Reference Guide

MACHEREY-NAGEL

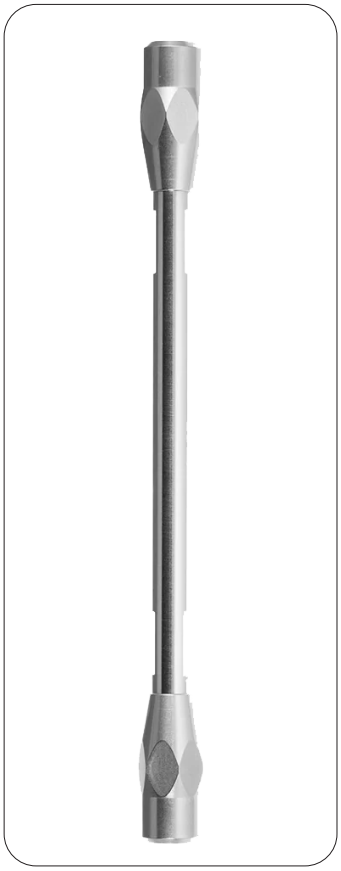
HPLC Columns

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.



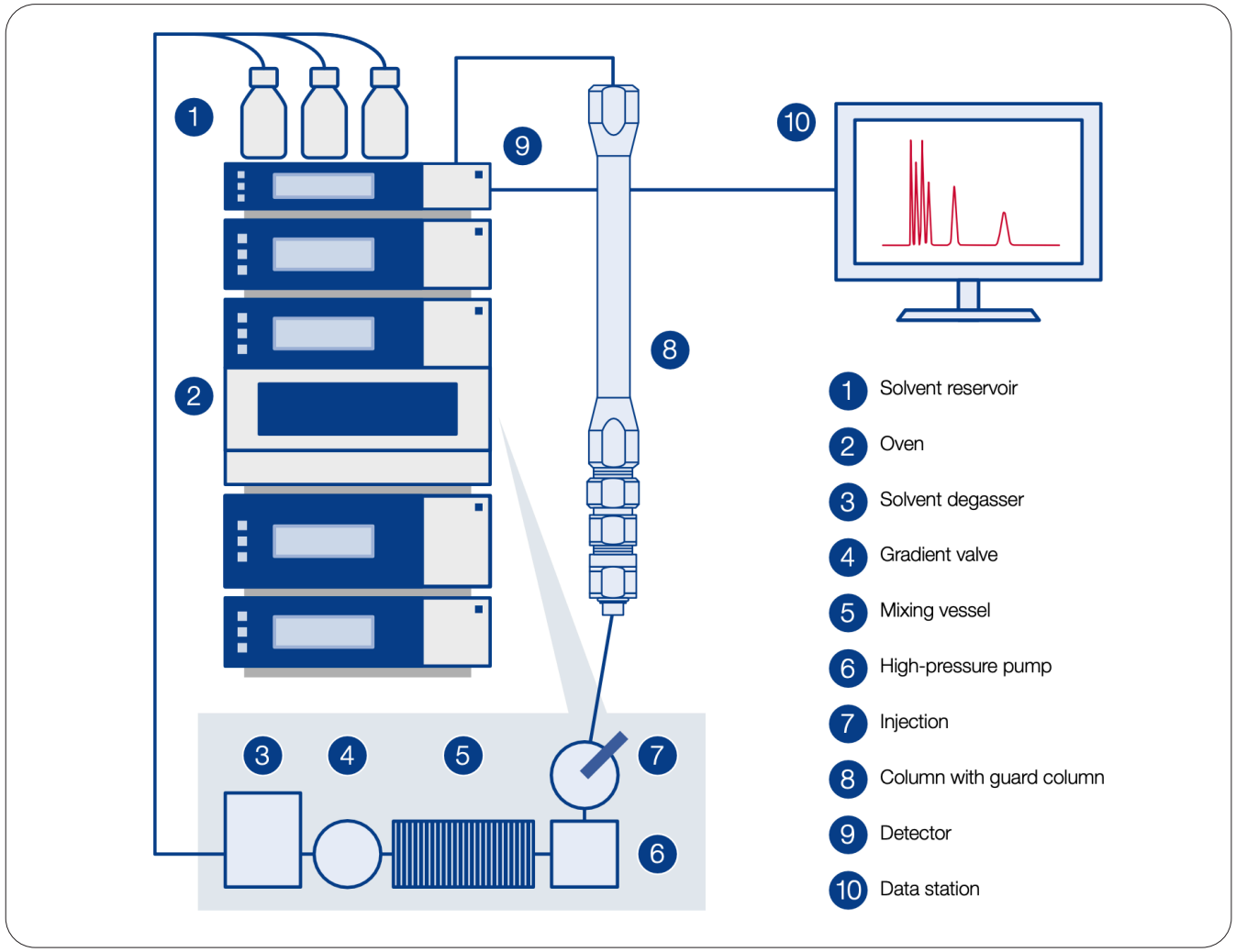
Basic Principle

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.

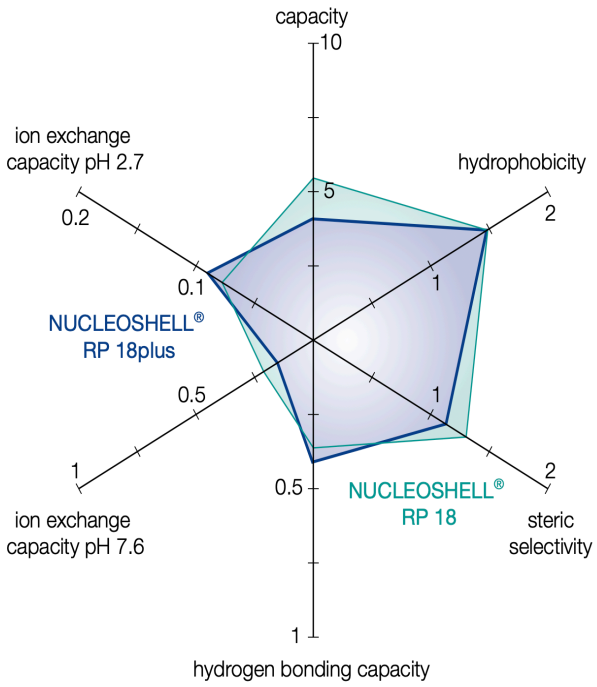
Selectivity

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].

Characteristic Parameters

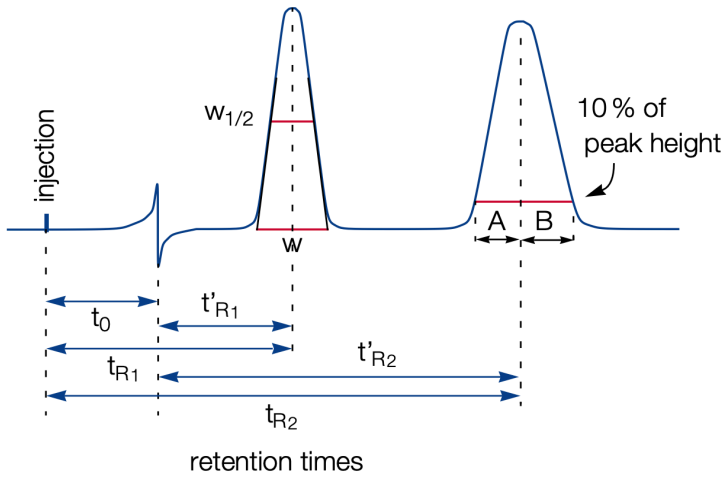
The success of a chromatographic separation depends apart from the stationary and mobile phase also on other characteristics like the quality of the separating column or the linear flow rate. The following schematic chromatogram illustrates the most important parameters which characterize a separation.



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® RP18 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.



Schematic Chromatogram Legend	
Peak Width:	
$W_{1/2}$	Peak width at half height
W	Peak width of the peak (intersection point of the inflectional tangents with the zero line)
Peak Symmetry:	
A	Peak front to peak maximum at 10 % of peak height
B	Peak maximum to peak end at 10 % of peak height
Retention Time:	
t_0	Dead time of a column = retention time of a non-retarded substance
t_{R1}, t_{R2}	Retention times of components 1 and 2
t'_{R1}, t'_{R2}	Net retention times of components 1 and 2

In a chromatographic system the substances differ from each other in their retention time in or on the stationary phase. The time, which is needed by a sample component to migrate from column inlet (sample injection) to the column end (detector) is the retention time t_{R1} or t_{R2}. The dead time t_0 is the time required by an inert compound to migrate from column inlet to column end without any retardation by the stationary phase. Consequently, the dead time is identical with the retention time of the sample component remaining in the stationary phase. The difference of total retention time and dead time yields the net retention time t'_{R1} or $t'R_2$, which is the time a sample component remains in the stationary phase.

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

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 $w_{1/2}$ is also included.

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

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

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

Instead of the mobile phase volumetric flow rate [mL/min], which is controlled at the HPLC instrument, it is advantageous to use the linear velocity u [cm/sec]. The linear velocity is independent of the column cross section and proportional to the pressure drop in the column. The linear velocity can be calculated by means of the dead time, where L is the column length in cm and t0 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.

