USP listing

USP specification of MN HPLC phases

Code	Specification	MN HPLC Phases	Page
USP L1	octadecyl silane chemically bonded to porous silica particles 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® C_{18} ec	181
		NUCLEODUR® C_{18} Gravity	158
		NUCLEODUR® C_{18} Gravity-SB	162
		NUCLEODUR® C_{18} HTec	178
		NUCLEODUR® C_{18} Isis	164
		NUCLEODUR® C_{18} Pyramid	166
		NUCLEODUR® PolarTec	168
		NUCLEODUR® Sphinx RP	176
		NUCLEOSHELL® RP 18	200
		NUCLEOSHELL® RP 18plus	202
		NUCLEOSIL® C_{18}	220
		NUCLEOSIL® C_{18} AB	220
		NUCLEOSIL® C_{18} HD	220
		NUCLEOSIL® Nautilus	221
		NUCLEOSIL® C_{18} MPN	250
		NUCLEOSIL® C_{18} PPN	251
USP L3	porous silica particles, 1.5 to 10 µm diameter, or monolithic silica gel	NUCLEODUR® SiOH	190
		NUCLEOSIL® SiOH	230
USP L7	octyl silane chemically bonded to totally porous silica particles, 1.8 to 10 µm diameter	NUCLEODUR® C_8 ec	181
		NUCLEODUR® C_8 Gravity	158
		NUCLEOSIL® C_8	224
		NUCLEOSIL® C_8 HD	224
USP L8	an essentially monomolecular layer of aminopropyl silane chemically bonded to totally porous silica gel support, 1.5 to 10 µm diameter	NUCLEODUR® NH_2 / NH_2-RP	188
		NUCLEOSIL® Carbohydrate	254
		NUCLEOSIL® NH_2 / NH_2-RP	227
USP L9	irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm diameter	NUCLEOSIL® SA	229
USP L10	nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® CN / CN-RP	186
		NUCLEOSIL® CN / CN-RP	228
USP L11	phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm diameter	NUCLEODUR® Phenyl-Hexyl	170
		NUCLEODUR® π^2	172
		NUCLEOSHELL® Phenyl-Hexyl	207
		NUCLEODUR® Sphinx RP	176
		NUCLEOSIL® C_6H_5	226
USP L14	silica gel having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm diameter	NUCLEOSIL® SB	229
USP L16	dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm diameter	NUCLEOSIL® C_2	225
USP L17	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the H form, 6 to 12 µm diameter	NUCLEOGEL® ION 300 OA	256
		NUCLEOGEL® SUGAR 810 H	255
USP L19	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the Ca form, 5 to 15 µm particle size	NUCLEOGEL® SUGAR 810 Ca	255
		NUCLEOGEL® SUGAR Ca	256
USP L20	dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm diameter	NUCLEOSIL® OH (Diol)	226
USP L21	a rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm diameter	NUCLEOGEL® RP	252
USP L22	a cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size	NUCLEOGEL® SCX	247
USP L23	an anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, about 10 µm in size	NUCLEOGEL® SAX	247
USP L26	butyl silane chemically bonded to totally porous silica particles, 5 to 10 µm diameter	NUCLEODUR® C_4 ec	248
		NUCLEOSIL® C_4	225
		NUCLEOSIL® C_4 MPN	250
USP L32	a chiral ligand-exchange resin packing · L-proline copper complex covalently bonded to irregular shaped silica particles, 5 to 10 µm diameter	NUCLEOSIL® CHIRAL-1	242
USP L34	strong cation-exchange resin consisting of sulfonated cross-linked PS-DVB copolymer in the Pb form, 5 to 7 µm particle size	NUCLEOGEL® SUGAR Pb	256
USP L36	a 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5 µm aminopropyl silica	NUCLEOSIL® CHIRAL-3	243
USP L40	cellulose tris-(3,5-dimethylphenylcarbamate) coated porous silica particles, 5 to 20 µm diameter	NUCLEOCEL *DELTA*	240

USP listing

USP specification of MN HPLC phases

Code	Specification	MN HPLC Phases	Page
USP L43	pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm diameter	NUCLEODUR® PFP	174
		NUCLEOSHELL® PFP	212
USP L45	beta-cyclodextrin bonded to porous silica particles, R,S-hydroxypropyl ether derivative, 3 to 10 µm diameter	NUCLEODEX β-OH, β-PM	238
USP L58	strong cation-exchange resin consisting of sulfonated cross-linked PS/DVB copolymer in the Na form, 6 to 30 µm diameter	NUCLEOGEL® SUGAR Na	256
USP L60	spherical porous silica gel, particle size of 10 µm diameter or smaller, the surface of which has been covalently modified with alkyl amide groups and endcapped	NUCLEODUR® PolarTec	168
		NUCLEOSIL® C_{18} Nautilus	220
USP L75	A chiral-recognition protein, bovine serum albumin (BSA), chemically bonded to silica particles, about 7 µm in diameter, with a pore size of 300 Angstrom	RESOLVOSIL BSA-7	241
USP L118	Aqueous polymerized C_{18} groups on silica particles, 1.2 to 5 µm in diameter	NUCLEODUR® C_{18} PAH	234
		NUCLEOSIL® C_{18} PAH	236

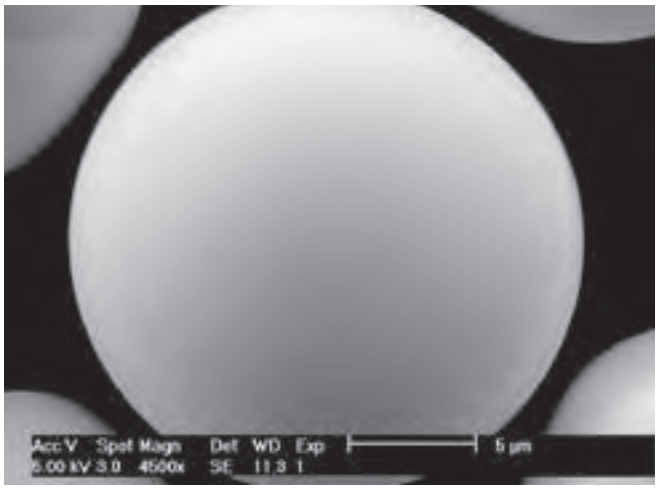
NUCLEODUR® high purity silica for HPLC

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

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

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

Particle shape and surface symmetry



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

Purity

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

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

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

Pressure stability

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

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

Physical data of NUCLEODUR®

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

NUCLEODUR® modifications

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

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

1.8 µm particles for increased separation efficiency

Key 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 fractionated to limit the increase in back pressure.

Availability

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

Advantages of 1.8 µm particle size

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

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

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

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

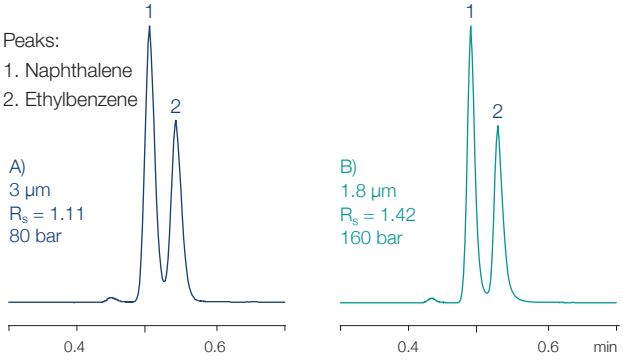
Significant improvement in resolution

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

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

Resolution as a function of particle size

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



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

Column back pressure

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

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

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

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

Comparison of back pressures

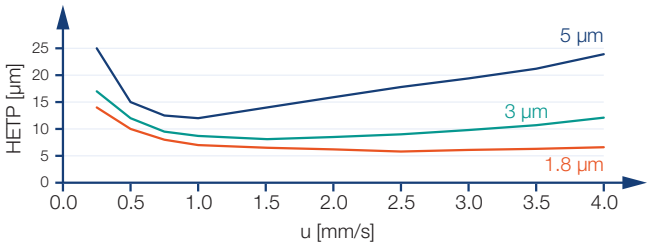
Eluent 100 % methanol, flow rate 1.5 mL/min
temperature 22 °C, column dimensions 50 × 4.6 mm

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

Higher flow rates and shorter run times

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

Van Deemter curves



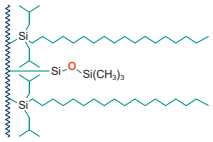
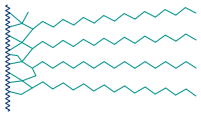
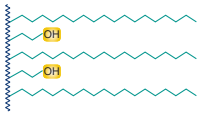
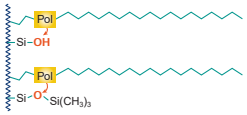
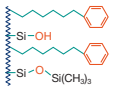
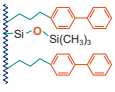
Column 50 x 4.6 mm, acetonitrile – water (50:50, v/v), analyte toluene

Technical requirements

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

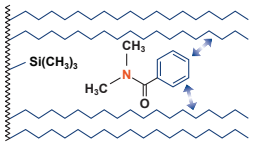
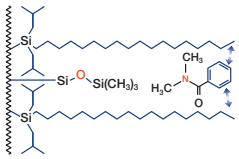
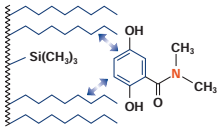
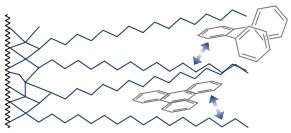
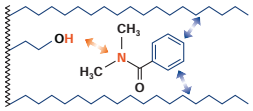
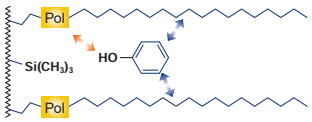
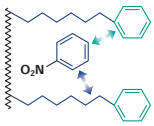
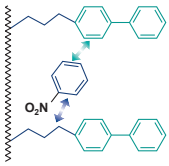
NUCLEODUR® phase overview

