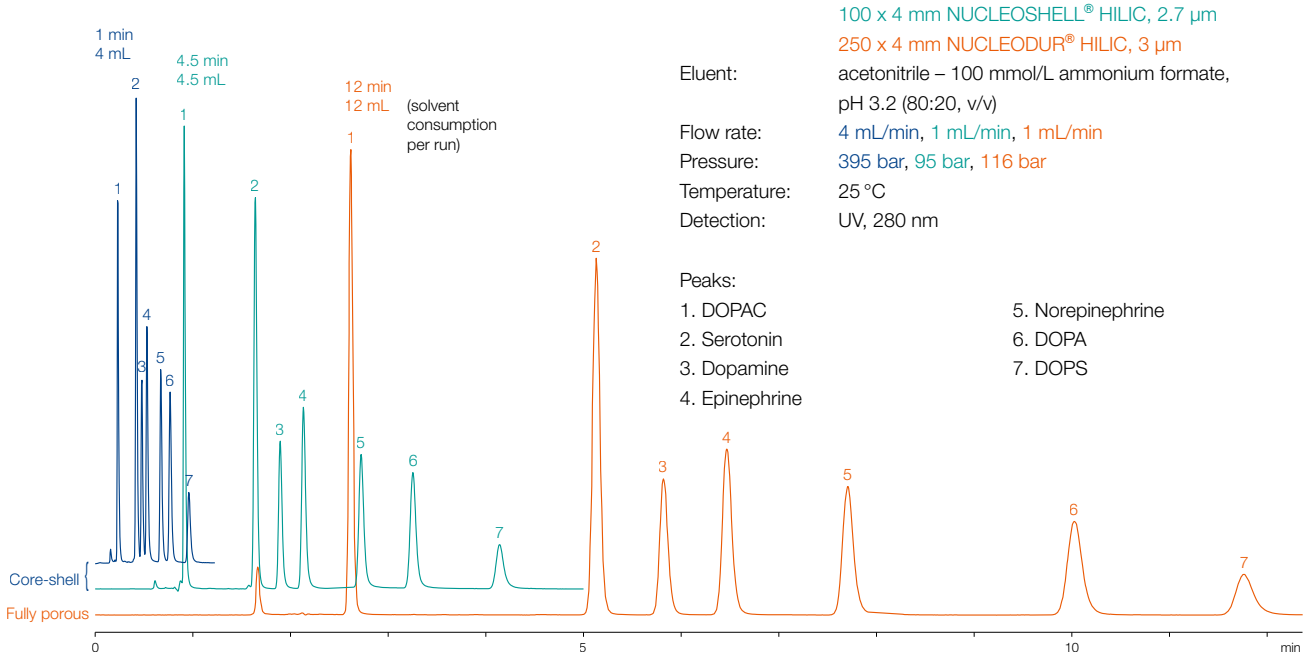
Flow rate: 4 mL/min, 1 mL/min, 1 mL/min

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



Ordering information

NUCLEOSHELL® HILIC

Analytical EC columns NUCLEOSHELL® HILIC (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
150	4.6	2.7	763336.46	763338.30
150	4	2.7	763336.40	763338.30
150	3	2.7	763336.30	763338.30
150	2	2.7	763336.20	763338.20
100	4.6	2.7	763334.46	763338.30
100	3	2.7	763334.30	763338.30
100	2	2.7	763334.20	763338.20
50	4	2.7	763332.40	763338.30
50	3	2.7	763332.30	763338.30
50	2	2.7	763332.20	763338.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

For more products and information
Or visit *www.mn-net.com*

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 × 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® spherical silica and NUCLEOSHELL® spherical core-shell silica particles

Good to know

NUCLEODUR® and NUCLEOSHELL® column heads must not be removed!

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 note that not all phase modifications and particle sizes are available in every possible dimension.

Guard columns for EC columns

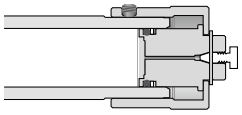
EC column with ID	EC guard column*
2 mm	4/2
3 mm	4/3
4 mm	4/3
4.6 mm	4/3
Pack of 3 cartridges	

*Information about the Column Protection System on page 90.