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

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 hmin and uopt 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

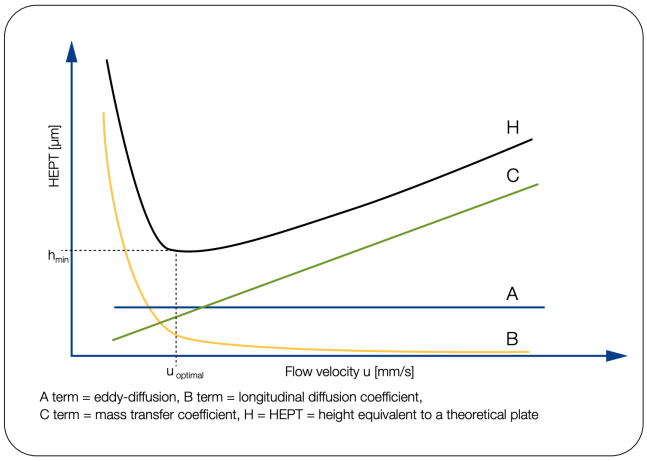
NUCLEODUR® High Purity Silica for HPLC

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.

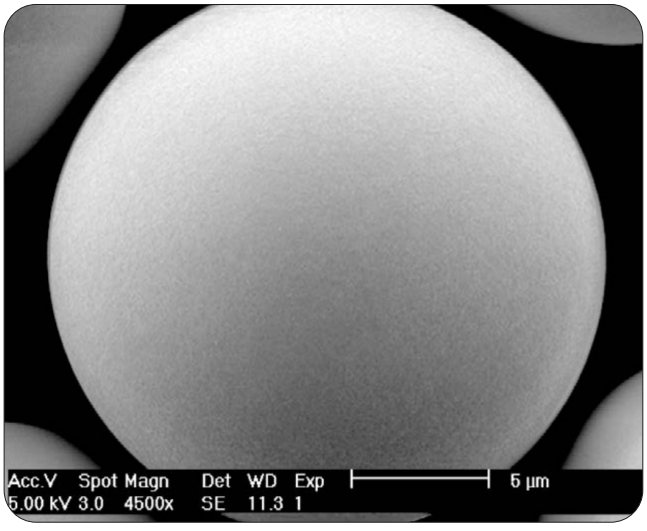
NUCLEODUR® as a state-of-the-art silica is the ideal base material for modern HPLC phases. It is the result of MACHEREY-NAGEL's pioneering research in chromatography for more than 40 years.

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.

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.



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



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.

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.

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.

NUCLEODUR® High Purity Silica for UHPLC

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.

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

Physical Data of NUCLEODUR®		
	Standard	Widepore
Pore size	110 Å	300 Å
Surface area (BET)	340 m2/g	100 m2/g
Pore volume	0.9 mL/g	0.9 mL/g
Density	0.47 g/mL	0.47 g/mL

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

Significant Improvement in Resolution

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

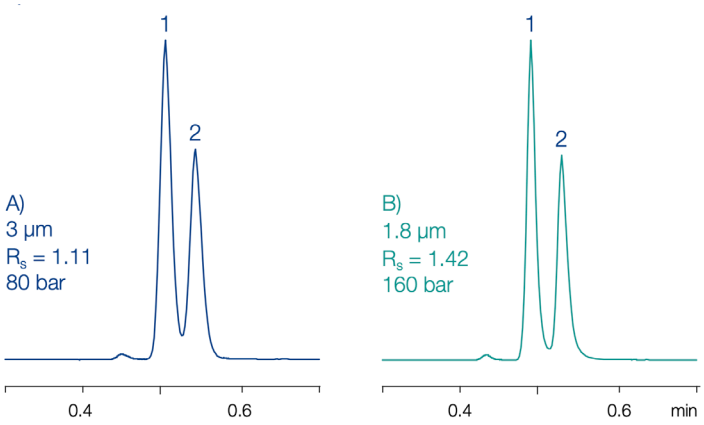
Rs = resolution, **α** = selectivity (separation factor),
$\mathbf{k_i'}$ = retention N = plate number with N∏/d_p,
$\mathbf{d_p}$ = particle diameter

Resolution as Function of Particle Size

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

Peaks:

1. Naphthalene
2. Ethylbenzene



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

NUCLEODUR® High Purity Silica for UHPLC

Column Back Pressure

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

$$\Delta_P = \frac{\Phi \cdot L_c \cdot \eta \cdot \mu}{d_p^2}$$

Δ_P = pressure drop, Φ = flow resistance (non-dimensional),
L_C = column length, η = viscosity, μ = 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.

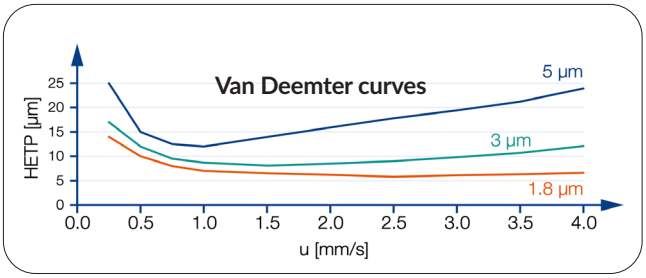
Comparison of back pressures

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

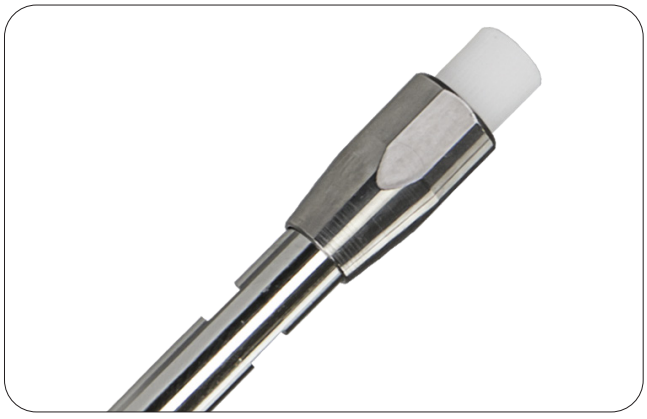
The optimal flow rate for 1.8 µm particles is higher than for 3 and 5 µm particles (see figure – the flow rate should be at the van Deemter minimum).



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® C_{18} Gravity · C_8 Gravityy

Base deactivation

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

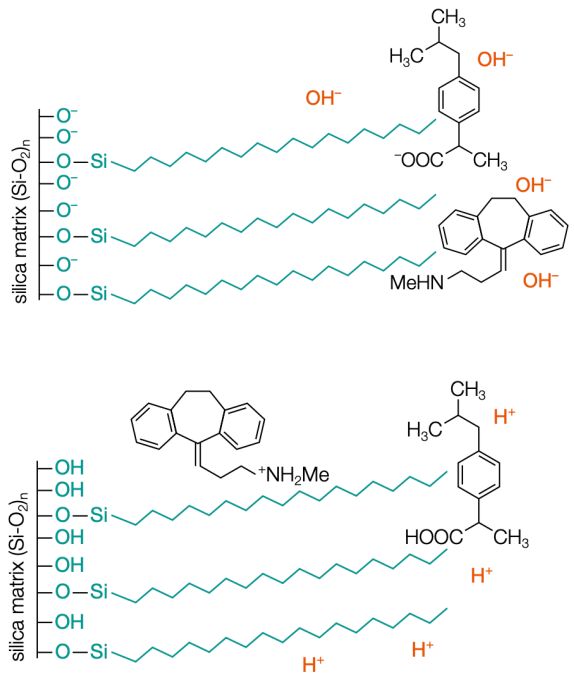
Enhanced pH Stability

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

Benefits of Enhanced pH Stability

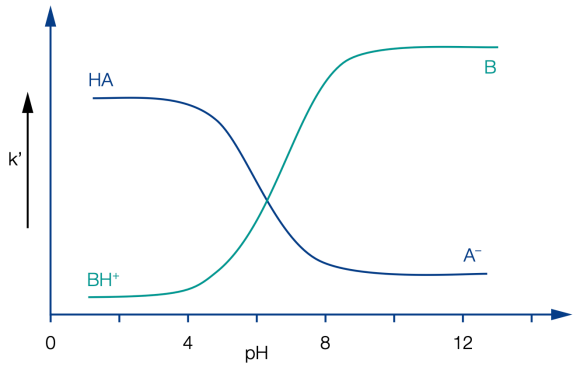
An expanded pH range is often required in method development. Many nitrogen containing compounds like basic drugs are protonated at acidic or neutral pH and exhibit poor retention on a standard C_{18} phase. The retention behavior can be improved by working at a higher pH, where the analyte is no longer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

Surface Silanols at Different pH Values



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

Correlation between retention and pH for basic and acidic compounds



An example how selectivity can be controlled by pH is the separation of the acid ketoprofen, the base lidocaine and benzamide. Under acidic conditions the protonated lidocaine is eluted very fast due to lack of sufficiently strong hydrophobic interactions.