Overview of NUCLEODUR® HPLC phases

Phase	Specification	Page	Characteristic*	Stability	Structure
C_{18} Gravity	octadecyl, high density coating, multi-endcapping 18 % C · USP L1	158	A ●●●●● B ◖ C ●●◖	pH 1 – 11, suitable for LC/MS	
C_{18} Gravity-SB	octadecyl (monomeric), extensive endcapping 13 % C · USP L1	162	A ●●●● B ●●◖ C -	pH 1 – 9, suitable for LC/MS	
C_8 Gravity	octyl, high density coating, multi-endcapping 11 % C · USP L7	158	A ●●◖ B ● C ●◖	pH 1 – 11, suitable for LC/MS	
C_{18} Isis	octadecyl phase with specially crosslinked surface modification endcapping 20 % C · USP L1	164	A ●●●●◖ B ●● C ●●●●●	pH 1 – 10, suitable for LC/MS	
C_{18} Pyramid	octadecyl with polar endcapping 14 % C · USP L1	166	A ●●●● B ●●◖ C ●●	stable in 100 % aqueous eluent, pH 1 – 9, suitable for LC/MS	
PolarTec	octadecyl with embedded polar group 17 % C · USP L1 and L60	168	A ●●●● B ●●◖ C ●●●●	stable in 100 % aqueous eluent, pH 1 – 9, suitable for LC/MS	
Phenyl-Hexyl	phenylhexyl, multi-endcapping 10 % C · USP L11	170	A ●● B ●●● C ●	pH 1 – 10, suitable for LC/MS	
π^2	biphenylpropyl, multi-endcapping 17 % C · USP L11	172	A ●● B ●●●● C ●●●	pH 3 – 10	

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

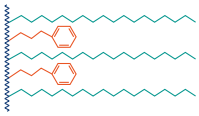
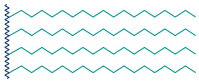
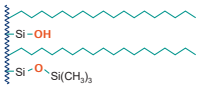
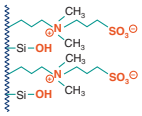
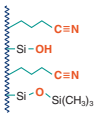
NUCLEODUR® phase overview

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

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

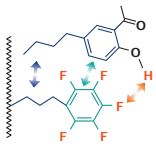
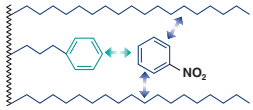
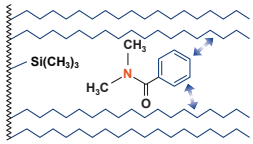
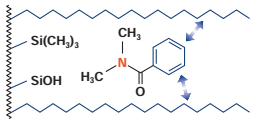
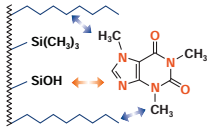
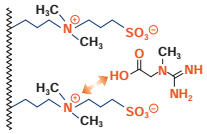
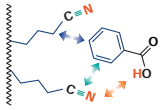
NUCLEODUR® phase overview

Overview of NUCLEODUR® HPLC phases

Phase	Specification	Page	Characteristic*	Stability	Structure
PFP	pentafluorophenylpropyl, multi-endcapping 8 % C · USP L43	174	A ●● B ●●●● C ●●●●	pH 1 – 9, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
Sphinx RP	bifunctional, balanced ratio of propylphenyl and octadecyl, endcapping 15 % C · USP L1 and L11	176	A ●●● B ●●◖ C ●	pH 1 – 10, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C_{18} HTec	octadecyl, high density coating, high capacity, multi-endcapping 18 % C · USP L1	178	A ●●●●● B ◖ C ●●◖	pH 1 – 11, suitable for LC/MS	NUCLEODUR® (Si-O$_2$)$_n$ 
C_{18} ec	octadecyl, medium density, endcapping available in 110 Å and 300 Å pore size 17.5 % / 4 % C · USP L1	181	A ●●●● B ● C ●●●◖	pH 1 – 9	NUCLEODUR® (Si-O$_2$)$_n$ 
C_8 ec	octyl, medium density, endcapping 10.5 % C · USP L7	181	A ●● B ●◖ C ●●◖	pH 1 – 9	NUCLEODUR® (Si-O$_2$)$_n$ 
C_4 ec	butyl, medium density, endcapping, 300 Å pore size 2.5 % C · USP L26	181	A ● B ●● C ●◖	pH 1 – 9	NUCLEODUR® (Si-O$_2$)$_n$ 
HILIC	zwitterionic ammonium – sulfonic acid phase 7 % C	184	A ● B ●●●●● C -	pH 2 – 8.5	NUCLEODUR® (Si-O$_2$)$_n$ 
CN / CN-RP	cyano (nitrile) for NP and RP separations 7 % C · USP L10	186	A ● B ●●●● C -	pH 1 – 8, stable towards highly aqueous mobile phases	NUCLEODUR® (Si-O$_2$)$_n$ 

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

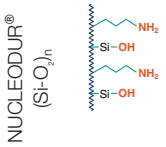
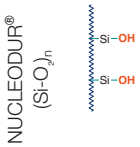
NUCLEODUR® phase overview

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

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

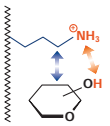
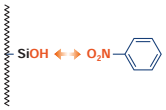
NUCLEODUR® phase overview

Overview of NUCLEODUR® HPLC phases

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

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

NUCLEODUR® phase overview

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

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

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

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

Base deactivation

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

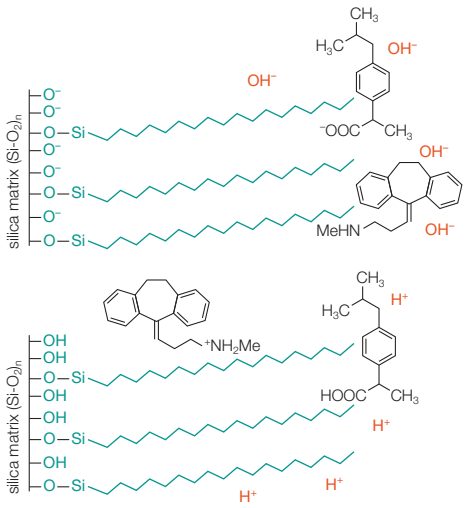
Enhanced pH stability

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

Benefits of enhanced pH stability

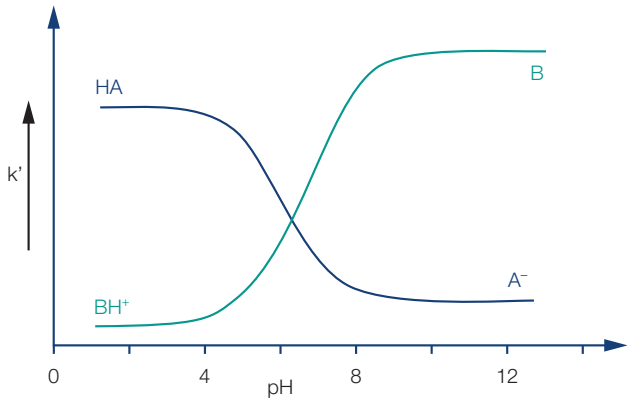
An expanded pH range is often required in method development. Many nitrogen containing compounds like basic drugs are protonated at acidic or neutral pH and exhibit poor retention on a standard C_{18} phase. The retention behavior can be improved by working at a higher pH, where the analyte is no longer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

Surface silanols at different pH values



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

Correlation between retention and pH for basic and acidic compounds



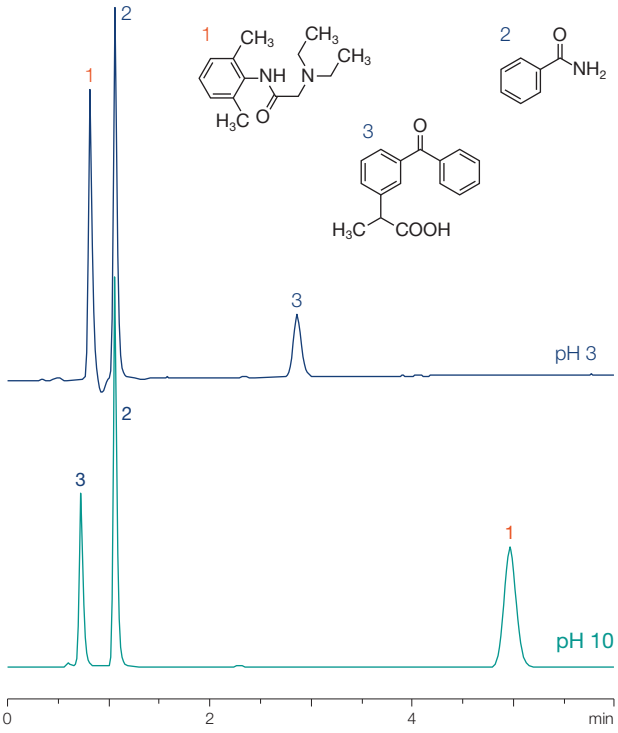
An example how selectivity can be controlled by pH is the separation of the acid ketoprofen, the base lidocaine and benzamide. Under acidic conditions the protonated lidocaine is eluted very fast due to lack of sufficiently strong hydrophobic interactions between analyte and C_{18} chains, while the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

Influence of the pH value on selectivity

MN Appl. No. 120860

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

Peaks:
1. Lidocaine
2. Benzamide
3. Ketoprofen



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

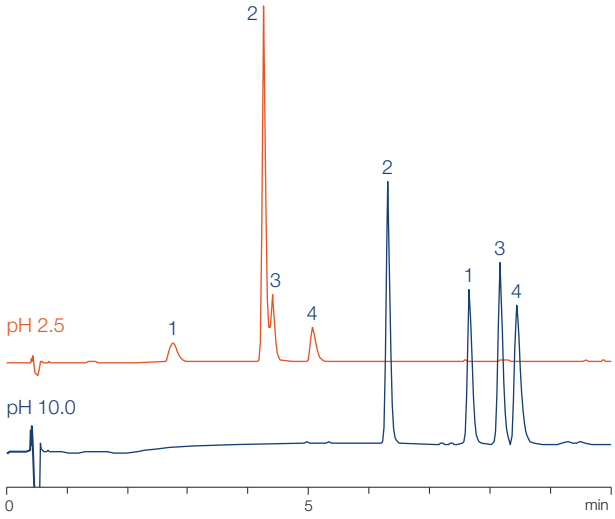
At pH 2.5 the protonated analytes exhibit poor retention (early elution) and in addition an inadequate resolution for papaverine and noscapine, whilst the formally non ionized molecules can be baseline separated due to the better retention pattern at alkaline pH.