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
- Can be packed with all NUCLEODUR® spherical silicas
- Stainless steel columns are most frequently used in HPLC.

Available standard dimensions of VarioPrep columns

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

* 10 × 8, 10 × 16, 15 × 32 and 15 × 50 mm ID columns are used as guard columns and require the respective holders, see page 91.

Basics of preparative HPLC

In principal for preparative HPLC the same rules apply than for analytic HPLC. However, both differ significantly in their aim. The aim of analytic HPLC is a preferably complete separation of the single components of a mixture with subsequent peak identification. In contrast the goal of preparative HPLC is isolation of the desired product in defined purity, maximum amount while having a cost effective method of operating.

Upscaling table for common MN column dimensions

ID × Length [mm]	4 × 250	8 × 250	10 × 250	16 × 250	21 × 250	32 × 250	40 × 250	50 × 250	80 × 250
Linear scale-up factor	1	4	6.25	16	27.6	64	100	156.3	400
Typical amount of sample* [mg]	0.02–2	0.08–8	0.13–13	0.3–35	0.6–60	1.3–130	2–210	3–350	10–850
Typical flow rate [mL/min]	0.5–1.5	2–6	3–9	8–24	14–40	32–96	50–150	80–250	200–600

* based on RP material; the herein stated maximum amounts of sample are dependent on the separation problem and the sample. In some cases half the maximum amount of sample can already lead to a drastic overload of the column, in other cases the maximum amount of sample still leads to an acceptable separation.

Column protection system for analytical HPLC columns

Innovative and universal guard column holder system

- Suitable for all analytical HPLC columns with 1/16" fittings
- Cartridges filled with special NUCLEODUR® 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)
- 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

Good to know

UNIVERSAL RP guard columns are suitable for all HPLC columns under RP conditions

Ordering information

Product	Pack of	REF
Column Protection System, consisting of 1 × guard column holder, 2 × capillaries (ID 0.12 mm), 3 × ferrules (for HPLC columns with particle size > 2 µm), 3 × ferrules (for HPLC columns with particle size < 2 µm), 2 × wrenches (wrench size: 12 and 14 mm)	1	718966
Replacement parts for the Column Protection System		
Special ferrules made of PEEK for HPLC columns with particle size > 2 µm	5	718967
Special ferrules made of PEEK for HPLC columns with particle size < 2 µm	5	718963
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 low rates), nuts and metal ferrules	3	718971
Wrench (size 12 and 14 mm)	1	718970
Universal RP guard columns		
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

Column protection systems for preparative HPLC columns

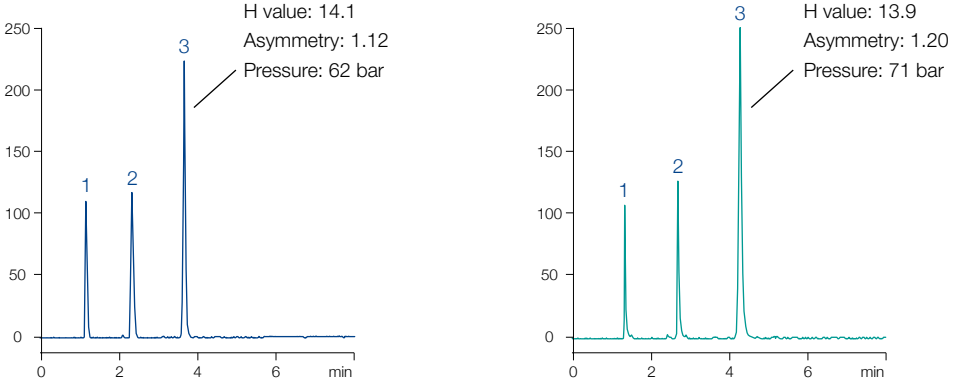
Improved guard column systems for (semi-)preparative HPLC

- Easy handling and cartridge exchange
- Robust stainless steel hardware with 1/16" thread
- 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® C18 HTec, 5 µm
125 x 16 mm NUCLEODUR® C18 HTec, 5 µm + 10 x 16 mm NUCLEODUR® C18 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

Guard column holders for VarioPrep columns

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

	8, 10 mm	16, 21 mm	32, 40 mm	≥ 50 mm	Holder ID	Guard columns pack of	Holder REF	Replacement O-Ring REF
VP	10/8				8 mm	2	718251	718975
VP		10/16			16 mm	2	718256	718976
VP			15/32		32 mm	1	718253	718977
VP				15/50	50 mm	1	718255	718978

For REF numbers of individual VP guard column cartridges and VP columns please visit our website: *www.mn-net.com/chromatography*.