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

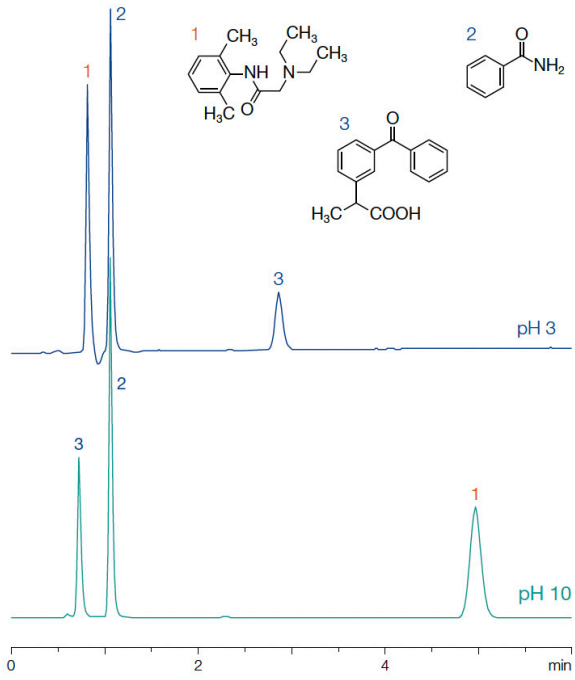
Recommended Applications

- 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.

Infl uence of the pH value on selectivity

MN Appl. No. 120860



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

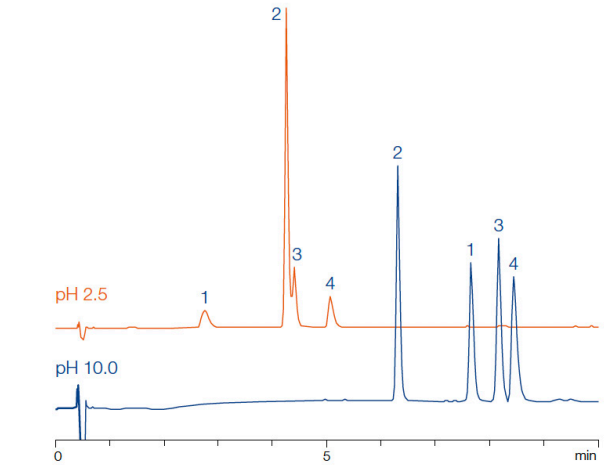
Peaks:

1. Lidocaine
2. Benzamide
3. Ketoprofen

As mentioned above, pH stability of the stationary phase can be helpful for improving selectivity in method development. The following figure shows the separation of 4 basic drugs under acidic and basic conditions. At pH 2.5 the protonated analytes exhibit poor retention (early elution) and in addition an inadequate resolution for papaverine and noscapine, whilst the formally non ionized molecules can be baseline separated due to the better retention pattern at alkaline pH.

Separation of basic alkaloids

MN Appl. No. 118010



Column:	125 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Solvent	A) acetonitrile B) 20 mmol/L (NH4)2HPO4, 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

NUCLEODUR® C_{18} Gravity · C_8 Gravityy

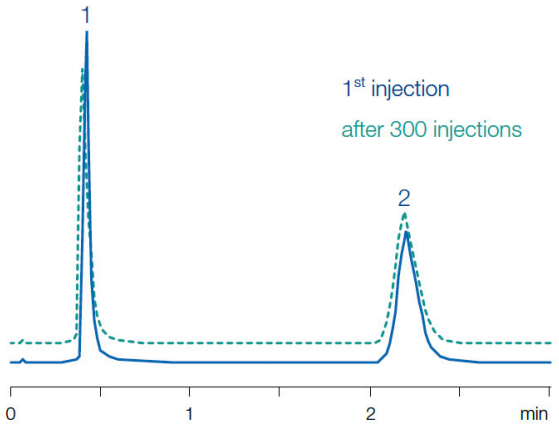
The following chromatogram demonstrates the stability of NUCLEODUR® C_{18} Gravity under alkaline conditions. The ultra-pure Gravity with its unique high density surface bonding technology withstands strong alkaline mobile phase conditions.

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

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

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

Separation of Basic Alkaloids
MN Appl. No. 118010

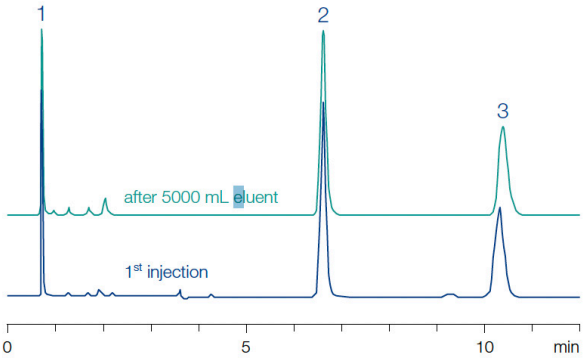


Column:	50 x 4.6 mm NUCLEODUR® C_{18} Gravity, 5 µm
Solvent	Methanol – water – ammonia (20:80:0.5, v/v/v), pH 11
Flow rate	1.3 mL/min
Temperature	30 °C
Detection	UV, 254 nm
Injection	2.0 µL

Peaks:

1. Theophylline
2. Caffeine

Stability of NUCLEODUR® C_{18} Gravity at pH 1.5
MN Appl. No. 120840



Column:	125 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Solvent	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

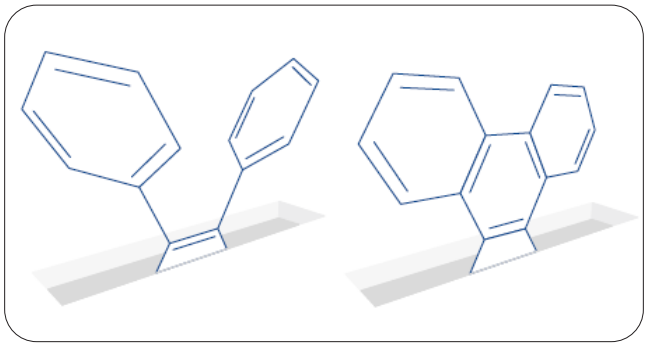
NUCLEODUR® C_{18} Isis

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® C_{18} Isis shows a carbon load of 20%. The target crosslinking of the C_{18} chains on the surface enables the separation of compounds with similar molecular structure but different stereochemical properties. The technical term for this feature is steric selectivity.

Slot Model

Sander and Wise[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).



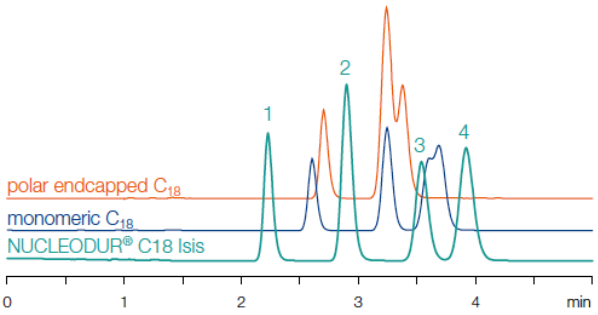
Steric Selectivity

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

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® C_{18} Isis compared to a conventional C_{18} column.

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

Steric Selectivity of NUCLEODUR® C_{18} Isis



Column:	125 x 4 mm NUCLEODUR® C_{18} Isis monomerically coated C_{18} phase polar endcapped phase CC_{18} phase
Solvent	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

Key Features

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

Technical Data

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

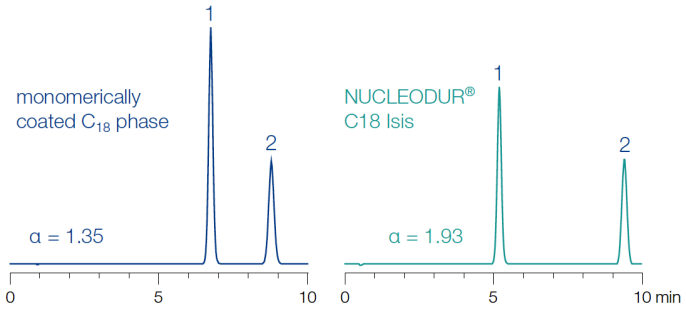
Recommended Applications

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

NUCLEODUR® C_{18} Isis

Steric Selectivity of NUCLEODUR® C_{18} Isis

Column:	125 x 4 mm
Solvent	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® C_{18} Isis				
Analytical EC columns NUCLEODUR® C_{18} 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	3.6	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.

NUCLEODUR® C_{18} 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.

Stability Features

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

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

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

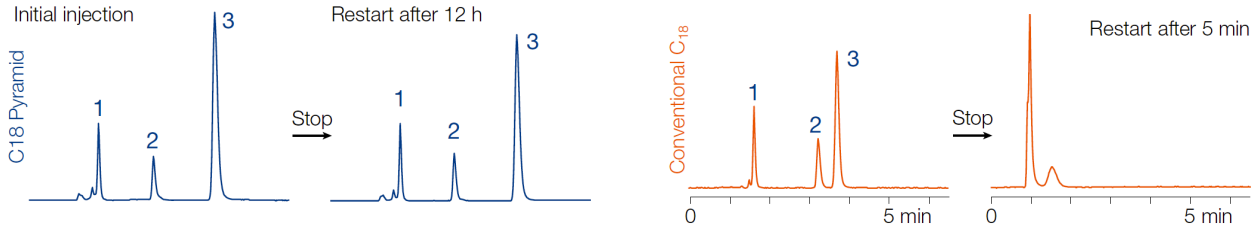
- Special C18 phase; polar endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm (7 and 10 µm particles for preparative purposes on request); carbon content 14 %; pH stability 1–9

Recommended Applications

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

Stability Test

MN Appl. No. 120870



Column:	125 x 4 mm
Solvent	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:

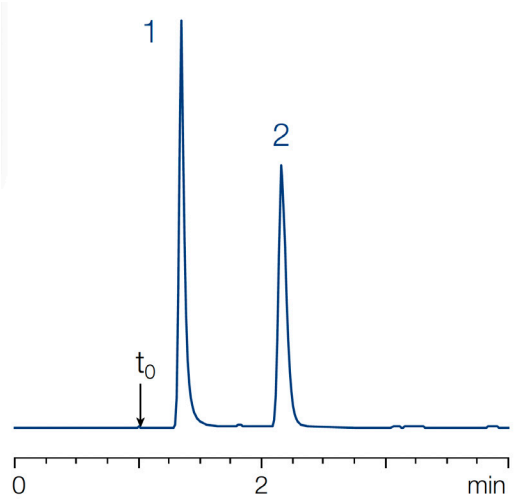
1. Tartaric acid
2. Acetic acid
3. Maleic acid

NUCLEODUR® C_{18} Pyramid

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® C^{18} 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.