Separation of basic alkaloids

MN Appl. No. 118010

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

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



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

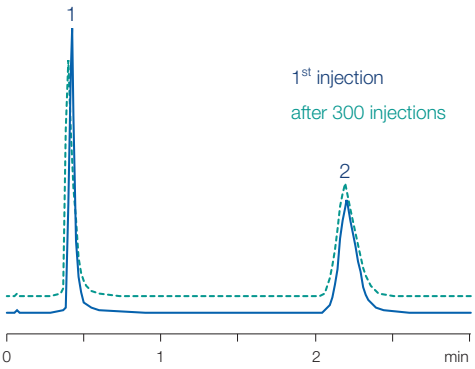
NUCLEODUR® columns

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

MN Appl. No. 120850

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

Peaks:
1. Theophylline
2. Caffeine



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

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

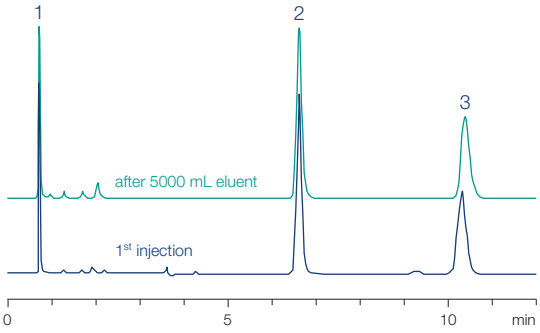
The following chromatograms show the excellent column stability of NUCLEODUR® C_{18} Gravity in acidic conditions. Retention times of all three compounds in the column performance test remain consistent and virtually unchanged, even after the column is run with 5000 mL eluent. Due to the extremely stable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

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

MN Appl. No. 120840

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

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



Eluent in column acetonitrile – water

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

Eluent in column acetonitrile – water

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

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_8 Gravity, 1.8 µm; octyl phase, particle size 1.8 µm, 11 % C · UHPLC							
Analytical EC columns							
2 mm	760756.20	760755.20	760760.20	760757.20		760759.20	
3 mm	760756.30	760755.30		760757.30			
4 mm	760756.40	760755.40		760757.40			
4.6 mm	760756.46	760755.46		760757.46			
EC guard columns*		4 × 2 mm: 761905.20		4 × 3 mm: 761905.30			
NUCLEODUR® C_8 Gravity, 5 µm; octyl phase, particle size 5 µm, 11 % C							
Analytical EC columns							
2 mm		760750.20		760754.20	760751.20	760752.20	760753.20
3 mm		760750.30		760754.30	760751.30	760752.30	760753.30
4 mm		760750.40		760754.40	760751.40	760752.40	760753.40
4.6 mm		760750.46	760749.46	760754.46	760751.46	760752.46	760753.46
EC guard columns*		4 × 2 mm: 761907.20		4 × 3 mm: 761907.30			
Preparative VarioPrep columns							
10 mm		762081.100			762071.100		762070.100
21 mm		762081.210			762071.210	762082.210	762070.210
VP guard columns **		10 × 8 mm: 762097.80		10 × 16 mm: 762097.160			
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

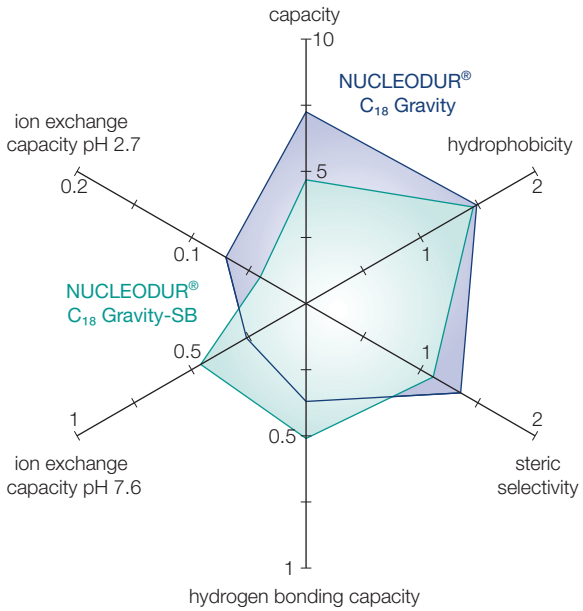
Recommended application

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

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

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

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



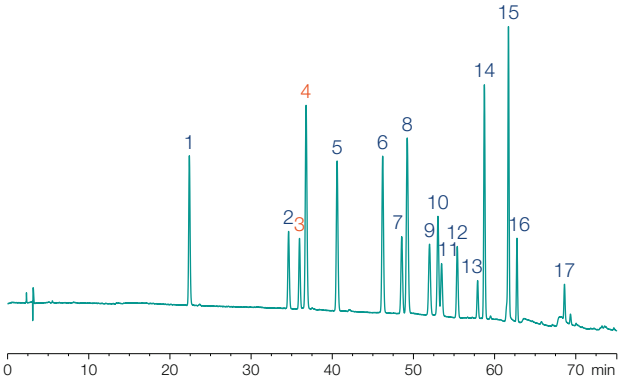
Pesticide mix (Ehrenstorfer, 17 components)

MN Appl. No. 127330

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

Peaks:

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



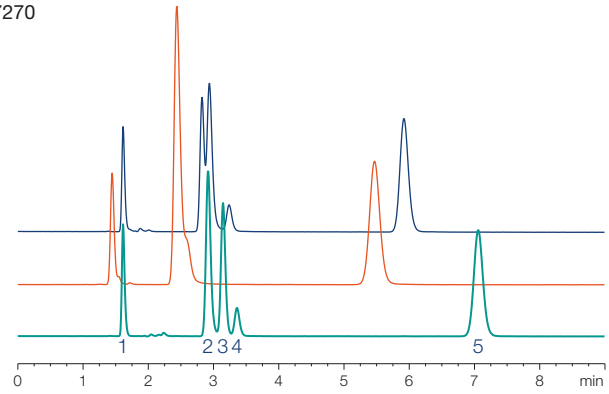
Good separation of the critical pair hexazinone/simazine

Selectivity comparison of nucleobases

MN Appl. No. 127270

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

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



Better resolution of early eluting analyte

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Gravity-SB, 1.8 µm; particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760591.20	760593.20	760595.20	760596.20		760598.20	
3 mm	760591.30	760593.30		760596.30			
4 mm	760591.40	760593.40		760596.40			
4.6 mm	760591.46	760593.46		760596.46			
EC guard columns*		4 × 2 mm: 761990.20		4 × 3 mm: 761990.30			
NUCLEODUR® C_{18} Gravity-SB, 3 µm; particle size 3 µm							
Analytical EC columns							
2 mm		760603.20		760606.20	760607.20	760608.20	760609.20
3 mm		760603.30		760606.30	760607.30	760608.30	760609.30
4 mm		760603.40		760606.40	760607.40	760608.40	760609.40
4.6 mm		760603.46	760605.46	760606.46	760607.46	760608.46	760609.46
EC guard columns*		4 × 2 mm: 761991.20		4 × 3 mm: 761991.30			
NUCLEODUR® C_{18} Gravity-SB, 5 µm; particle size 5 µm							
Analytical EC columns							
2 mm		760613.20		760616.20	760617.20	760618.20	760619.20
3 mm		760613.30		760616.30	760617.30	760618.30	760619.30
4 mm		760613.40		760616.40	760617.40	760618.40	760619.40
4.6 mm		760613.46	760615.46	760616.46	760617.46	760618.46	760619.46
EC guard columns*		4 × 2 mm: 761992.20		4 × 3 mm: 761992.30			
Preparative VarioPrep columns							
10 mm		762350.100			762351.100		762353.100
21 mm		762350.210			762351.210		762353.210
32 mm							762353.320
40 mm						762352.400	762353.400
VP guard columns **		10 × 8 mm: 762354.80		10 × 16 mm: 762354.160		15 × 32 mm: 762355.320	
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

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

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

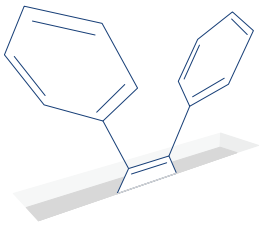
Recommended application
- Steroids, (*o*,*p*,*m*-)substituted aromatics, fat-soluble vitamins

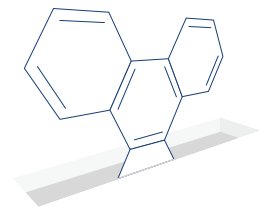
Surface modification

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

Slot Model

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





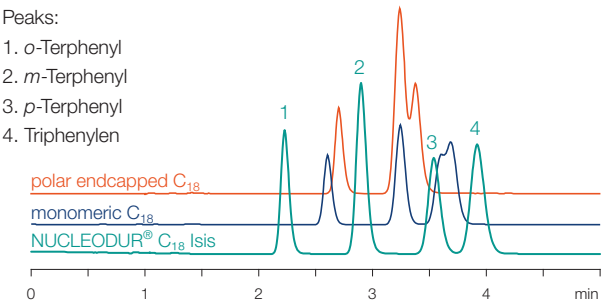
Steric selectivity

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

Steric selectivity of NUCLEODUR® C_{18} Isis

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

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

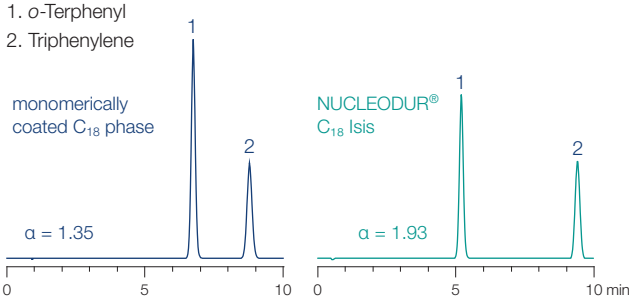


The separation of o-terphenyl and triphenylene is a good example to evaluate the selectivity of a RP column in terms of the shape of two molecules. The phenyl rings of *o*-terphenyl are twisted out of plane while triphenylene has a planar geometry. The separation factor α is a measure for the steric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

