Chromatography

MACHEREY-NAGEL NUCLEODUR® and NUCLEOSHELL®



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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.

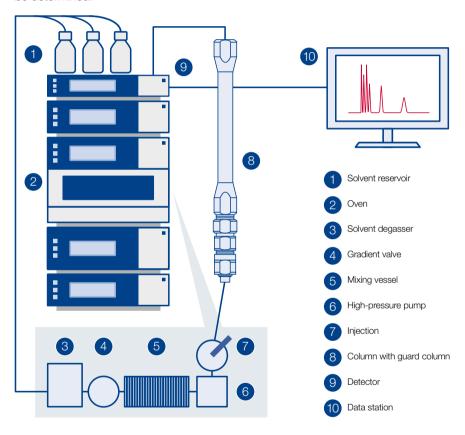
Basic principle

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

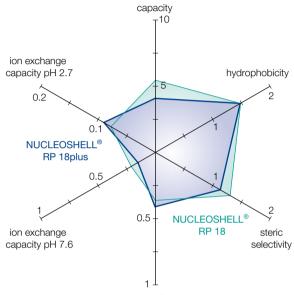
While flowing through the column each component of the solved mixture interacts differently with the stationary phase. According to the characteristics of the substance (hydrophobic, polar, ionic, aromatic, sterically hindered etc.) the strength of the interactions vary and thus the compounds are retained 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, NH₂) non-polar eluents like *n*-heptane, hexane, dichloromethane or 2-propanol are applicable. While for reversed phases (e.g., C₁₈, C₈, C₄, C₂, C₆H₅) 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.

Basics

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].



hydrogen bonding capacity

Parameter of the Tanaka diagram:

Capacity = k' (pentylbenzene)

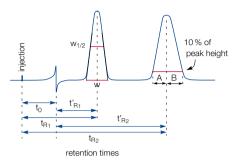
Hydrophobicity = α (pentylbenzene, butylbenzene) Steric selectivity = α (triphenyl, o-terphenyl)

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® 18 plus for example shows a lower ion exchange capacity at pH 7.6 for the monomeric NUCLEOSHELL® RP 18 plus. 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_{1/6} peak width at half height peak width of the peak (intersection point of the inflectional tangents with the zero line) Peak symmetry: Α peak front to peak maximum at 10% of peak height В peak maximum to peak end at 10 % of peak height Retention time: t_{o} 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 $t_{\rm R1}$ or $t_{\rm R2}$. The dead time $t_{\rm 0}$ 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 $t'_{\rm R1}$ or $t'_{\rm R2}$, which is the time a sample component remains in the stationary phase.

$$t'_{B1} = t_{B1} - t_0$$
 bzw. $t'_{B2} = t_{B2} - t_0$

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'.

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

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.

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

Basics

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

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

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

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.

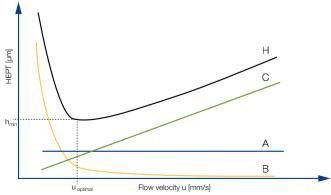
$$N = 5.54 \cdot \left(\frac{t_{R1}}{w_{1/2}}\right)^2$$

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

Basics

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

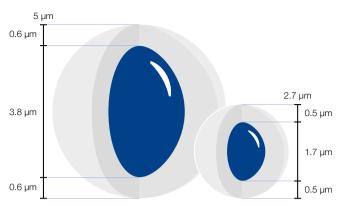
- Our facilities are ISO 9001 certified
- Perfect reproducibility from batch-to-batch and within each lot
- Individually tested columns, supplied with test chromatogram and conditions



USP listing

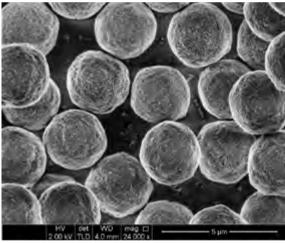
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Code	Specification	MN HPLC Phases	Page
USP L1	octadecyl silane chemically bonded to porous silica particles 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® C18 ec	38
	monolithic sinca ger	NUCLEODUR® C18 Gravity	18
		NUCLEODUR® C18 Gravity-SB	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 μ m 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	NUCLEODUR® PFP	32
	diameter	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

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 $< 2 \, \mu m$ 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 μ m diameter and a porous outer shell of 0.5 μ m thickness. Accordingly, the total diameter of the particle is 2.7 μ m.