Separation of Very Polar Compounds

MN Appl. No. 119170



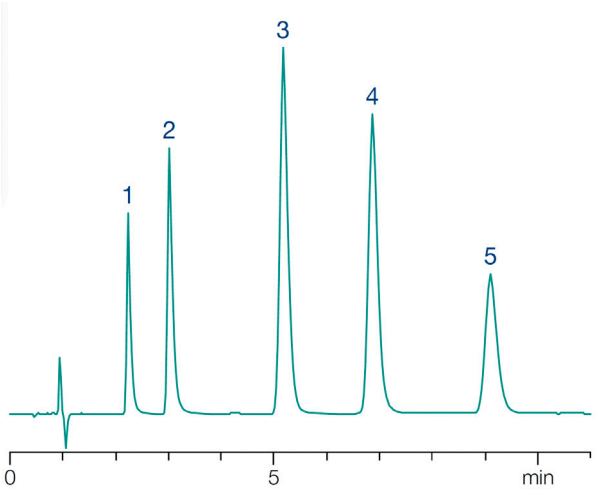
Column:	125 x 4 mm NUCLEODUR® C_{18} Pyramid, 5 µm
Solvent	0.2 % H_3PO_4
Flow rate	1.0 mL/min
Temperature	22 °C
Detection	UV, 202 nm
Injection	1 µL

Peaks:

1. Formic acid
2. Acetic acid

Separation of Basic Alkaloids

MN Appl. No. 118010



Column:	125 x 4 mm NUCLEODUR® C_{18} Pyramid, 5 µm
Solvent	methanol – 20 mM $NH_4H_2PO_4$, pH 6.95 (70:30, v/v)
Flow rate	40 °C
Temperature	5 µL
Detection	UV, 254 nm
Injection	2 µL

Peaks:

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

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.

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.

Key Features

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

Technical Data

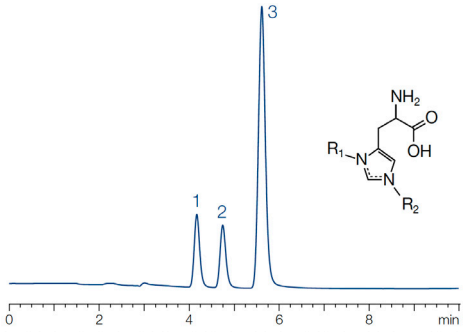
- Phase with embedded polar group; endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 17 %; pH stability 1–9

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.

Separation of Histidines

MN Appl. No. 125140



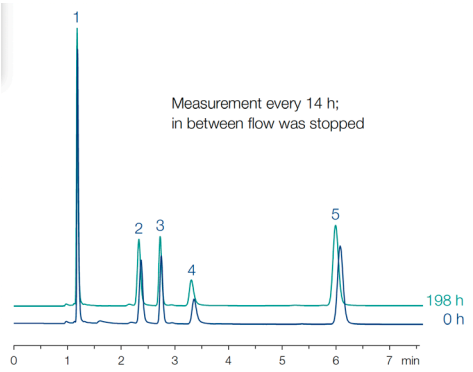
Column:	150 x 3 mm NUCLEODUR® PolarTec, 3 µm
Solvent	1.0 mmol/L perfl uoropentanoic acid in water – 0.5 mmol/L perfl uoropentanoic acid in acetonitrile (99.5:0.5, v/v)
Flow rate	0.4 mL/min
Temperature	20 °C
Detection	UV, 230 nm

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

Stability of NUCLEODUR® PolarTec

MN Appl. No. 124610



Column:	1150 x 3 mm NUCLEODUR® PolarTec, 3 µm
Solvent	30 mmol/L KH_2PO_4, pH 3.0
Flow rate	0.5 mL/min
Detection	30 °C
Injection	UV, 220 nm

Peaks:

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

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

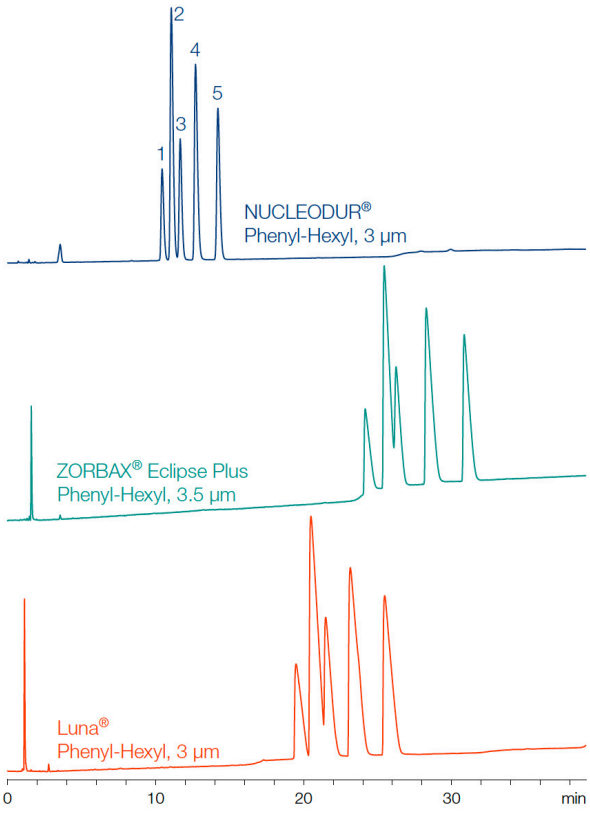
- Phase with phenylhexyl modification; multi-endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 10 %; pH stability 1–10

Recommended Applications

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

Separation of basic alkaloids

MN Appl. No. 118010



Column:	150 x 3 mm NUCLEODUR® Phenyl-Hexyl, 3 µm Agilent ZORBAX® Eclipse Phenyl-Hexyl, 3.5 µm Phenomenex Luna® Phenyl-Hexyl, 3 µm
Solvent	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 solvent

Peaks:

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

NUCLEODUR® C_{18} ec · C_8 ec · C_4 ec

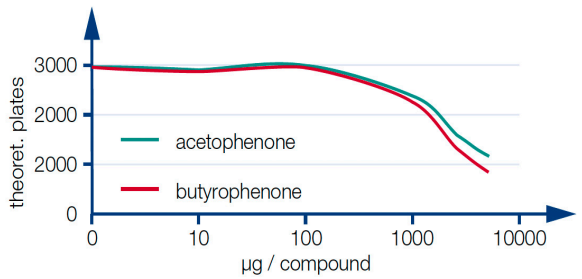
NUCLEODUR® C_{18} ec for daily routine analysis

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

Loading Curve



Column:	250 x 4.6 mm NUCLEODUR® 100-20 C18 ec
Solvent	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 n Ideal and reliable standard RP phase for daily routine analysis and up-scaling for preparative HPLC
- Medium density octadecyl (C_{18}) and octyl (C_{18}) 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

NUCLEODUR® C_{18} ec · C_8 ec · C_4 ec

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.

NUCLEODUR® Octyl Phases

In addition to NUCLEODUR® C_{18} phases MACHEREY-NAGEL offers octyl modified NUCLEODUR® C_8 Gravity and NUCLEODUR® C_8ec columns to expand the RP tool box. Based on the same spherical high purity silica the C_8 phases exhibit the same chemical and mechanical stability as the C_{18} counterparts. Indeed, NUCLEODUR® C_8 Gravity can also be run at pH extremes (pH 1–11) by choosing appropriate elution parameters. Due to the shorter chain and less hydrophobic properties of the stationary phase the retention of 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® C_8 ec and NUCLEODUR® C_8 Gravity are most suitable for the development of new methods but also for robust routine analyses.

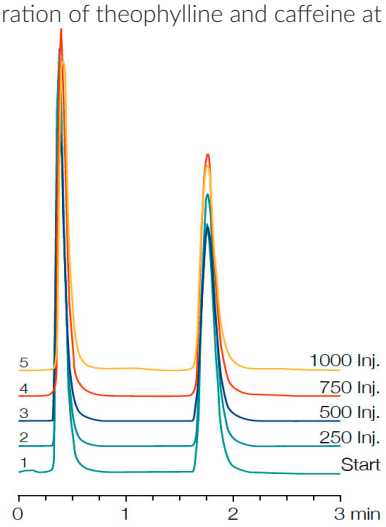
There are no general guidelines which could make the choice between C_8 and C_{18} phases easier but it will always be beneficial to add both phases to the existing pool of RP columns in the laboratory. Comparative studies reveal some different selectivity patterns of NUCLEODUR® C_8 ec and C_{18} ec. The separation of phenols 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.