Steric selectivity of NUCLEODUR® C_{18} Isis

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

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



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

Surface deactivation

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

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Isis, 1.8 µm; particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760406.20	760405.20	760396.20	760407.20		760409.20	
	3 mm	760406.30	760405.30		760407.30			
	4 mm	760406.40	760405.40		760407.40			
	4.6 mm	760406.46	760405.46		760407.46			
EC guard columns*			4 × 2 mm: 761910.20		4 × 3 mm: 761910.30			
NUCLEODUR® C_{18} Isis, 3 µm; particle size 3 µm								
Analytical EC columns								
	2 mm		760400.20		760401.20	760402.20	760403.20	760404.20
	3 mm		760400.30		760401.30	760402.30	760403.30	760404.30
	4 mm		760400.40		760401.40	760402.40	760403.40	760404.40
	4.6 mm		760400.46	760397.46	760401.46	760402.46	760403.46	760404.46
EC guard columns*			4 × 2 mm: 761911.20		4 × 3 mm: 761911.30			
NUCLEODUR® C_{18} Isis, 5 µm; particle size 5 µm								
Analytical EC columns								
	2 mm		760410.20		760415.20	760412.20	760413.20	760414.20
	3 mm		760410.30		760415.30	760412.30	760413.30	760414.30
	4 mm		760410.40		760415.40	760412.40	760413.40	760414.40
	4.6 mm		760410.46	760416.46	760415.46	760412.46	760413.46	760414.46
EC guard columns*			4 × 2 mm: 761912.20		4 × 3 mm: 761912.30			
Preparative VarioPrep columns								
	10 mm		762404.100			762405.100		762403.100
	21 mm		762404.210			762405.210		762403.210
	32 mm							762403.320
	40 mm						762406.400	762403.400
VP guard columns **			10 × 8 mm: 762420.80		10 × 16 mm: 762420.160		15 × 32 mm: 762422.320	
EC and VarioPrep columns in packs of 1, guard columns see below.								

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

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

RP-HPLC with highly aqueous mobile phases

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

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

Stability features

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

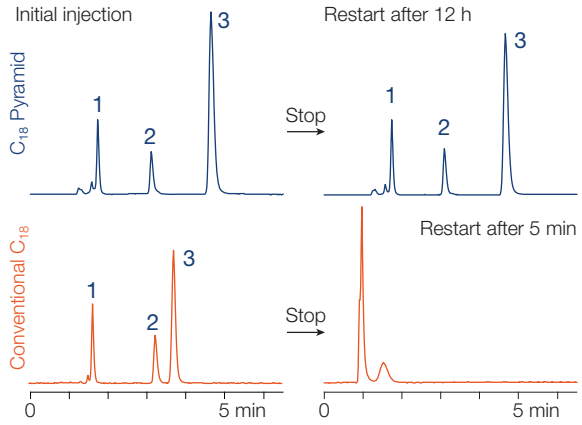
It can be shown that the retention times for NUCLEODUR® C_{18} Pyramid remain nearly unchanged between initial injection and restart after the flow has been stopped for 12 h, whilst the performance of the conventional RP column already collapsed totally after 5 min.

Stability test

MN Appl. No. 120870

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

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



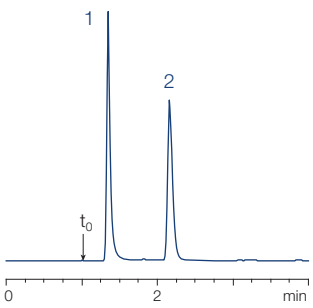
Retention characteristics

Separation of very polar compounds

MN Appl. No. 119170

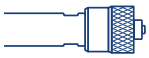
Column:	125 x 4 mm NUCLEODUR® C_{18} Pyramid, 5 µm
Eluent:	0.2 % H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	22 °C
Detection:	UV, 202 nm
Injection:	2 µL

Peaks:
1. Formic acid
2. Acetic acid



The polar surface exhibits retention characteristics different from conventional C_{18} phases. Application 119170 shows the improved retention behavior of the very polar short chain organic acids, which are insufficiently retained on RP columns with predominantly hydrophobic surface properties. In addition to the exceptional polar selectivity NUCLEODUR® C_{18} Pyramid also provides adequate hydrophobic retention (see application No. 19190 at *https://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 (see application 119200 at *https://chromaappdb.mn-net.com*).

Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} Pyramid, 1.8 µm; particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760271.20	760272.20	760275.20	760273.20		760274.20	
	3 mm	760271.30	760272.30		760273.30			
	4 mm	760271.40	760272.40		760273.40			
	4.6 mm	760271.46	760272.46		760273.46			
EC guard columns*			4 × 2 mm: 761915.20		4 × 3 mm: 761915.30			
NUCLEODUR® C_{18} Pyramid, 3 µm; particle size 3 µm								
Analytical EC columns								
	2 mm		760263.20		760264.20	760260.20	760261.20	760262.20
	3 mm		760263.30		760264.30	760260.30	760261.30	760262.30
	4 mm		760263.40		760264.40	760260.40	760261.40	760262.40
	4.6 mm		760263.46	760259.46	760264.46	760260.46	760261.46	760262.46
EC guard columns*			4 × 2 mm: 761916.20		4 × 3 mm: 761916.30			
NUCLEODUR® C_{18} Pyramid, 5 µm; particle size 5 µm								
Analytical EC columns								
	2 mm		760200.20		760204.20	760201.20	760203.20	760202.20
	3 mm		760200.30		760204.30	760201.30	760203.30	760202.30
	4 mm		760200.40		760204.40	760201.40	760203.40	760202.40
	4.6 mm		760200.46	760205.46	760204.46	760201.46	760203.46	760202.46
EC guard columns*			4 × 2 mm: 761917.20		4 × 3 mm: 761917.30			
Preparative VarioPrep columns								
	10 mm		762271.100			762273.100		762272.100
	21 mm		762271.210			762273.210		762272.210
	32 mm							762272.320
	40 mm						762269.400	762272.400
VP guard columns **			10 × 8 mm: 762291.80		10 × 16 mm: 762291.160		15 × 32 mm: 762293.320	
EC and VarioPrep columns in packs of 1, guard columns see below.								

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

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

RP-HPLC under 100 % aqueous conditions

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

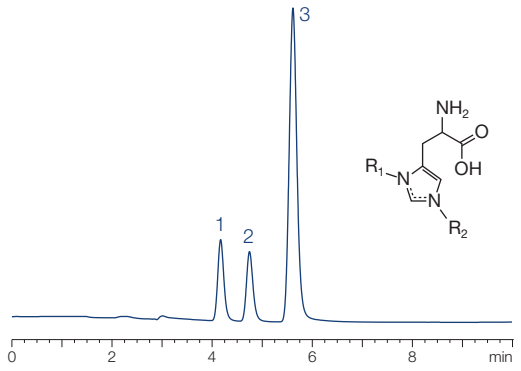
Separation of histidines

MN Appl. No. 125140

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

Peaks:

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



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

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

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

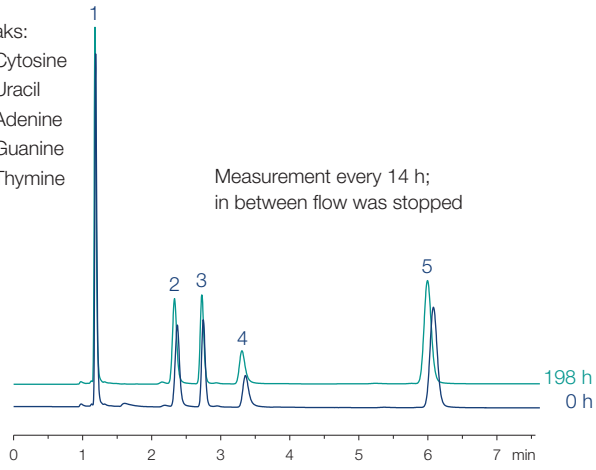
Even after days or weeks of operation in purely aqueous eluents the C_{18} chains of NUCLEODUR® PolarTec are neither folded nor show any collapsing. A significant reduction of retention time cannot be observed.

Stability of NUCLEODUR® PolarTec

MN Appl. No. 124610

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

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



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

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® PolarTec, 1.8 µm; particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760461.20	760463.20	760465.20	760466.20		760468.20	
3 mm	760461.30	760463.30		760466.30			
4 mm	760461.40	760463.40		760466.40			
4.6 mm	760461.46	760463.46		760466.46			
EC guard columns*		4 × 2 mm: 761980.20		4 × 3 mm: 761980.30			
NUCLEODUR® PolarTec, 3 µm; particle size 3 µm							
Analytical EC columns							
2 mm		760473.20		760476.20	760477.20	760478.20	760479.20
3 mm		760473.30		760476.30	760477.30	760478.30	760479.30
4 mm		760473.40		760476.40	760477.40	760478.40	760479.40
4.6 mm		760473.46	760475.46	760476.46	760477.46	760478.46	760479.46
EC guard columns*		4 × 2 mm: 761981.20		4 × 3 mm: 761981.30			
NUCLEODUR® PolarTec, 5 µm; particle size 5 µm							
Analytical EC columns							
2 mm		760483.20		760486.20	760487.20	760488.20	760489.20
3 mm		760483.30		760486.30	760487.30	760488.30	760489.30
4 mm		760483.40		760486.40	760487.40	760488.40	760489.40
4.6 mm		760483.46	760485.46	760486.46	760487.46	760488.46	760489.46
EC guard columns*		4 × 2 mm: 761982.20		4 × 3 mm: 761982.30			
Preparative VarioPrep columns							
10 mm		762220.100			762221.100		762223.100
21 mm		762220.210			762221.210		762223.210
32 mm							762223.320
40 mm						762222.400	762223.400
VP guard columns **		10 × 8 mm: 762224.80		10 × 16 mm: 762224.160		15 × 32 mm: 762226.320	
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

NUCLEODUR® Phenyl-Hexyl suitable for polar / aromatic compounds · USP L11

Key feature

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

Technical data

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

Recommended application

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

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

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

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

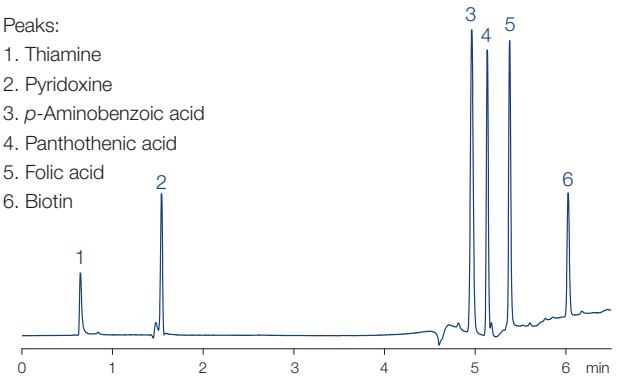
With NUCLEODUR® Phenyl-Hexyl e. g., tricyclic antidepressants or water soluble vitamins can be separated in good resolution.