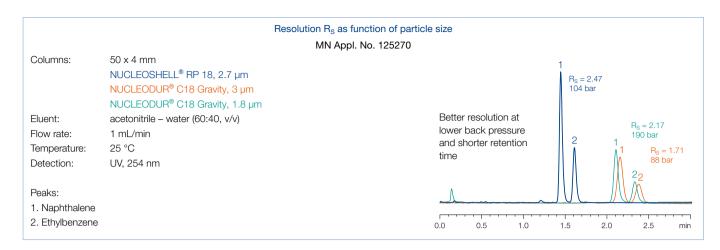
Utilizing a proprietary process of synthesis, NUCLEOSHELL® particles exhibit a distinct narrow particle size distribution (d90/d10 ~ 1.1). Columns packed with NUCLEOSHELL core shell particles feature exceptional separation efficiencies with theoretical plate numbers easily comparable to totally porous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

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

 $\begin{aligned} R_s &= \text{resolution, } \alpha = \text{selectivity (separation factor), } k_i \\ ^\prime &= \text{retention} \\ N &= \text{plate number with } N \propto 1/d_P, \ d_P = \text{particle diameter} \end{aligned}$

Key features

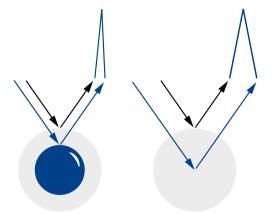
- Solid core of silicon dioxide, homogeneous shell of porous silica
- Highest efficiency compared to traditional totally porous materials
- Pore size 90 Å; particle size 2.7 µm (core 1.7 µm) and 5 µm (core 3.8 µm); specific surface 130 (2.7 µm) and 90 (5 µm) m²/g lower back pressure enables use on conventional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



Theoretical column efficiency (optimal conditions)								
Silica	d _ρ [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.

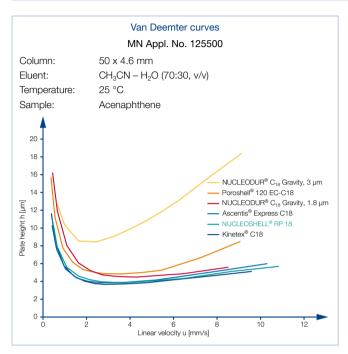
In comparison with fully porous silicas, core-shell particles from various manufacturers maintain the efficiency optimum (max. plates/m) over a long range of increasing linear mobile phase velocity.

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 $(d_{90}/d_{10} \sim 1.1)$
- Stable packing
- High heat transfer
 - · Minimized influence of frictional heat
- Efficiency of NUCLEOSHELL®
- $\sim 250~000~m^{-1}~(HETP \sim 4~\mu m)$

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

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



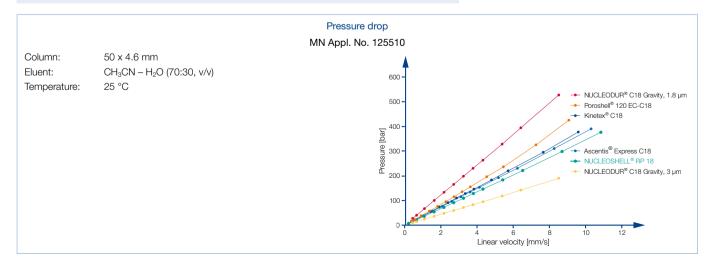
In direct comparison with conventional sub 2 micron phases, NUCLEOSHELL® columns only generate about 60 % of the back pressure and can be operated with the majority of conventional HPLC systems. In order to develop the maximum performance of NUCLEOSHELL® columns, we recommend reducing extra column voids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

$\Delta_{p} = \frac{\Phi \cdot L_{C} \cdot \eta \cdot u}{d_{p}^{2}}$

 $\Delta_P = \text{pressure drop}, \ \Phi = \text{flow resistance (non-dimensional)}, \ LC = \text{column length}, \ \eta = \text{viscosity}, \ u = \text{linear velocity}, \ d_P = \text{particle diameter}$

Good to know

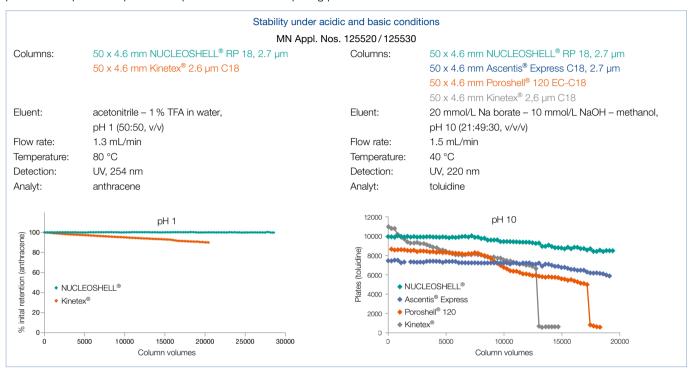
Core-shell particle technology from MACHEREY-NAGEL is an alternate route to gain highest column efficiency and resolution in HPLC at short run time, but with moderate back pressure.