Accessories

Accessories for stainless steel HPLC columns

- Stainless steel accessories are corrosion resistant, pressure stable and easy to work mechanically
- Suitable for HPLC columns with 1/16″ connections

Ordering information

Description	Pack of	REF
Capillary accessories		
1/16″ column end caps (plastic)	4	718582
1/16″ nut for connecting 1/16″ capillaries	5	718583
1/16″ ferrule	5	718584
Capillary unions		
Typ 1: 100 mm × 1/16″ × 0.25 mm	1	718637
Typ 2: 100 mm × 1/16″ × 0.12 mm	1	719489
Cutter for 1/16″ capillary tubing	1	706290

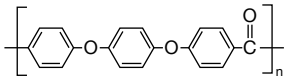
For accessories and replacement parts for EC columns see page 90, for accessories and replacement parts for VarioPrep columns see page 91.

Accessories

PEEK accessories

- PEEK (polyether ether ketone) is a high performance polymer belonging to the group of polyarylether ketones (PAEK), which meets all requirements of HPLC columns with respect to chemical resistance and mechanical stability. In some fields of application in HPLC like, e.g., ion chromatography and chromatography of biopolymers, PEEK fulfils the requirements for a nonmetallic material
- All fittings can be tightened by hand.

PEEK



Ordering information

PEEK fittings	Pack of	REF
1/16″ PEEK fingertight fitting 1-part combination nut + ferrule with MACHEREY-NAGEL logo	5	718778
1/16″ PEEK fitting 1-part combination nut + ferrule	1	718770
1/16″ PEEK fingertight Nut	1	718771
1/16″ PEEK ferrule for REF 718771	1	718772
1/16″ PEEK double ferrule	1	718775
1/16″ PEEK union, both sides inner threads, equipped with 2 fingertight nuts and double ferrules	1	718766
1/16″ PEEK union, both sides inner threads, without nuts and without ferrules	1	718767
1/16″ PEEK union, both sides outer diameters	1	718768

PEEK standard capillaries				
AD	ID (mm)	Length (m)	Pack of	REF
1/16″	0.13	1	1	718765
1/16“	0.17	1	1	718760
1/16“	0.25	1	1	718761
1/16“	0.5	1	1	718762
1/16“	0.75	1	1	718763

Tools for PEEK capillaries	Pack of	REF
Guillotine cutter for PEEK and PTFE	1	718769
Clean-Cut cutter for different capillary outer diameters	1	718755

List of abbreviations and trademarks

List of abbreviations

%C	carbon content in percent
Å	angstrom = 0.1 nm = 1.0 × 10^{-10} m
ACN	acetonitrile
BDS	base deactivated octadecylsilan (C_{18})
BET	analytical method for determination of surfaces size (developer: Stephen Brunauer, Paul Hugh Emmett and Edward Teller)
BTEX	aromatic hydrocarbons: benzene, toluene, ethyl benzene and xylene
BTX	sum parameter for volatile aromatic hydrocarbons
DIN	German Institute for Standardization
EC	column hardware for analytical columns in HPLC
ec	endcapping or endcapped
EP	European Pharmacopoeia (Ph. Eur., PharmEurl., etc.)
EPA	US Environmental Protection Agency
HEPT	height equivalent to a theoretical plate
HILIC	hydrophylic interaction chromatography
HPLC	high performance liquid chromatography
ID	internal diameter
ISO	International Organization for Standardization
MS	mass spectrometry (suitable)
nm	nanometer = 1.0 × 10^{-9} m
NP	normal phase
ODS	octadecylsilan (C_{18})
PA	polyamide, nylon
PAH	polycyclic aromatic hydrocarbons
ppb	parts per billion (1 per 1000000000 = 10^{-9})
ppm	parts per million (1 per 1000000 = 10^{-6})
REF	reference number, article number, product number, ordering number
RI	refractive index
RP	reversed phase
SiOH	silanol, unmodiied silica
SPE	solid phase extraction
THC	tetrahydrocannabinol
THF	tetrahydrofuran
TLC	thin layer chromatography
TOC	total organic carbon
UHPLC	ultra HPLC, high separation performance by < 2 µm particles or core-shell technology
UPLC	see UHPLC, but protected term of the company Waters Corporation (USA)
USP	United States Pharmacopeia
VP	column hardware for preparative columns in HPLC