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

MN Appl. No. 125920

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

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

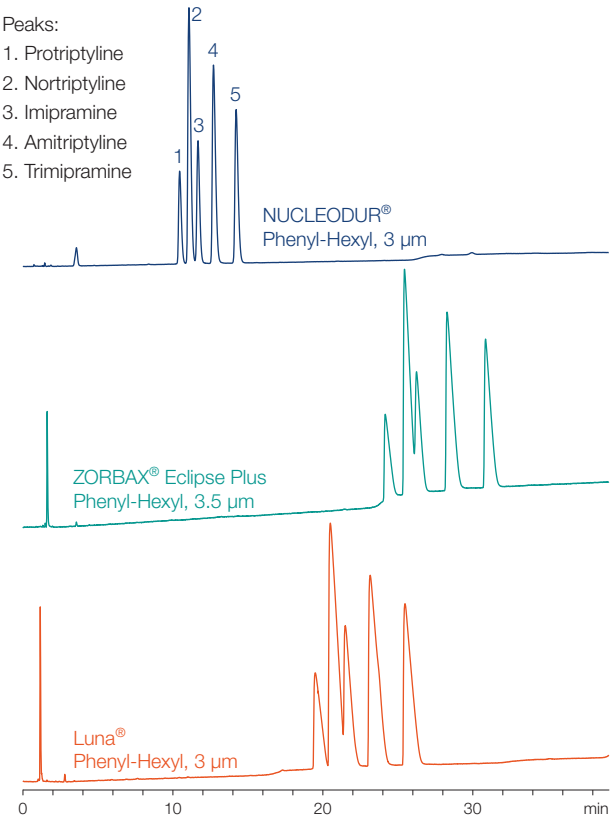


Tricyclic antidepressant (TCA)

MN Appl. No. 126020

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

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



Eluent in column acetonitrile – water

	ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® Phenyl-Hexyl, 1.8 µm; particle size 1.8 µm · UHPLC								
Analytical EC columns								
	2 mm	760561.20	760563.20	760565.20	760566.20		760568.20	
	3 mm	760561.30	760563.30		760566.30			
	4 mm	760561.40	760563.40		760566.40			
	4.6 mm	760561.46	760563.46		760566.46			
EC guard columns*			4 × 2 mm: 761985.20		4 × 3 mm: 761985.30			
NUCLEODUR® Phenyl-Hexyl, 3 µm; particle size 3 µm								
Analytical EC columns								
	2 mm		760573.20		760576.20	760577.20	760578.20	760579.20
	3 mm		760573.30		760576.30	760577.30	760578.30	760579.30
	4 mm		760573.40		760576.40	760577.40	760578.40	760579.40
	4.6 mm		760573.46	760575.46	760576.46	760577.46	760578.46	760579.46
EC guard columns*			4 × 2 mm: 761986.20		4 × 3 mm: 761986.30			
NUCLEODUR® Phenyl-Hexyl, 5 µm; particle size 5 µm								
Analytical EC columns								
	2 mm		760583.20		760586.20	760587.20	760588.20	760589.20
	3 mm		760583.30		760586.30	760587.30	760588.30	760589.30
	4 mm		760583.40		760586.40	760587.40	760588.40	760589.40
	4.6 mm		760583.46	760585.46	760586.46	760587.46	760588.46	760589.46
EC guard columns*			4 × 2 mm: 761987.20		4 × 3 mm: 761987.30			
Preparative VarioPrep columns								
	10 mm		762230.100			762231.100		762233.100
	21 mm							762233.210
	32 mm							762233.320
	40 mm							762233.400
VP guard columns **			10 × 8 mm: 762234.80		10 × 16 mm: 762234.160		15 × 32 mm: 762236.320	
EC and VarioPrep columns in packs of 1, guard columns see below.								

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

NUCLEODUR® π² hydrophobic biphenylpropyl phase · USP L11

Key feature

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

Technical data

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

Recommended application

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

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

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

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

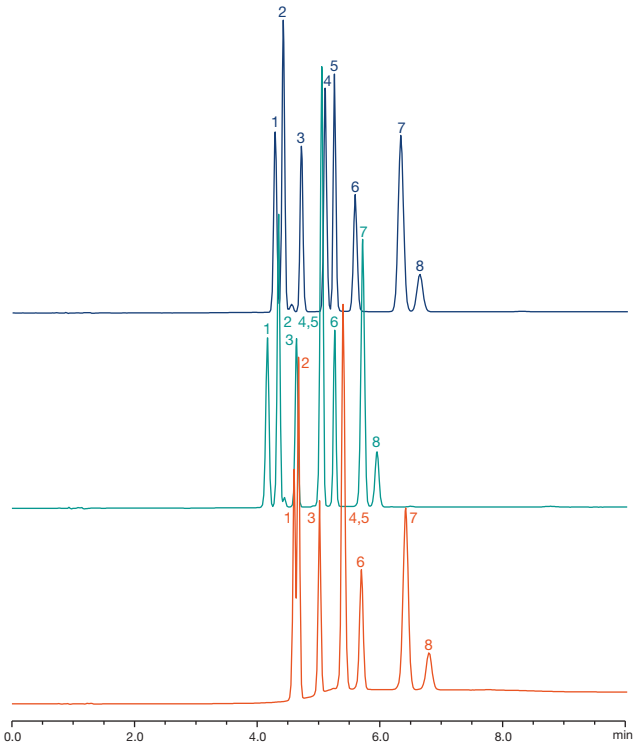
NUCLEODUR® π² exceeds other aryl phases in terms of stability under strongly aqueous conditions. Therefore i. a. steroids, sulfonamides and acidic pharmaceuticals are separated in good resolution with NUCLEODUR® π². NUCLEODUR® π² is the stationary phase with the highest aromatic analyte selectivity.

Sulfonamide antibiotics

MN Appl. No. 127920

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

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

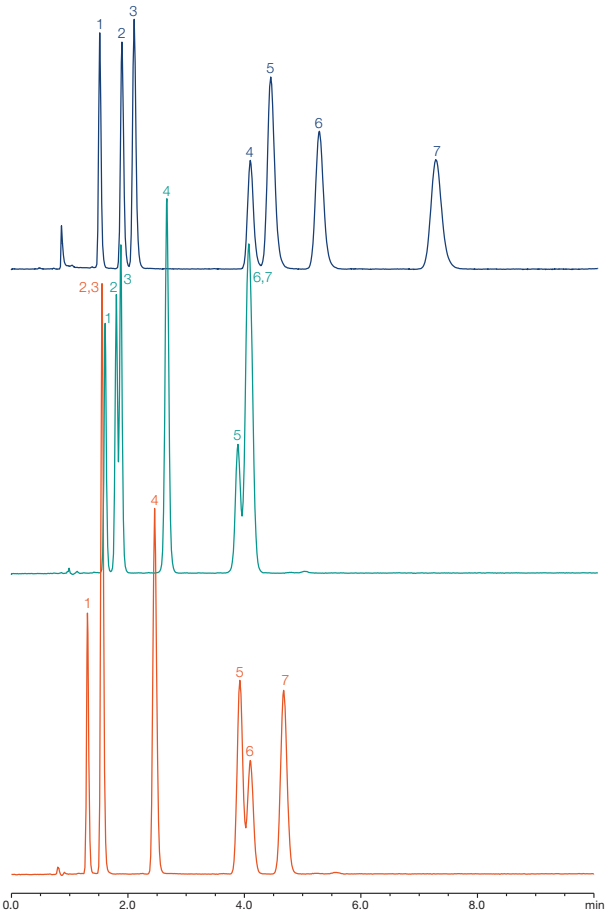


Steroids

MN Appl. No. 127910

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

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



Eluent in column acetonitrile – water

ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® π2, 5 µm; particle size 5 µm						
Analytical EC columns						
2 mm	760620.20	760621.20	760622.20	760623.20	760624.20	760625.20
3 mm	760620.30	760621.30	760622.30	760623.30	760624.30	760625.30
4 mm	760620.40	760621.40	760622.40	760623.40	760624.40	760625.40
4.6 mm	760620.46	760621.46	760622.46	760623.46	760624.46	760625.46
EC guard columns*	4 × 2 mm: 761810.20		4 × 3 mm: 761810.30			
EC columns in packs of 1, guard columns in packs of 3.						

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

NUCLEODUR® PFP hydrophobic pentafluorophenyl phase · USP L43

Key feature

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

Technical data

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

Recommended application

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

Orthogonality in selectivity

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

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

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

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

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

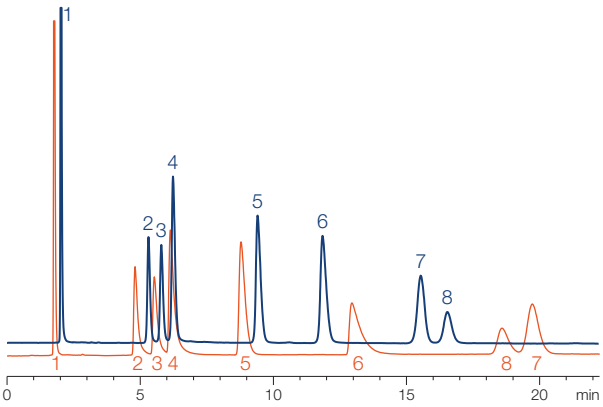
Applications in the areas of (bio-)pharma, natural compounds and environment show the broad applicability of this phase.