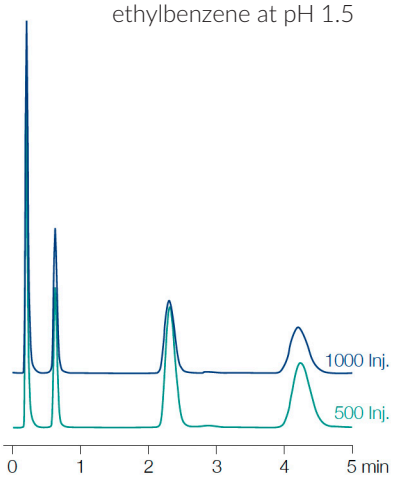
pH Stability of NUCLEODUR® C_{18} ec

Separation of theophylline and caffeine at pH 10



Column:	30 x 3 mm NUCLEODUR® 100-5 C18 ec
Solvent	methanol – aq. NH_3 (20:80, v/v), pH 10
Flow rate	0.5 mL/min
Temperature	25 °C
Detection	UV, 254 nm

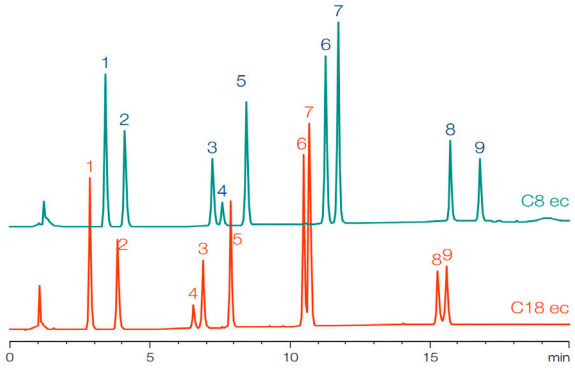
Separation of uracil, veratrol, toluene and ethylbenzene at pH 1.5



Column:	30 x 3 mm NUCLEODUR® 100-5 C18 ec
Solvent	acetonitrile – H_2O (65:35, v/v), TFA, pH 1.5
Flow rate	1.0 mL/min
Temperature	25 °C
Detection	UV, 254 nm

Separation of Phenols

MN Appl. Nos. 12089 / 120891



Column:	250 x 4 mm NUCLEODUR® 100-5 C8 ec /C18 ec
Solvent	A) water, B) methanol C_8: 20% B (2 min) ☐ 60% B in 12 min C_{18}: 25% B (2 min) ☐ 65% B in 12 min
Flow rate	1.0 mL/min, temperature 25 °C
Detection	UV, 275 nm, injection 10 µL

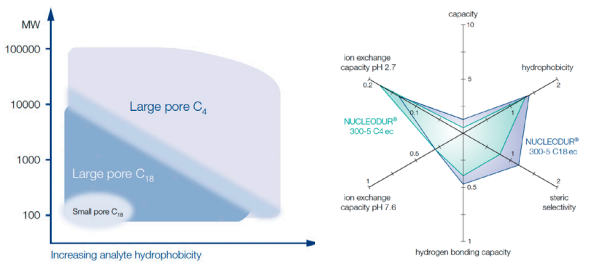
Peaks:

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

NUCLEODUR® Phases For Biochromatography

A description and applications for C_{18} and C_4 modified 300 Å NUCLEODUR® widepore materials for the separation of biopolymers, like peptids and proteins can be seen on the following pages.

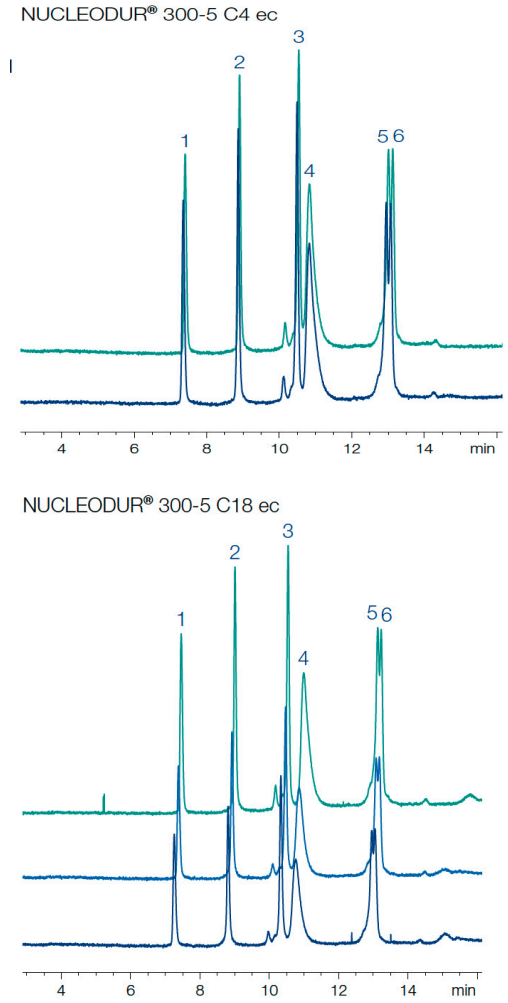
Column Selection by Analyte Characteristics



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



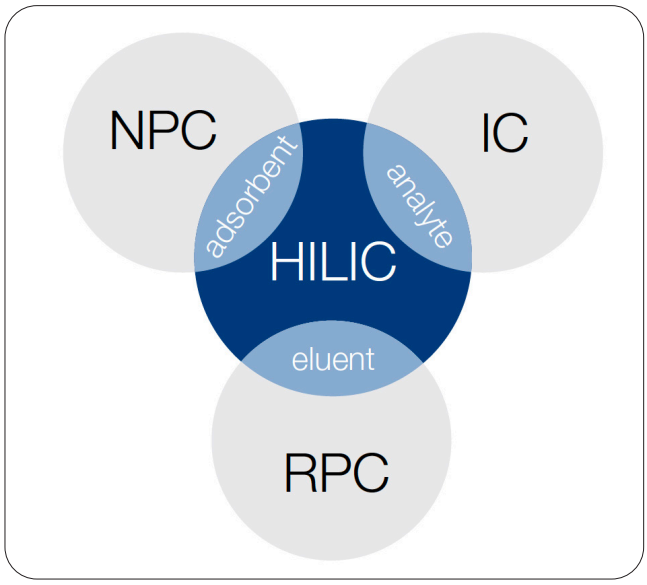
Column:	250 x 4 mm
Solvent	A) 0.1 % TFA in water B) 0.08 % TFA in acetonitrilel 20–60 % B in 15 min
Flow rate	1 mL/min
Temperature	25 °C
Detection	UV, 280 nm

Peaks:

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

NUCLEODUR® HILIC

Hydrophilic Interaction Chromatography



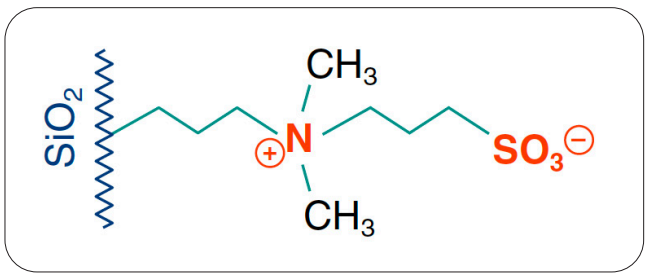
Especially for polar compounds reversed phase HPLC – the most common analytical method – is often limited. Here, hydrophilic stationary phases provide an additional tool for the separation of polar analytes in HPLC.

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 ammoniumsulfonic 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 High batch-to-batch reproducibility

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))

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.

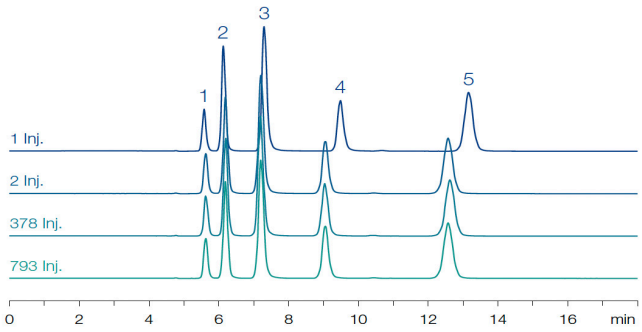
Stability and Equilibration

MN Appl. No. 123100

Column:	250 x 4 mm NUCLEODUR® HILIC, 5 µm
Solvent	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



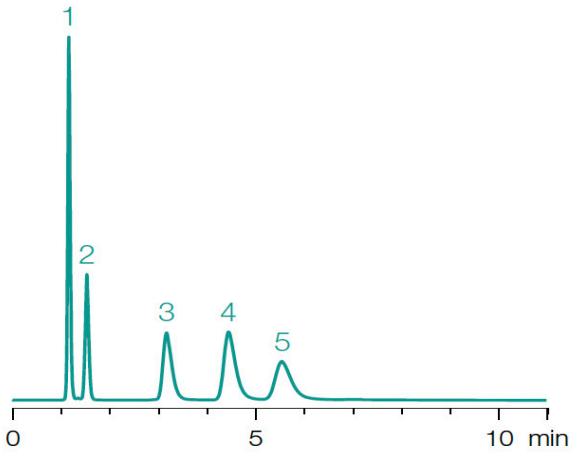
Separation of Adenosine and Phosphates

MN Appl. No. 123100

Column:	125 x 4 mm NUCLEODUR® HILIC, 5 µm
Solvent	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