Trademarks

MACHEREY-NAGEL Trademarks	
CHROMABOND	columns for solid phase extraction (SPE)
CHROMAFIL	syringe filters (membrane filters)
NUCLEODUR	spherical high purity silica for HPLC
NUCLEOSHELL	core-shell silica phases for HPLC
NUCLEOSIL	spherical standard silica for HPLC
OPTIMA	fused silica high performance capillary columns with immobilized phases

Registered trademarks (®)	
Acquity	Waters Corp. (USA)
Agilent	Agilent Technologies Inc. (USA)
Allure	Restek Corp. (USA)
Aqua	Phenomenex Inc. (USA)
Ascentis	Sigma-Aldrich Co. (USA)
Atlantis	Waters Corp. (USA)
Gemini	Phenomenex Inc. (USA)
HALO	Advanced Material Technology Inc. (USA)
Hypersil	Thermo Fisher Scientific Inc. (USA)
HyPurity	Thermo Fisher Scientific Inc. (USA)
Inertsil	GL Sciences (Japan)
Kromasil	Eka Chemicals AB (Sweden)
LiChrospher	Merck KGaA (Germany)
Luna	Phenomenex Inc. (USA)
Polaris	Agilent Technologies Inc. (USA)
ProntoSil	Bischof Chromatography (Germany)
Purospher	Merck KGaA (Germany)
Shim-pack Velox	Shimadzu Corp. (Japan)
Spherisorb	Waters Corp. (USA)
Superspher	Merck KGaA (Germany)
Symmetry	Waters Corp. (USA)
Synergi	Phenomenex Inc. (USA)
Xterra	Waters Corp. (USA)
YMC	YMC Co. Ltd. (Japan)
ZIC Merck	Sequant AB (Sweden)
Zorbax	Agilent Technologies Inc. (USA)

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Hypersil	Thermo Fisher Scientific Inc. (USA)
HyPURITY	Thermo Fisher Scientific Inc. (USA)
Kinetex	Phenomenex Inc. (USA)
Obelisc	Sielc Technologies (USA) Ostro Waters Corp. (USA)
Poroshell	Agilent Technologies Inc. (USA)
Sequant	Merck Sequant AB (Sweden)
SunFire	Waters Corp. (USA)
SymmetryShield	Waters Corp. (USA)

Disclaimer and product use restriction

Disclaimer

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Regarding these products or services we can not grant any guarantees regarding selection, efficiency or operation.

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MACHEREY-NAGEL chromatography products are intended, developed, designed and sold for research and development purposes and analytical quality control / routine measurements only, except, however, any other function of the product being expressly set forth in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for general laboratory use only!

MACHEREY-NAGEL products are suited for qualified personnel only!

MACHEREY-NAGEL products shall in any event be used wearing adequate protective clothing.

For detailed information please refer to the respective Material Safety Data Sheet of the product!

MACHEREY-NAGEL products shall exclusively be used in an adequate test environment.

MACHEREY-NAGEL does not assume any responsibility for damages due to improper application, abuse, misuse, storage or maintance of our products. Prior to application the user has to read carefully and understand the instruction or product leaflets included in the product package (if applicable or available on the webpage) - in case of any doubts the customer has to contact MACHEREY-NAGEL.

Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

The user has to ensure that the products used are suitable for the intended application.

MACHEREY-NAGEL does not warrant the reproducibility of published applications.

Literature

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