Separation of antihistamines

MN Appl. No. 124861

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

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



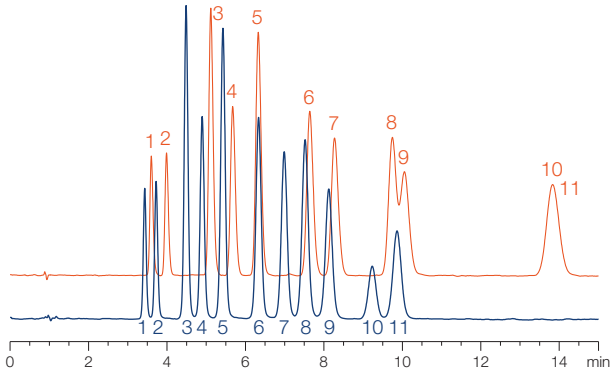
Separation of phenol isomers

MN Appl. No. 124531

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

Peaks:

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



Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® PFP, 1.8 µm; particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760431.20	760433.20	760435.20	760436.20		760438.20	
3 mm	760431.30	760433.30		760436.30			
4 mm	760431.40	760433.40		760436.40			
4.6 mm	760431.46	760433.46		760436.46			
EC guard columns*		4 × 2 mm: 761975.20		4 × 3 mm: 761975.30			
NUCLEODUR® PFP, 3 µm; particle size 3 µm							
Analytical EC columns							
2 mm		760443.20		760446.20	760447.20	760448.20	760449.20
3 mm		760443.30		760446.30	760447.30	760448.30	760449.30
4 mm		760443.40		760446.40	760447.40	760448.40	760449.40
4.6 mm		760443.46	760445.46	760446.46	760447.46	760448.46	760449.46
EC guard columns*		4 × 2 mm: 761976.20		4 × 3 mm: 761976.30			
NUCLEODUR® PFP, 5 µm; particle size 5 µm							
Analytical EC columns							
2 mm		760453.20		760456.20	760457.20	760458.20	760459.20
3 mm		760453.30		760456.30	760457.30	760458.30	760459.30
4 mm		760453.40		760456.40	760457.40	760458.40	760459.40
4.6 mm		760453.46	760455.46	760456.46	760457.46	760458.46	760459.46
EC guard columns*		4 × 2 mm: 761977.20		4 × 3 mm: 761977.30			
Preparative VarioPrep columns							
10 mm		762210.100			762211.100		762213.100
21 mm		762210.210			762211.210		762213.210
32 mm							762213.320
40 mm						762212.400	762213.400
VP guard columns **		10 × 8 mm: 762214.80		10 × 16 mm: 762214.160		15 × 32 mm: 762216.320	
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

- Quinolone antibiotics, sulfonamides, xanthines, substituted aromatics

Alternative RP selectivity

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

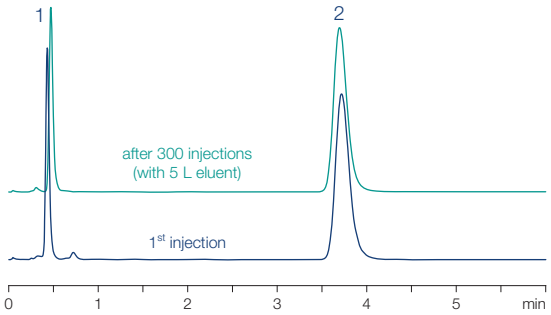
For the separation of polar compounds NUCLEODUR® Sphinx RP can be especially recommended and can also outperform many customary C_{18} phases. In addition, exhaustive endcapping steps minimize unwanted surface silanol activity and guarantee excellent peak shapes even for strong basic analytes.

Stability of NUCLEODUR® Sphinx RP at pH 10

MN Appl. No. 120900

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

Peaks:
1. Theophylline
2. Caffeine

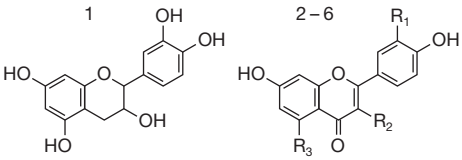


Different from standard phenyl phases, NUCLEODUR® Sphinx RP is far more stable towards hydrolysis and is also suggested for LC/MS applications. Due to the additional intermolecular interactions NUCLEODUR® Sphinx RP is an interesting replenishment to the high density bonded phases NUCLEODUR® C_8 / C_{18}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

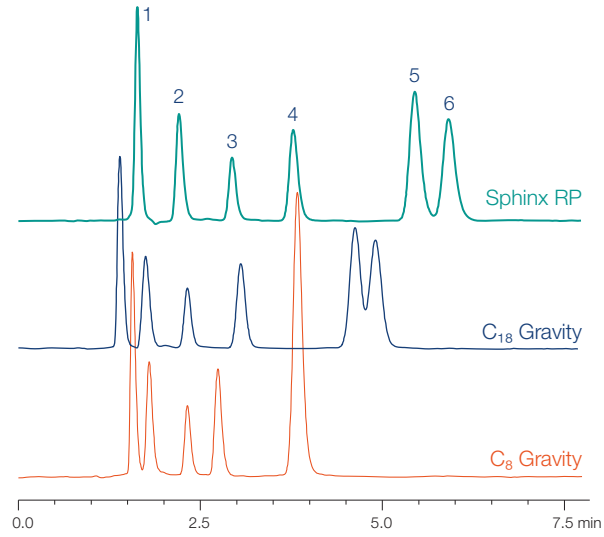
Separation of flavonoids on three different NUCLEODUR® phases

MN Appl. No. 119830

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



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



Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® Sphinx RP, 1.8 µm; particle size 1.8 µm · UHPLC							
Analytical EC columns							
2 mm	760821.20	760822.20	760825.20	760823.20		760824.20	
3 mm	760821.30	760822.30		760823.30			
4 mm	760821.40	760822.40		760823.40			
4.6 mm	760821.46	760822.46		760823.46			
EC guard columns*		4 × 2 mm: 761920.20		4 × 3 mm: 761920.30			
NUCLEODUR® Sphinx RP, 3 µm; particle size 3 µm							
Analytical EC columns							
2 mm		760806.20		760812.20	760807.20	760805.20	760808.20
3 mm		760806.30		760812.30	760807.30	760805.30	760808.30
4 mm		760806.40		760812.40	760807.40	760805.40	760808.40
4.6 mm		760806.46	760813.46	760812.46	760807.46	760805.46	760808.46
EC guard columns*		4 × 2 mm: 761921.20		4 × 3 mm: 761921.30			
NUCLEODUR® Sphinx RP, 5 µm; particle size 5 µm							
Analytical EC columns							
2 mm		760800.20		760809.20	760801.20	760802.20	760803.20
3 mm		760800.30		760809.30	760801.30	760802.30	760803.30
4 mm		760800.40		760809.40	760801.40	760802.40	760803.40
4.6 mm		760800.46	760815.46	760809.46	760801.46	760802.46	760803.46
EC guard columns*		4 × 2 mm: 761922.20		4 × 3 mm: 761922.30			
Preparative VarioPrep columns							
10 mm		762372.100			762375.100		762373.100
21 mm		762372.210			762375.210		762373.210
32 mm							762373.320
40 mm						762371.400	762373.400
VP guard columns **		10 × 8 mm: 762390.80		10 × 16 mm: 762390.160		15 × 32 mm: 762392.320	
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

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

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

Selectivity and base deactivation

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

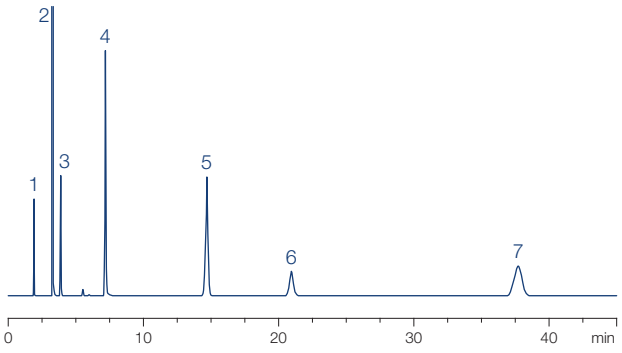
Engelhardt test

MN Appl. No. 123580

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

Peaks:

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



Stability and lifetime

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

pH stability test

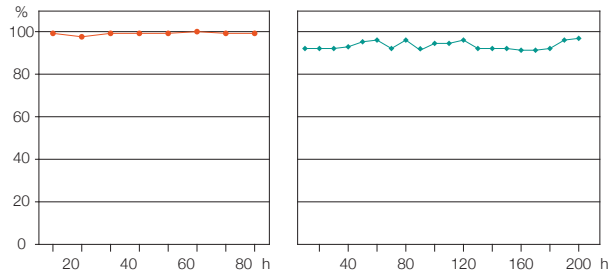
Column:	150 x 4 mm NUCLEODUR® C_{18} HTec, 5 µm
Flow rate:	1 mL/min
Detection:	UV, 254 nm
Injection:	5 µL

• pH 1:

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

• pH 10:

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



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

Up-scaling

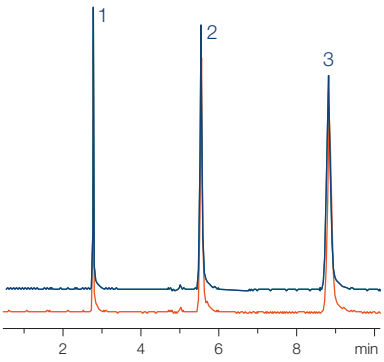
Due to highest quality standards in silica production and phase chemistry combined with optimized packing technology, NUCLEODUR® C_{18} HTec allows exceptional transferability from analytical to preparative scale with respect to different particle sizes (e. g., 5, 7 or 10 µm) as well as column dimensions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

Up-scaling with NUCLEODUR® C_{18} HTec

MN Appl. No. 123780

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

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



Capacity

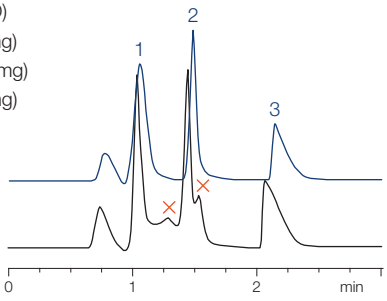
A vital criterion for efficiency in preparative HPLC is the capacity of the separation medium. NUCLEODUR® C_{18} HTec is characterized by a notably high loading capacity under both basic and acidic conditions, while competitor columns show overload effects even at lower loads (×).