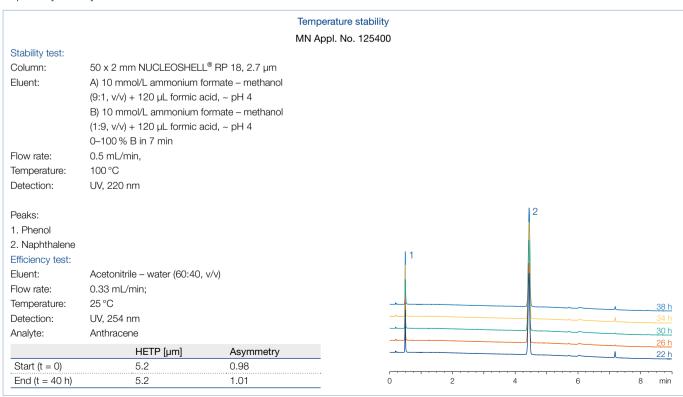
Features of NUCLEOSHELL® particles

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

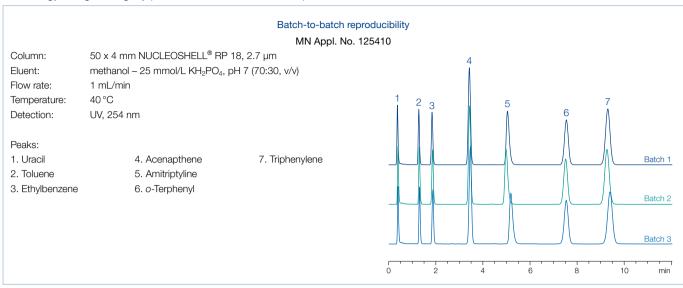
The following figure shows a column stability test of NUCLEOSHELL® RP 18 at mobile phase levels pH 1 and pH 10 compared with three competing phases.



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



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



CHROMAFIL® syringe filters Protecting your columns from solid contamination



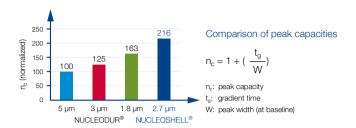
Ideal for filtration of GC and (U)HPLC sample solutions

- 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.



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

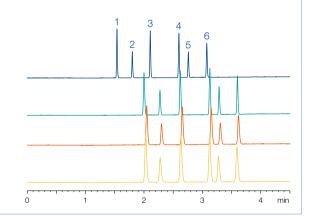
Eluent: A) acetonitrile, B) water, 40-100 % A in 4 min

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

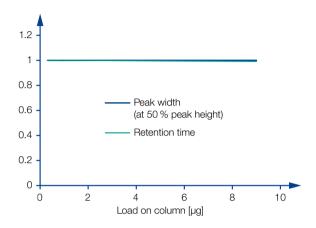
	Max. pressure [bar]	Resolution (4, 5)
NUCLEOSHELL®, 2.7 μm	255	5.45
NUCLEODUR®, 1.8 μm	450	4.14
NUCLEODUR®, 3 μm	214	2.97
NUCLEODUR®, 5 μm	142	2.30



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



Column: 50 x 3 mm NUCLEOSHELL $^{\otimes}$ RP 18, 2.7 μ m

Eluent: acetonitrile – 25 mmol/L KH_2PO_4 , pH 3

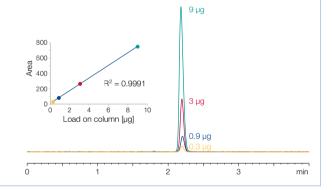
(70:30, v/v)

Flow rate: 0.66 mL/min
Temperature: 30 °C
Detection: UV, 285 nm

Peaks:

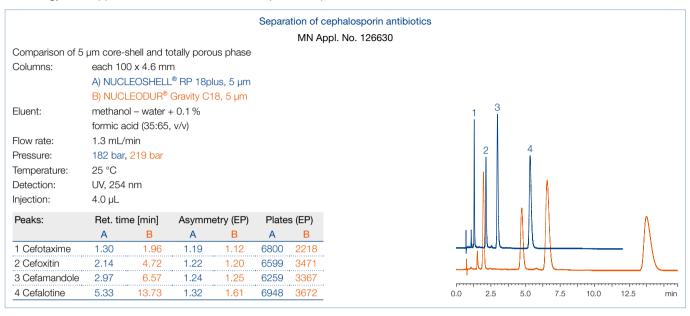
1. Valerophenone

Loading capacity



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.



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	Structi	ure
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	NOOLOGAHELL HP 78 HP
RP 18plus	octadecyl (monomeric), multi-endcapping 5.7 % C (2.7 µm particles) 4.4 % C (5 µm particles) USP L1	72	A	pH 2–9, suitable for LC/MS	NUCLEOSHELL® (Si-O ₂) _n	NUOLEOSHELL® PRINCEOSHELL® PRINCEOSHELL®
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	NOCEOGRAFIEL® Business Services Servic
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	NUCLEOSHELL® PRENYHOWI SE SE THE SE
Biphenyl	biphenylpropyl, multi-endcapping 5.2 % C (2.7 μm particles) USP L11	81	A	stable in 100 % aqueous eluent, pH 1.5–8.5, suitable for LC/MS	NUCLEOSHELL (Si-O ₂) _n	NOCLEGS-FELL® Behrery
PEP	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	NVOLEOSKELL® P P P P P P P P P P P P P P P P P P P
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	NOOLOG Self. -S-O-H

^{*} A = \bigcirc hydrophobic selectivity, B = \bigcirc polar/ionic selectivity, C = \bigcirc steric selectivity *** phases which provide a similar selectivity based on chemical and physical properties

NUCLEOSHELL® phase overview

Application	Similar phases**	Interactions · retention med	chanism
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)	Si(CH ₃) ₃ H ₃ C N N N N N N N N N N N N N
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)	Si-O _{Si(CH₃)₃ H₃C - O₄}
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)	OH CH ₃
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	O ₂ N
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	O ₂ N
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	F F H
hydrophilic compounds such as organic polar acids and bases, polar natural compounds	-	ionic/ hydrophilic and electrostatic	CH ₃ SO ₃ CH ₃ NH H ₃ C CH ₃ SO ₃ NH NH ₂ CH ₃ SO ₃ NH

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.