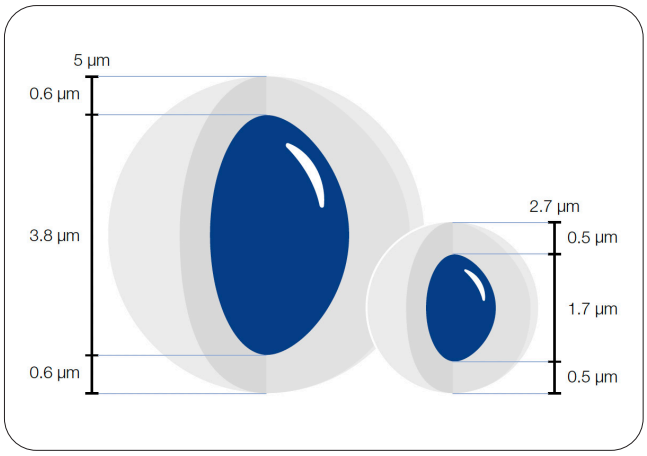
Peaks:

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

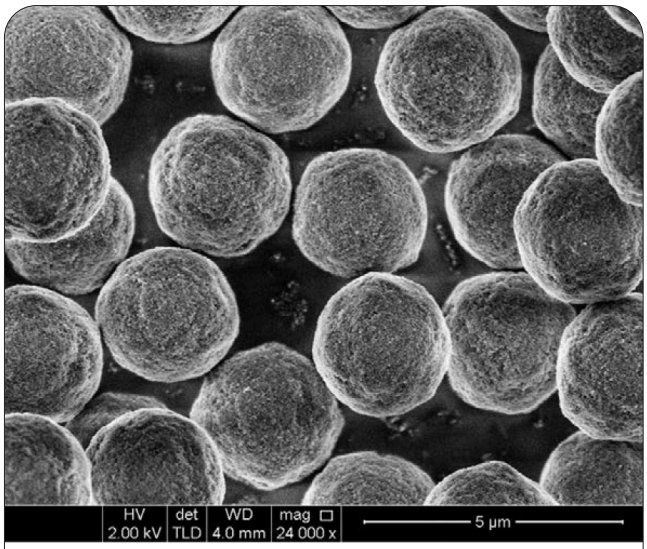


NUCLEODUR® Core-Shell Silica for Hplc

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.

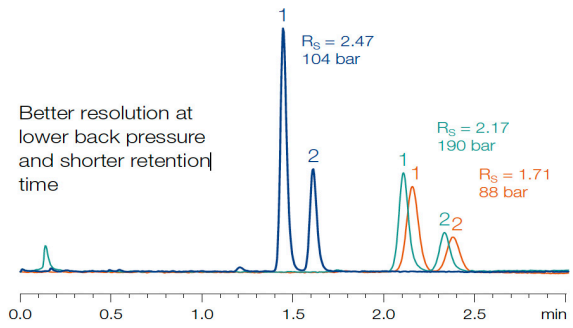
Utilizing a proprietary process of synthesis, NUCLEOSHELL® particles exhibit a distinct narrow particle size distribution (d90 / d10 ~ 1.1). Columns packed with NUCLEOSHELL core shell particles feature exceptional separation efficiencies with theoretical plate numbers easily comparable to totally porous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

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

Rs = resolution, **α** = selectivity (separation factor),
k_i' = retention N = plate number with N1/d_p,
d_p = particle diameter

Resolution RS as Function of Particle Size

MN Appl. No. 125270



Column:	50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm NUCLEODUR® C_{18} Gravity, 3 µm NUCLEODUR® $C1_{18}$ Gravity, 1.8 µm
Solvent	acetonitrile – water (60:40, v/v)
Flow rate	1 mL/min
Temperature	25 °C
Detection	UV, 254 nm

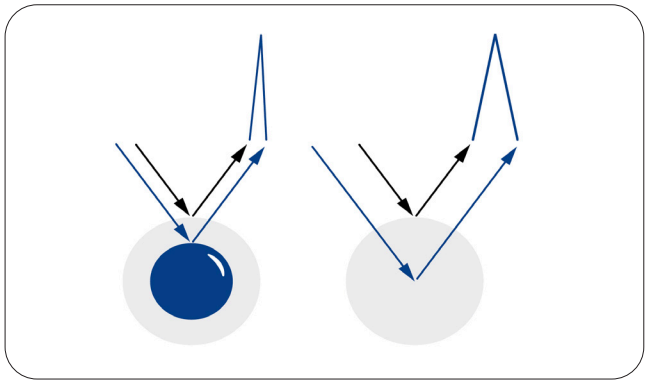
Peaks:

1. Naphthalene
2. Ethylbenzene

Theoretical column efficiency (optimal conditions)								
Silica	d_p [µm]	L [m]	HETP [µm]	Efficiency [plates/m]	L [mm]	N	Rs	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

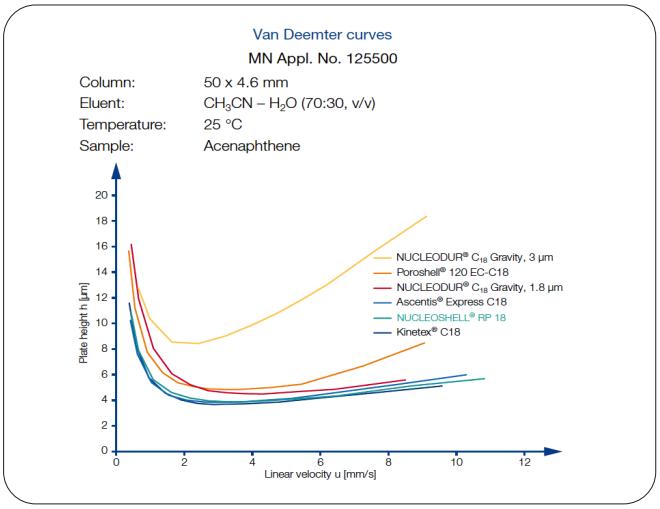
High heat transfer

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

NUCLEOSHELL® Core-Shell Silica for HPLC

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

A term = eddy-diff usion, B term = longitudinal diff usion coeffi cient, C term = mass transfer coeffi cient



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.

$$\Delta_P = \frac{\Phi \cdot L_c \cdot \eta \cdot \mu}{d_p^2}$$

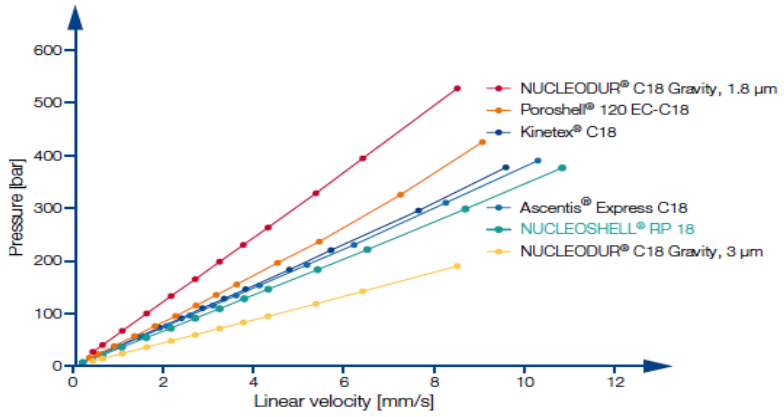
Δ_P = pressure drop, Φ = flow resistance (non-dimensional), L_C = column length, η = viscosity, μ = linear velocity, d_P = particle diameter

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.

Pressure drop

MN Appl. No. 125510



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

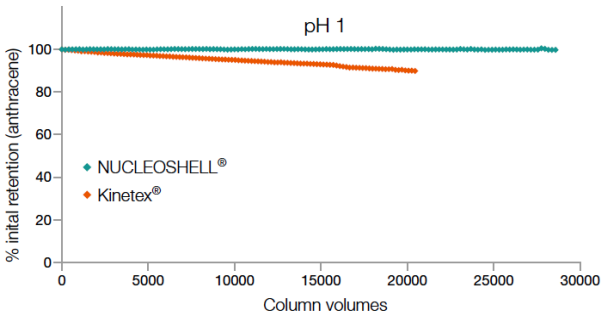
Features of NUCLEOSHELL® Particles

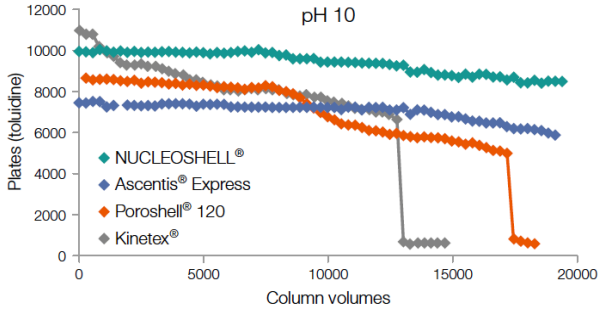
A criterion for the long-term stability of the column at pH extremes is the percentage decrease of initial retention and initial plates, respectively.