Loading capacity under acidic conditions

MN Appl. No. 123890

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

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



Eluent in column acetonitrile – water

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

NUCLEODUR® columns

Eluent in column acetonitrile – water

ID	Length → 30 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® C_{18} HTec, 5 µm; particle size 5 µm							
Analytical EC columns							
2 mm		760311.20		760313.20	760314.20	760315.20	760316.20
3 mm		760311.30		760313.30	760314.30	760315.30	760316.30
4 mm		760311.40		760313.40	760314.40	760315.40	760316.40
4.6 mm		760311.46	760312.46	760313.46	760314.46	760315.46	760316.46
EC guard columns*		4 × 2 mm: 761927.20		4 × 3 mm: 761927.30			
Preparative VarioPrep columns							
10 mm		762551.100			762554.100		762556.100
21 mm		762551.210		762553.210	762554.210		762556.210
32 mm				762553.320		762555.320	762556.320
40 mm						762555.400	762556.400
50 mm				762553.500		762555.500	762556.500
VP guard columns **		10 × 8 mm: 762591.80		10 × 16 mm: 762591.160			
		15 × 32 mm: 762592.320		15 × 50 mm: 762592.500			
NUCLEODUR® C_{18} HTec, 7 µm; particle size 7 µm							
Preparative VarioPrep columns							
10 mm		762561.100			762564.100		762566.100
21 mm		762561.210		762563.210	762564.210		762566.210
32 mm				762563.320		762565.320	762566.320
40 mm						762565.400	762566.400
50 mm				762563.500		762565.500	762566.500
VP guard columns **		10 × 8 mm: 762591.80		10 × 16 mm: 762591.160			
		15 × 32 mm: 762592.320		15 × 50 mm: 762592.500			
NUCLEODUR® C_{18} HTec, 10 µm; particle size 10 µm							
Preparative VarioPrep columns							
10 mm		762571.100			762574.100		762576.100
21 mm		762571.210		762573.210	762574.210		762576.210
32 mm				762573.320		762575.320	762576.320
40 mm						762575.400	762576.400
50 mm				762573.500		762575.500	762576.500
VP guard columns **		10 × 8 mm: 762591.80		10 × 16 mm: 762591.160			
		15 × 32 mm: 762592.320		15 × 50 mm: 762592.500			
EC and VarioPrep columns in packs of 1, guard columns see below.							

Guard column systems

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

For details of our column systems see page 258.
NUCLEODUR® C_{18} HTec bulk material in 7 and 10 µm for self-packing of preparative columns see page 264.

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

Key feature

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

Technical data

- Pore size 110 Å:
  particle sizes 3 µm and 5 µm, 7 µm, 10 µm, 12 µm, 16 µm, 20 µm, 30 µm and 50 µm for preparative separations; carbon content 17.5 % for C_{18}, 10.5 % for C_8; pH stability 1 – 9; high reproducibility from lot to lot
- Pore size 300 Å:
  technical data and applications in chapter “HPLC column for biochemical separations” (see page 244)

Recommended application

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

NUCLEODUR® C_{18} ec for daily routine analysis

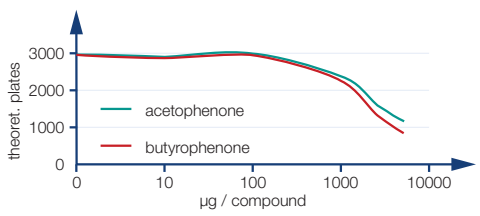
The efficiency of a separation is controlled by particle size and selectivity of the stationary phase. The exceptional surface coverage of monomeric bonded alkylsilanes, combined with an exhaustive endcapping, results in a surface with lowest silanol activity. This allows the tailing-free elution of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

Loading capacity

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

Loading curve

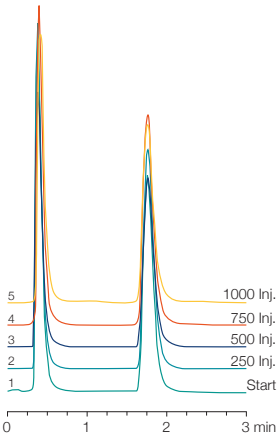
Column:	250 × 4.6 mm NUCLEODUR® 100 – 20 C_{18} ec
Eluent:	acetonitrile – H_2O 80:20 (v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 280 – 370 nm



pH stability of NUCLEODUR® C_{18} ec

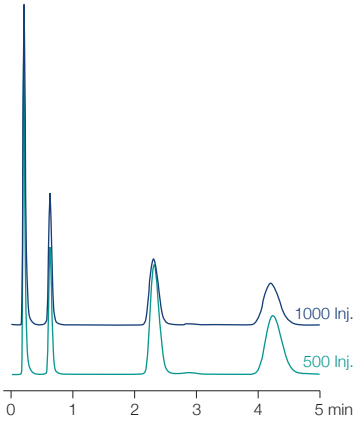
Separation of theophylline and caffeine at pH 10

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



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

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



NUCLEODUR® columns

Chemical stability

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

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

NUCLEODUR® octyl phases

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

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

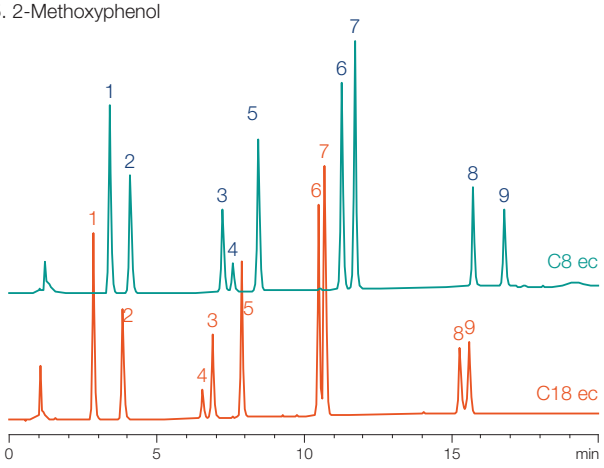
Separation of phenols

MN Appl. Nos. 120890 / 120891

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

Peaks:

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



NUCLEODUR® phases for biochromatography

A description and applications for C_{18} and C_4 modified 300 Å NUCLEODUR® widepore materials for the separation of biopolymers, like peptids and proteins can be found in chapter “HPLC column for biochemical separations” (see page 244).

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

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

Eluent in column acetonitrile – water

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

Eluent in column acetonitrile – water

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

Eluent in column acetonitrile – water

ID	Length → 50 mm	75 mm	100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 C_8 ec; octyl phase, particle size 3 µm, 10.5 % C						
Analytical EC columns						
2 mm	760063.20		760059.20	760060.20		760062.20
3 mm	760063.30		760059.30	760060.30		760062.30
4 mm	760063.40		760059.40	760060.40		760062.40
4.6 mm	760063.46	760064.46	760059.46	760060.46	760061.46	760062.46
EC guard columns*		4 × 2 mm: 761936.20		4 × 3 mm: 761936.30		
NUCLEODUR® 100-5 C_8 ec; octyl phase, particle size 5 µm, 10.5 % C						
Analytical EC columns						
2 mm	760700.20		760704.20	760701.20		760703.20
3 mm	760700.30		760704.30	760701.30		760703.30
4 mm	760700.40		760704.40	760701.40		760703.40
4.6 mm	760700.46	760706.46	760704.46	760701.46	760702.46	760703.46
EC guard columns*		4 × 2 mm: 761937.20		4 × 3 mm: 761937.30		
Preparative VarioPrep columns						
10 mm	762072.100			762061.100		762062.100
21 mm	762072.210			762061.210		762062.210
32 mm						762062.320
40 mm					762079.400	762062.400
VP guard columns **	10 × 8 mm: 762092.80		10 × 16 mm: 762092.160		15 × 32 mm: 762321.320	
EC and VarioPrep columns in packs of 1, guard columns see previous NUCLEODUR® phases.						

Guard column systems see previous NUCLEODUR® phases. For details of our column systems see page 258.
NUCLEODUR® C_{18} ec bulk material with 10 – 50 µm for self-packing of preparative columns see page 264.
The ordering information for C_{18} and C_4 modified 300 Å NUCLEODUR® widepore materials for the separation of biopolymers can be found in the chapter “HPLC column for biochemical separations” (see page 248).
* and ** for corresponding guard column systems see page 258.