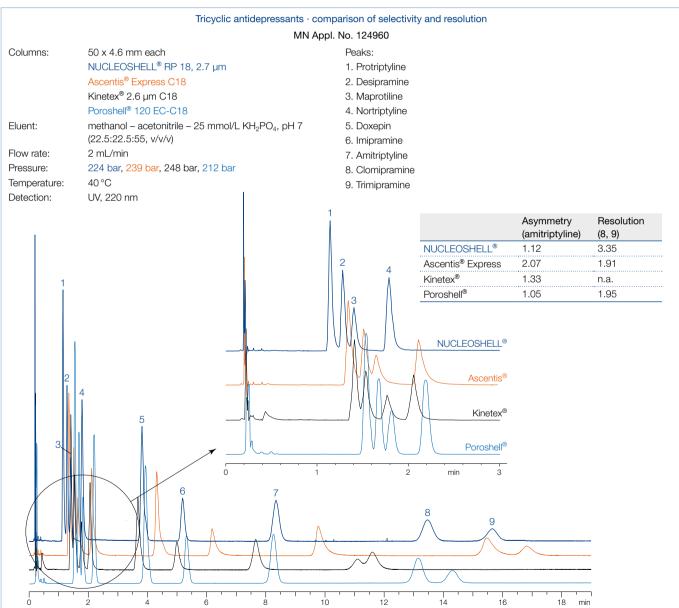
NUCLEOSHELL® RP 18 combines innovative silica technology and excellent surface deactivation that outperforms conventional C_{18} silicas in terms of efficiency, resolution, and speed.

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



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.



MN Appl. No. 124940

 $50 \times 4 \text{ mm NUCLEOSHELL}^{\$} \text{ RP } 18, 2.7 \text{ }\mu\text{m}$ Columns:

150 x 4 mm NUCLEODUR® C18 Gravity, 5 µm

Eluent: A) acetonitrile B) 20 mmol/L KH₂PO₄, 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)

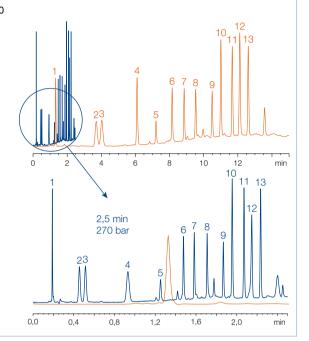
Flow rate: 2 mL/min, 1 mL/min 270 bar, 110 bar Pressure:

Temperature: 25 °C Detection: UV, 220 nm

Peaks:

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

7. Cephalothin



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.

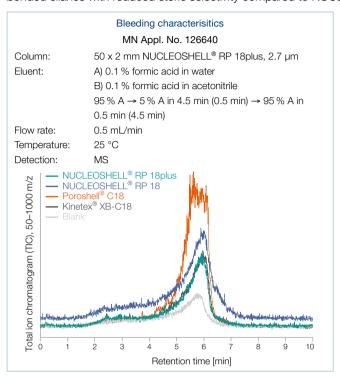
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NUCLEOSHELL® RP 18plus

Hydrophobic phase with polar selectivity

NUCLEOSHELL® RP 18 plus is a C₁₈ 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

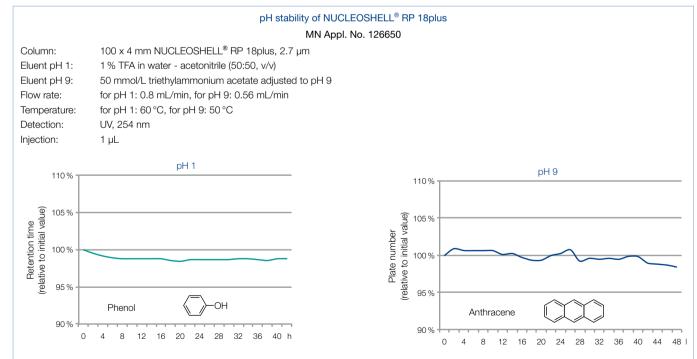
- C₁₈ phase with polar selectivity
- Hydrophobic C₁₈ phase with distinct polar selectivity ideal for method development and suitable for LC/MS
- Excellent performance under highly aqueous conditions

Technical data

- Monomeric octadecyl phase; multi-endcapped
- Pore size 90 Å, available particle sizes $2.7~\mu m$ and $5~\mu m$, carbon content 5.7~% for $2.7 \mu m$, 4.4 % for $5 \mu m$; 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



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

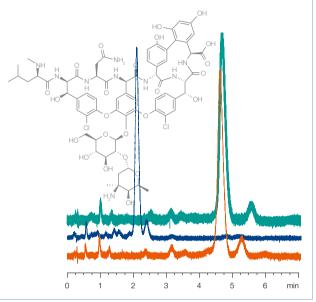
> NUCLEOSHELL® RP 18plus, 2.7 µm NUCLEOSHELL® RP 18, 2.7 µm

Kinetex® 2.6 µm C18

water - methanol - acetonitrile - glacial acetic acid (100:8:2:0.3, Eluent:

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 18 plus 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 18 plus 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.