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. No. 125510





Column:	50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm 50 x 4.6 mm Kinetex® 2.6 µm C_{18}
Solvent	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

Column:	50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm 50 x 4.6 mm Ascentis® Express C_{18}, 2.7 µm 50 x 4.6 mm Poroshell® 120 EC-C_{18} 50 x 4.6 mm Kinetex® 2,6 µm C_{18}
Solvent	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
Solvent	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

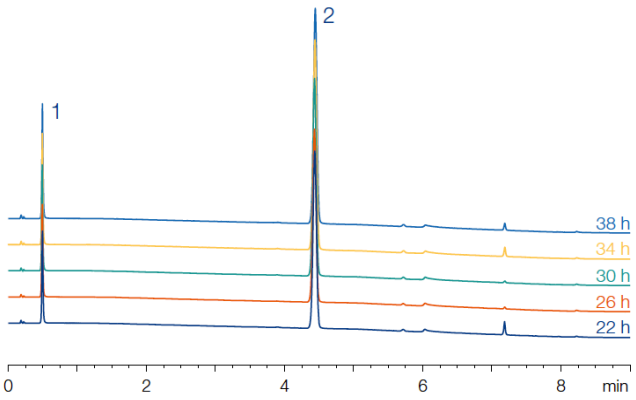
Efficiency Test:

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

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

Peaks:

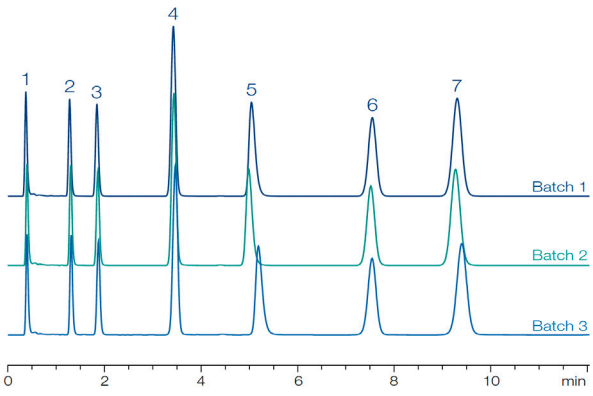
1. Phenol
2. Naphthalene



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
Solvent	methanol – 25 mmol/L KH_2PO_4, pH 7 (70:30, v/v)
Flow rate	1 mL/min
Temperature	40 °C
Detection	UV, 254 nm

Peaks:

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

NUCLEOSHELL® 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

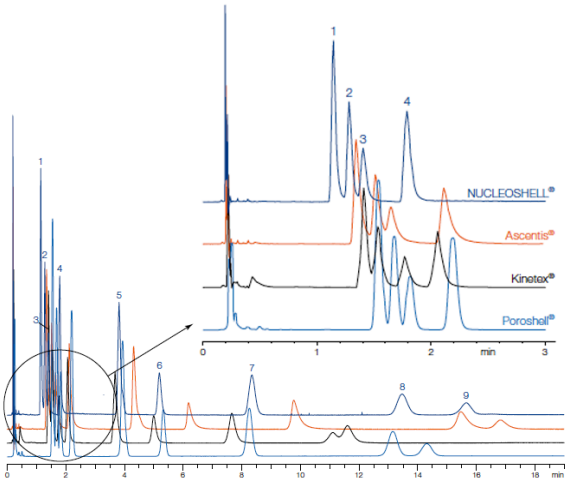
Recommended Applications

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

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.

Tricyclic Antidepressants · Comparison of Selectivity and Resolution

MN Appl. No. 124960



Column	50 x 4.6 mm each NUCLEOSHELL® RP 18, 2.7 µm Ascentis® Express C_{18} Kinetex® 2.6 µm C_{18} Poroshell® 120 EC-C_{18}
Solvent	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

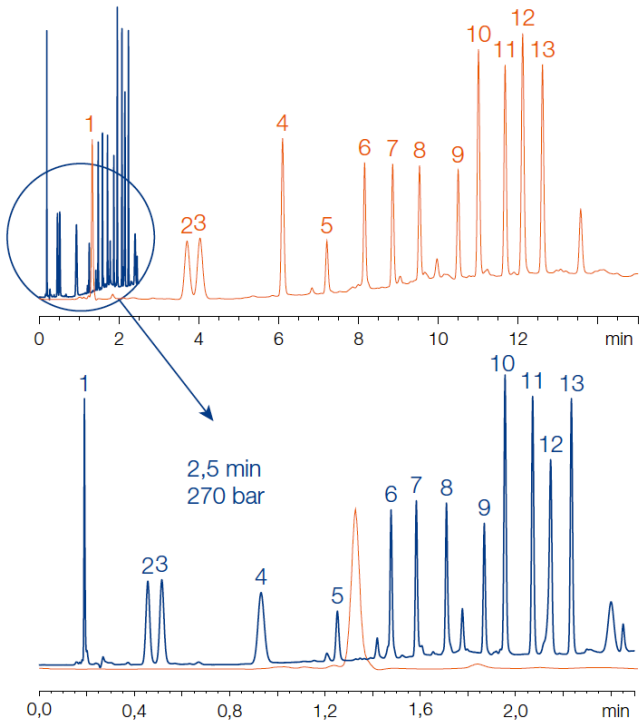
Peaks:

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

13 β-Lactam Antibiotics in Less Than 3 min

MN Appl. No. 124940



Column	50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm 150 x 4 mm NUCLEODUR® C_{18} Gravity, 5 µm
Solvent	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

NUCLEODUR® RP 18

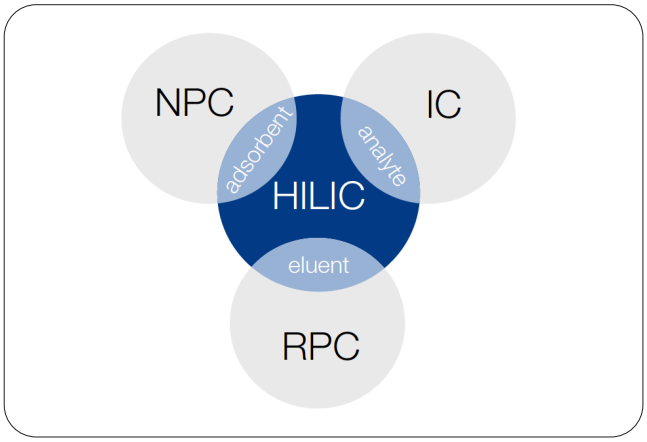
Analytical EC columns NUCLEOSHELL® RP 18 (pack of 1)

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.

NUCLEOSHELL® HILIC

Hydrophilic Interaction Chromatography

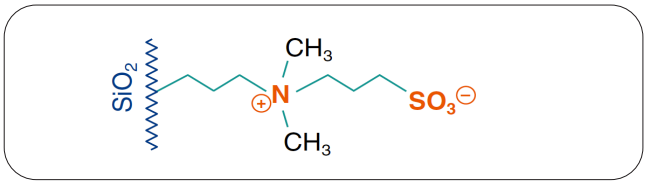


Hydrophilic interaction chromatography (HILIC) is a separation technique using polar stationary phases and organic-aqueous mobile phases. A minimum water content of at least 2 % is indispensable to provide a permanent water layer between the adsorbent surface and the organic fraction of the mobile phase. 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



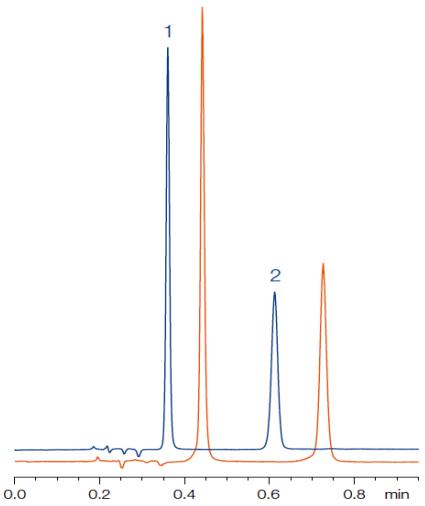
important substances creatine and creatinine can be achieved on NUCLEOSHELL® HILIC as well as on NUCLEODUR® HILIC, 1.8 µm at similar retention but much lower back pressure.

Good to know

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

Separation of Creatine and Creatinine

MN Appl. No. 124990



Column	50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm 50 x 4 mm NUCLEODUR® HILIC, 1.8 µm
Solvent	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

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

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.