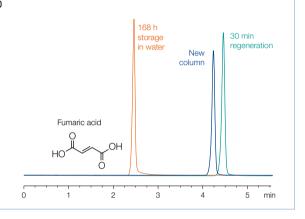
Phase collapse and regeneration

MN Appl. No. 126670

Column: 100 x 4 mm NUCLEOSHELL® RP 18plus, 2.7 µm

Eluent: 20 mmol/L KH₂PO₄, pH 2.6

0.5 mL/min Flow rate: Temperature: 20 °C Detection: UV, 215 nm Injection: 0.5 µL



Ordering information

NUCLEOSHELL® RP18 plus							
Analytical EC co	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.

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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 C₁₈ 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

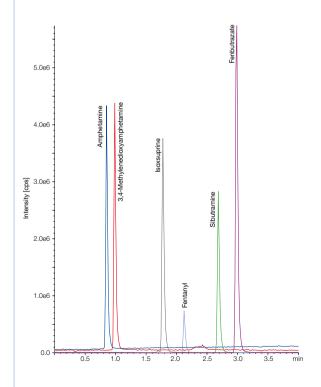
Gradient: in 4.5 min from 5 % to 90 % B, hold for 0.5 min, in 0.5 min

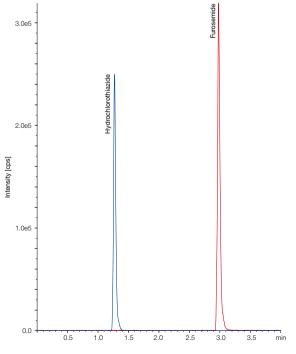
to 5 % B, hold 0 % B for 4.5 min

Flow rate: 1.3 mL/min 30 °C Temperature: MS, SMRM Detection: Injection:

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 $^{\odot}$ Bluebird RP 18, 2.7 μ m

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

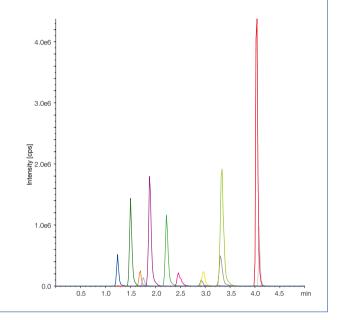
Flow rate: 1.3 mL/min
Temperature: 50 °C
Detection: MS, MRM
Injection: 5 µL

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

Analyte	RT [min]	[M+H] ⁺	Q ₁ (Quantifier)	Q ₂ (Qualifier)
Sulfadimethoxine	4.03	311.1	156.1	92.1
Sulfaquinoxaline	4.08	301.2	156.1	92.1

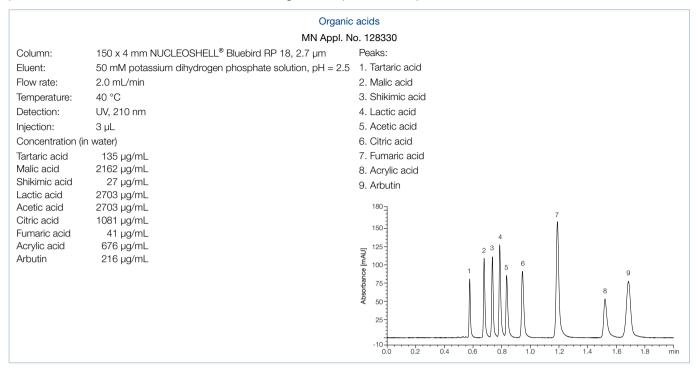


The reliable phase modification process leads to a high batch-to-batch reproducibility, where different batches show very consistent performance results. This can be shown in application 128610 with analytes of different polarities, which also demonstrate the hydrophobic properties of this C_{18} phase.

Batch-to-batch reproducibility MN Appl. No. 128610 50 x 4 mm NUCLEOSHELL® Bluebird RP 18, 2.7 µm Peaks: Column: Eluent: 25 mM ammonium dihydrogen phosphate 4. Naphthalene 1. Uracil solution - methanol (35:65, v/v), pH = 7.0 2. Ethyl benzoate 5. Biphenyl Flow rate: 1.0 mL/min 3. Lidocaine 6. Acenaphthene 40 °C Temperature: 1250 Detection: UV, 254 nm Injection: 5 μL 1000 Concentration: Absorbance [mAU] 800 Uracil 45 μg/mL batch 1 Ethyl benzoate 181 μg/mL 600 Lidocaine 1134 µg/mL Naphthalene 1134 µg/mL Biphenyl 45 µg/mL 200 Acenaphthene 227 µg/mL -10+ batch 3 The mixture was diluted to 4 mL with water

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.



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						

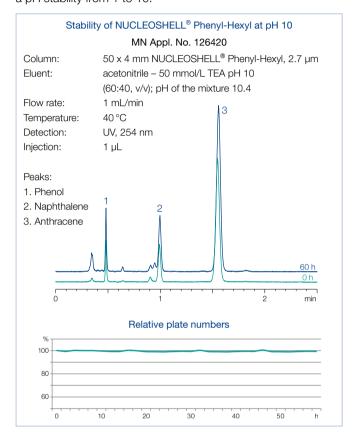
^{*}Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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NUCLEOSHELL® Phenyl-Hexyl

Alternative selectivity to C₁₈ phases

Phenylhexyl modified phases offer an excellent separation efficiency especially for aromatic and unsaturated compounds with electron-withdrawing groups. The combination of hydrophobic and $\pi\text{-}\pi$ 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.



Key features

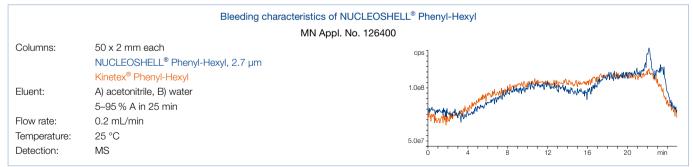
- Suitable for polar/aromatic compounds
- Hydrophobic phase with alternative selectivity compared to classical C₁₈ modifications
- Separation principle based on 2 retention mechanisms: π-π interactions and hydrophobic interactions
- Suitable for LC/MS

Technical data

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

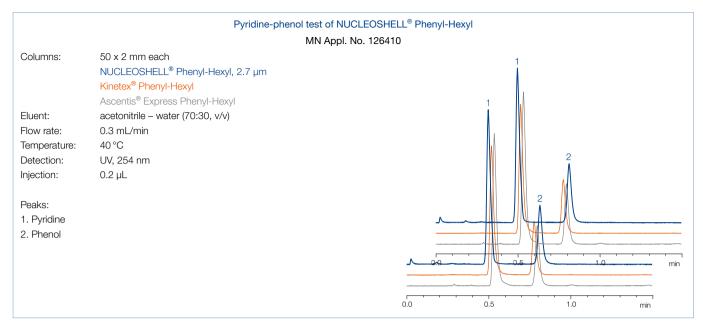
Recommended applications

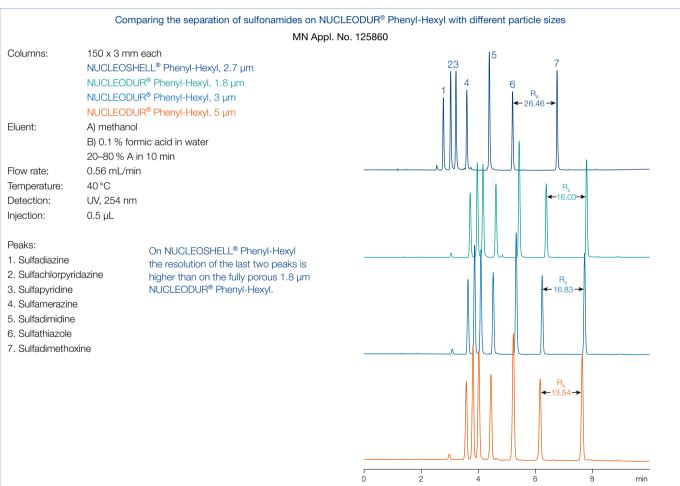
- USP listing L11
- Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics



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.

NUCLEOSHELL® 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	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

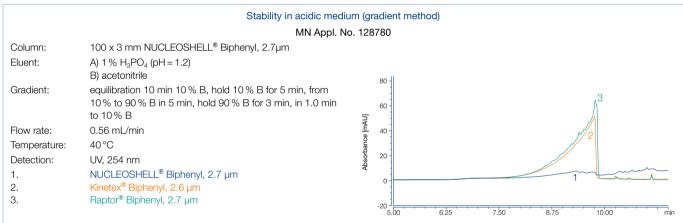
- Enhanced retention for aromatic and unsaturated substances due to a separation principle based on 2 retention mechanisms: $\pi\text{-}\pi$ interactions and hydrophobic
- Stable in 100 % aqueous mobile phase systems
- Suitable for LC/MS due to low bleeding characteristics