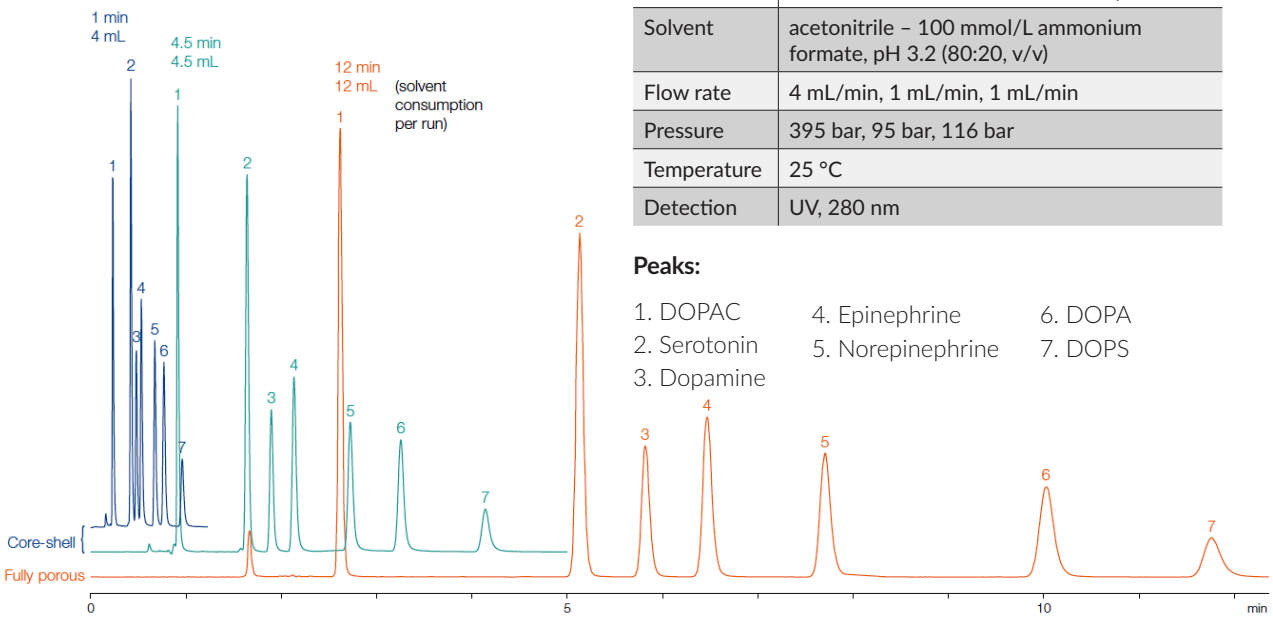
Run time has been cut down to 1 min. Column back pressure remains modest < 400 bar, while solvent demand is reduced to less than 35 %.

Good to know

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

Separation of Catecholamines

MN Appl. No. 125440



Column	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
Solvent	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

Accessories

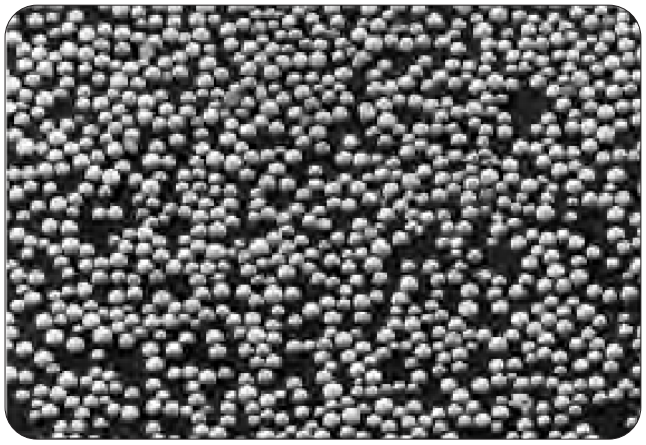
Accessories for stainless steel HPLC columns

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

Ordering Information

Capillary Accessories		
Description	**Pack of**	**REF**
1/16" column end caps (plastic)	4	718582
1/16" nut for connecting 1/16" capillaries	5	718583
1/16" ferrule	5	718584
Capillary Accessories		
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

NUCLEOSIL® Standard Silica for HPLC



Benefits of NUCLEOSIL® Silica

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

Physical Properties

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

NUCLEOSIL® Modifications

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

Key Features

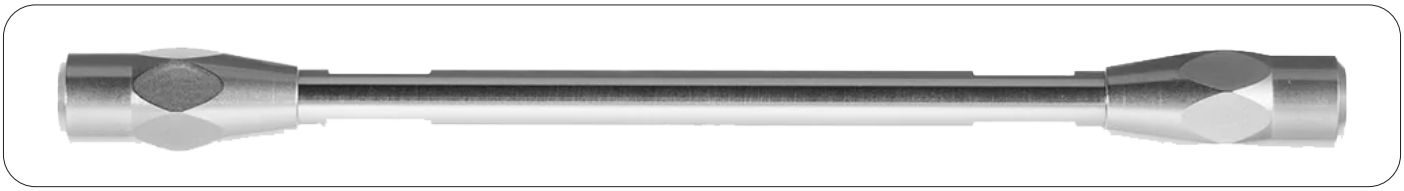
- NUCLEOSIL® is a family of totally porous spherical silicas. They feature a very pure and uniform SiO_2 structure and have gained wide acceptance as routine chromatographic packings for very different fields of modern chromatography.
- One of the first spherical silicas used in HPLC
- Developed in the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

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

* Maximum packing pressure of NUCLEOSIL® bulk packings

NUCLEODUR® HPLC C_{18} Columns

Description	Part No.
HPLC Column NUCLEODUR® 100-5 C_{18} ec 125x3 mm	CM-100000
HPLC Column NUCLEODUR® 100-5 C_{18} ec 125x4 mm	CM-100001
HPLC Column NUCLEODUR® 100-5 C_{18} ec 250x4,6 mm	CM-100002
HPLC Column NUCLEODUR® 100-5 C_{18} ec 150x4,6 mm	CM-100003
HPLC Column EC 50/4.6 NUCLEODUR® 100-5	CM-100004
HPLC Column EC 250/4.6 NUCLEODUR® 100-3 C18 HPLC Column, EC	CM-100005
HPLC Column EC 150/4.6 NUCLEODUR® C_{18} Gravity, 3 µm	CM-100006
HPLC Column EC 250/4.6 NUCLEODUR® C_{18} Gravity, 5 µm	CM-100007
HPLC Column EC 150/4.6 NUCLEODUR® C_{18} Gravity, 5 µm	CM-100008
HPLC Column EC 125/4 NUCLEODUR® 300-5 C4 125 mm, ID: 4 mm	CM-100009
HPLC Column EC 250/4 NUCLEODUR® C_{18} Pyramid, 5 µm	CM-100010
HPLC Column EC 150/4.6 NUCLEODUR® C_{18} Pyramid, 5 µm	CM-100011
HPLC Column EC 50/2 NUCLEODUR® C_{18} Pyramid, 1.8 µm	CM-100012
HPLC Column EC 100/2 NUCLEODUR® C_{18} HTHPLC Column, EC, 1.8 µm	CM-100013
HPLC Column EC 250/4.6 NUCLEODUR® C_{18} Isis, 3 µm	CM-100014
HPLC Column EC 100/3 NUCLEODUR® PolarTHPLC Column, EC, 3 µm	CM-100015
HPLC Column EC 150/3 NUCLEODUR® PolarTHPLC Column, EC, 3 µm	CM-100016
HPLC Column EC 150/2 NUCLEODUR® HILIC, 1.8 µm	CM-100017
HPLC Column EC 100/3 NUCLEODUR® HILIC, 3 µm	CM-100018
HPLC Column EC 150/2 NUCLEODUR® HILIC, 5 µm	CM-100019
HPLC Column EC 125/4.6 NUCLEODUR® 100-5 NH2	CM-100020
HPLC Guard Column EC 4/3 NUCLEODUR® C_{18} Pyramid, 5 µm, Pkg. 3	CM-100021
HPLC Guard Column EC 4/2 NUCLEODUR® 100-5 C_{18} ec, Pkg. 3	CM-100022
HPLC Guard Column EC 4/3 NUCLEODUR® 100-5 C_{18} ec, Pkg. 3	CM-100023
HPLC Cartridge Column CC 30/4 NUCLEODUR® Sugar 810-H	CM-100029
NUCLEOSHELL® HPLC Columns	
HPLC Column EC 50/2 NUCLEOSHELL® RP 18, 2.7 µm	CM-100024
HPLC Column EC 150/4.6 NUCLEOSHELL® RP 18 5 µm, 150 mm, ID: 4.6 mm	CM-100025
HPLC Column EC 100/2 NUCLEOSHELL® HILIC, 2.7 µm	CM-100026
NUCLEOGEL® HPLC Columns	
HPLC Column VA 300/7.8 NUCLEOGEL® Sugar 810 H	CM-100028



NUCLEOSIL® HPLC C_8 Columns	
HPLC Column EC NUCLEOSIL 100-5 C_8 HD, 5 µm, 150 x 4.6 mm	CM-100037
HPLC Column EC NUCLEOSIL 100-5 C8, 5 µm, 250 x 4.6 mm	CM-100038
HPLC Column EC NUCLEOSIL 120-5 C8, 5 µm, 150 x 4 mm	CM-100039
HPLC Column EC NUCLEOSIL 100-5 C8, 5 µm, 150 x 4.6 mm	CM-100040
HPLC Column EC NUCLEOSIL 100-5 C8 HD, 5 µm, 125 x 2 mm	CM-100041
HPLC Column EC NUCLEOSIL 120-3 C8, 3 µm, 125 x 4 mm	CM-100042
HPLC Column EC NUCLEOSIL 100-5 C8, 5 µm, 250 x 4 mm	CM-100043
Guard Columns and Holders	
HPLC Guard Column EC 4/3 UNIVERSAL RP, Pkg. 3	CM-100031
ChromCart Guard Column Holder 30 mm for Stand-alone	CM-100030
Column Protection System Guard Column Holder for EC 4x2 + 4x3 mm Guard Columns	CM-100027

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