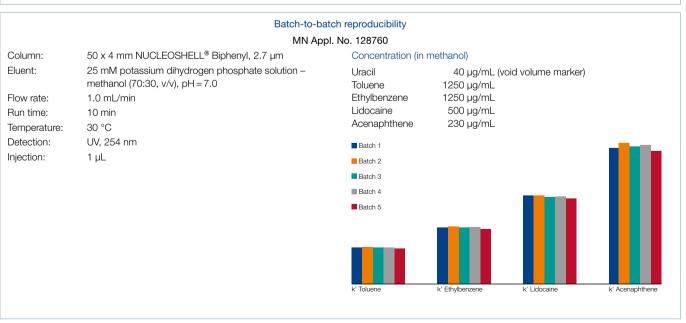
Technical data

- 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





Phthalates

MN Appl. No. 128830

100 x 3 NUCLEOSHELL® Biphenyl, 2.7 µm Columns:

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

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

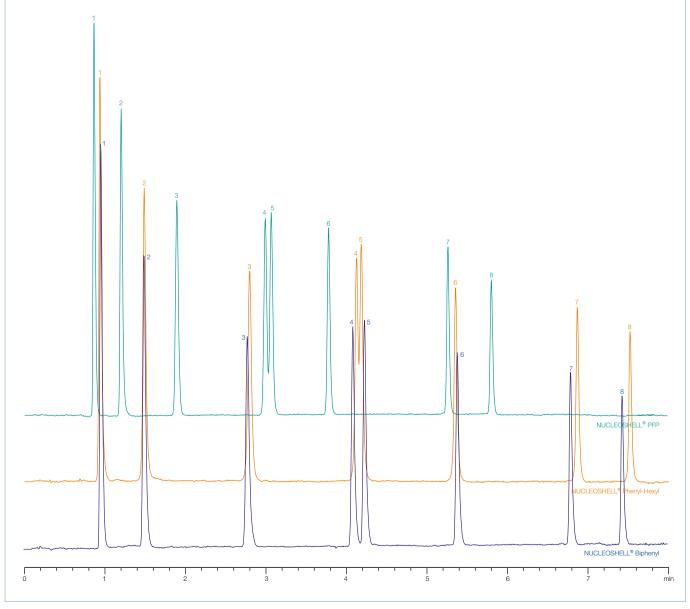
for 3.5 min, in 2.0 min to 50 % B, hold 50 % B for 4.5 min

Flow rate: 1.0 mL/min Temperature: 30 °C UV, 228 nm Detection: 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®	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							

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MN Application database with a new design



The MN chromatography application database

- Free access to more than 3,000 application examples from SPE, TLC, HPLC and GC
- Simple key word search results are obtained in seconds
- ChromaAppDB.mn-net.com



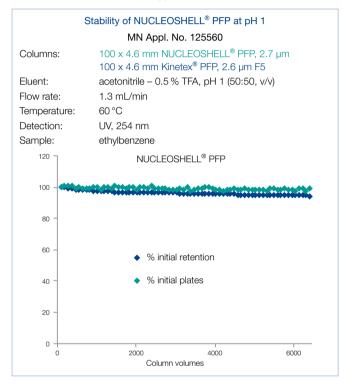
^{*}Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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 C₁₈ modifications
- Separation principle based on 4 retention mechanisms (polar interactions (H bonds), dipole-dipole, π-π, hydrophobic interactions)
- Suitable for LC/MS

Technical data

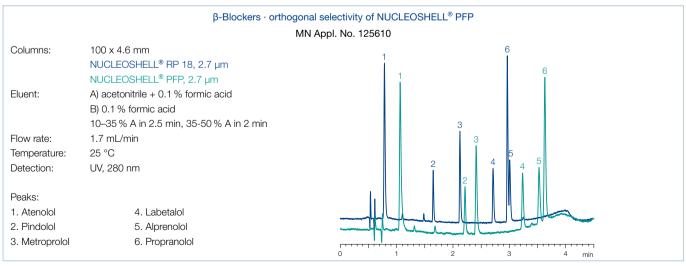
- Phase with pentafluorophenylpropyl phase; multi-endcapped
- Pore size 90 Å, particle size 2.7 μm; carbon content ~ 3 %; pH stability 1–9;

Recommended applications

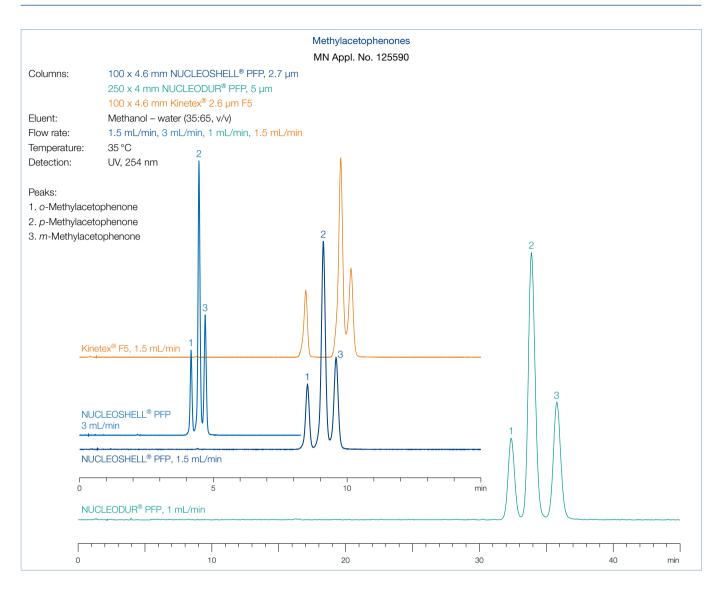
- USP listing L43
- Aromatic and unsaturated compounds, phenols, halogen compounds, isomers, polar compounds like pharmaceuticals, antibiotics; strong retention of basic compounds

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.



NUCLEOSHELL® PFP



Ordering information

NUCLEOSHELL	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							

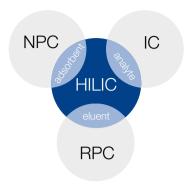
^{*}Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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



NUCLEOSHELL® HILIC

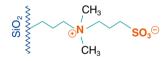
Hydrophilic interaction chromatography



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

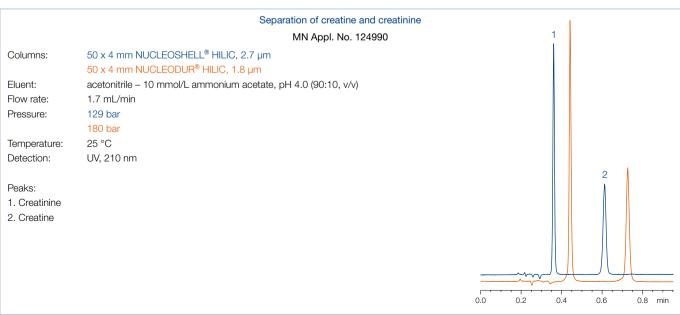
- Zwitterionic ammonium-sulfonic acid phase; not endcapped
- Pore size 90 Å, particle size 2.7 μm; carbon content 1.3 %; pH stability 2–8.5

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))



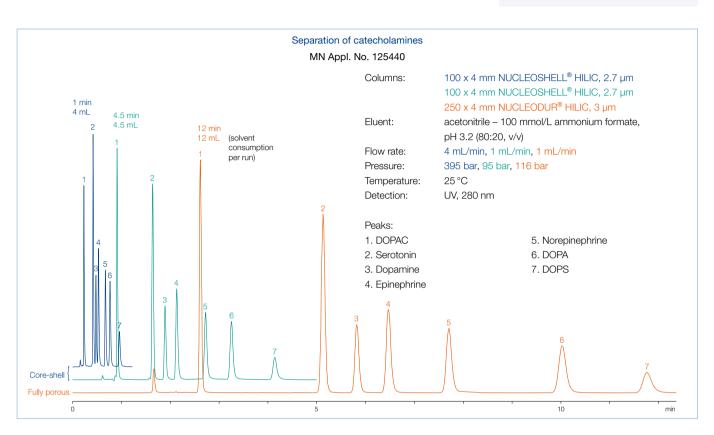
NUCLEOSHELL® HILIC

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-ofthe-art core-shell silica.



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



MN column systems

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

Availabl	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 pl	hase modificat	ions and partic	le sizes are av	ailable in every	possible dimer	nsion.							

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.

Good to know

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



MN column systems

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 dimension	is of VarioP	rep 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} x 8, 10 x 16, 15 x 32 and 15 x 50 mm ID columns are used as guard columns and require the respective holders, see page 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

Ordering information

Good to know

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

Product	Pack of	REF
Column Protection System, consisting of 1 \times guard column holder, 2 \times capillaries (ID 0.12 mm), 3 \times ferrules (for HPLC columns with particle size \times 2 μ m), 3 \times ferrules (for HPLC columns with particle size \times 2 μ m), 2 \times 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 $< 2 \ \mu m$	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 quard column

Eluent: acetonitrile - water (80:20, v/v)

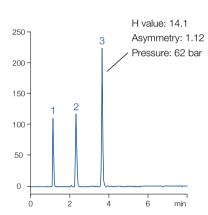
Flow rate: 16 mL/min 22 °C Temperature:

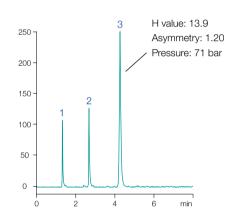
Peaks:

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

Gua	Guard column holders for VarioPrep columns							
VP G	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.

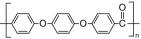


Accessories

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







1/16" PEEK fingertight Nut

1/16" PEEK double ferrule

1/16" PEEK ferrule for REF 718771

1/16" PEEK union, both sides outer diameters



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

1/16" PEEK union, both sides inner threads, equipped with 2 fingertight nuts and double ferrules

1/16" PEEK union, both sides inner threads, without nuts and without ferrules

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



1 1

1

1

1

1

718771

718772

718775

718766

718767

718768

List of abbreviations and trademarks

List of abbreviations

%C	carbon content in percent		
Å	angstrom = $0.1 \text{ nm} = 1.0 \times 10^{-10} \text{ m}$		
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		
ес	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 tradem	narks (®)
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)

Common law trademarks (™)			
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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)		

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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.

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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

- [1] Tanaka, N. et al., Journal of Chromatographic Science, 27 (1989), 721-728.
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