NUCLEODUR® columns

NUCLEODUR® HILIC zwitterionic phase

Key feature

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

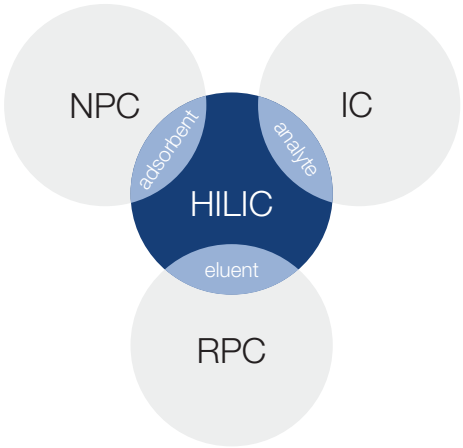
Technical data

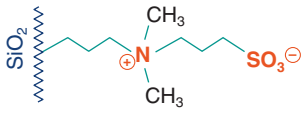
- Ammonium – sulfonic acid modified silica; pore size 110 Å; particle sizes 1.8, 3 and 5 µm; carbon content 7 %; pH stability 2 – 8.5

Recommended application

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

Hydrophilic interaction chromatography





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

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

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

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

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

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

Retention characteristic

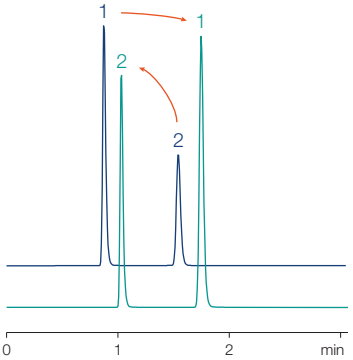
Commonly HILIC is described as partition chromatography or liquid-liquid extraction system between mobile and stationary phases. Versus a water-poor mobile phase a water-rich layer on the surface of the polar stationary phase is formed. Thus, a distribution of the analytes between these two layers will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

Separation of uracil and naphthalene

MN Appl. Nos. 122911 / 122912

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

Peaks:
1. Uracil
2. Naphthalene



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

Stability features

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

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

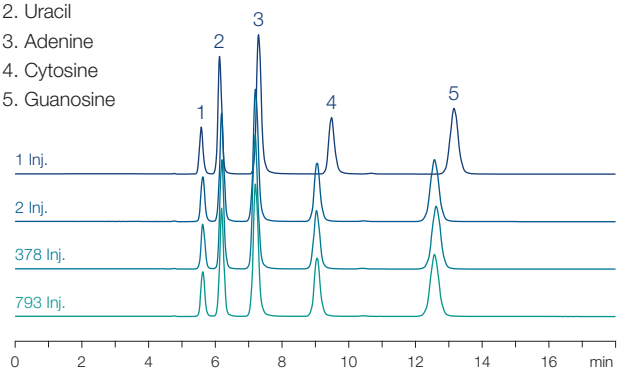
Overall NUCLEODUR® HILIC provides excellent chromatographic features and is hereby the perfect choice for separation of polar or charged compounds.

Stability and equilibration

MN Appl. No. 123100

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

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



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

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

Guard column system

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

- Tricyclic antidepressants, steroids, organic acids

Alternative bonded-phase functionality

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

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

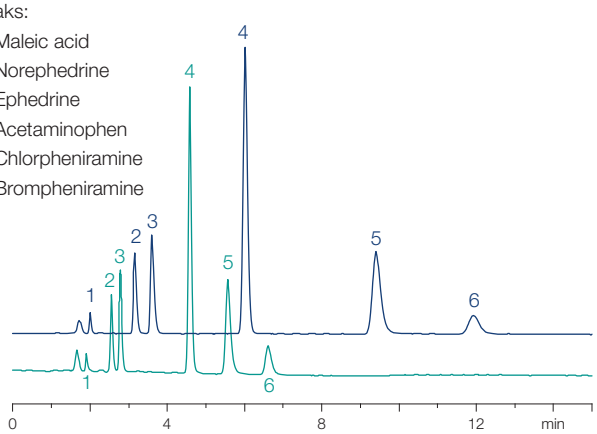
The fully endcapped and highly reproducible NUCLEODUR® 100-5 CN-RP phase has cyanopropyl groups on the surface able to generate a clearly recognizable different retention behavior compared to purely alkyl-functionalized surface modifications (see figure below).

Separation of cold medicine ingredients on two different NUCLEODUR® phases

MN Appl. No. 119340

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

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



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

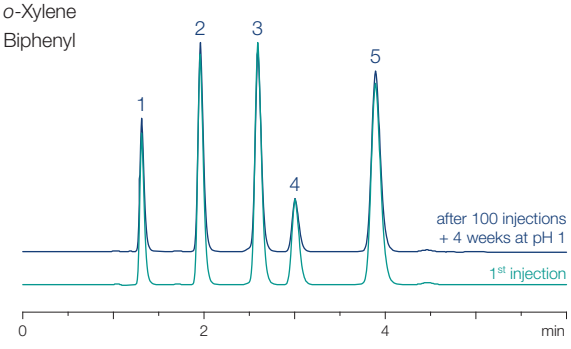
Short-chain bonded phases are sometimes suspected of revealing shortcomings in stability towards hydrolysis at low pH [10]. Application 119350 shows that even after 100 sample injections and four weeks storage at pH 1 (blue curve), neither a considerable shift in retention, nor a visible change in peak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

Stability of NUCLEODUR® CN-RP at pH 1

MN Appl. No. 119350

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

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



Multi-mode columns

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

Separation of steroids in normal phase and reversed phase mode

MN Appl. Nos. 119271 / 119272

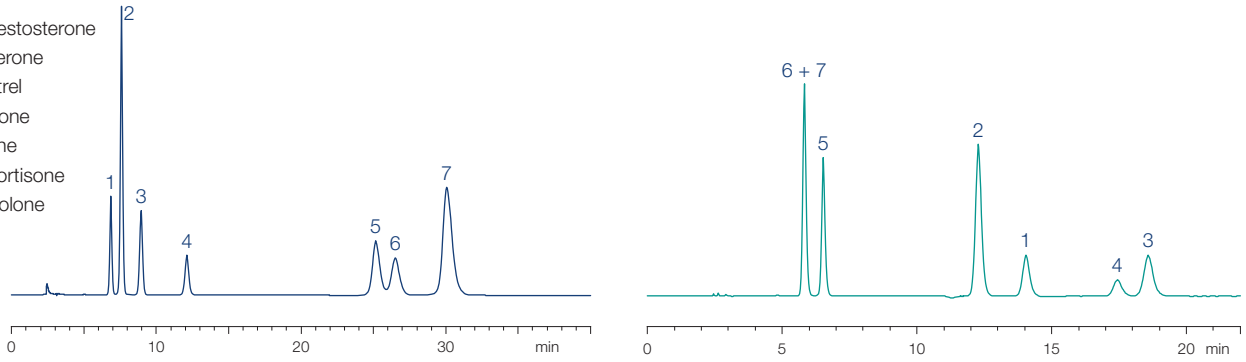
Normal phase mode

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

Reversed phase mode

Column: 250 x 4 mm NUCLEODUR® 100-5 CN-RP
Eluent: acetonitrile – water (25:75, v/v)
other conditions as for normal phase mode

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



ID	Length → 50 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 CN-RP; particle size 3 µm; eluent in column acetonitrile – water				
Analytical EC columns				
2 mm	760159.20	760157.20		
3 mm		760157.30		
4 mm			760156.40	
4.6 mm			760156.46	
EC guard columns*	4 × 2 mm: 761941.20		4 × 3 mm: 761941.30	
NUCLEODUR® 100-5 CN-RP; particle size 5 µm; eluent in column acetonitrile – water				
Analytical EC columns				
4 mm		760153.40		760152.40
4.6 mm		760153.46	760154.46	760152.46
EC guard columns*			4 × 3 mm: 761944.30	
NUCLEODUR® 100-5 CN; particle size 5 µm; eluent in column *n*-heptane				
Analytical EC columns				
4 mm		760151.40	760149.40	760150.40
4.6 mm		760151.46	760149.46	760150.46
EC guard columns*			4 × 3 mm: 761943.30	
EC columns in packs of 1, guard columns in packs of 3.				

Guard column system

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

For details of our column systems see page 258.

NUCLEODUR® columns

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

Key feature

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

Technical data

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

Recommended application

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

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

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

Multi-mode columns

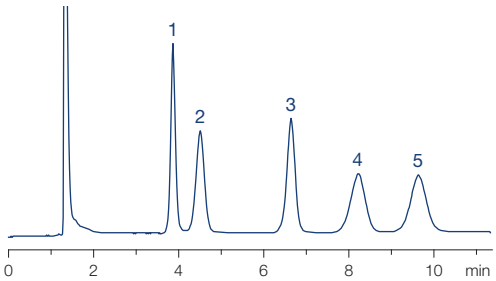
Besides cyano modifications, amino modifications belong to the most frequently used polar silica phases – both feature the important advantage, that they can be run in the RP mode using aqueous-organic eluent mixtures as well as in the NP mode, e. g., with hexane as mobile phase.

Reversed phase separation of sugars

MN Appl. No. 122160

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

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



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

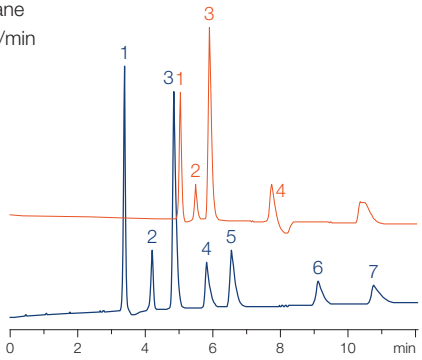
Main field of application of NUCLEODUR® NH_2 is the separation of simple and complex sugars, sugar alcohols and other hydroxy compounds under RP conditions as well as hydrocarbons under NP conditions.

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

MN Appl. No. 122180

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

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



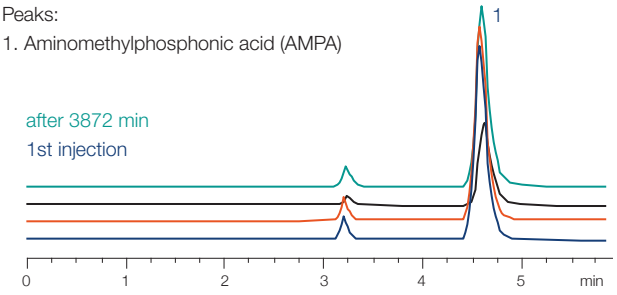
Due to the special method of surface modification NUCLEODUR® NH_2 features a pronounced stability at higher as well as at lower pH values. The following figure shows, that even after several days of exposure of the column material at pH 1.75 good separation efficiency and peak symmetry are maintained.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

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

Hydrolytical resistance of NUCLEODUR® NH_2-RP

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

Peaks:
1. Aminomethylphosphonic acid (AMPA)

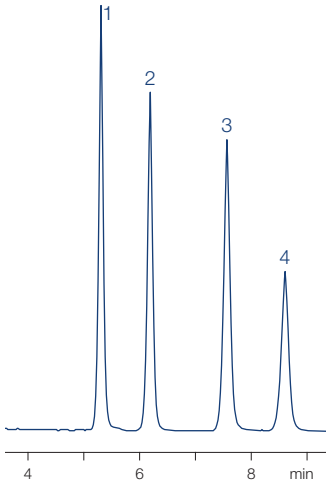


Separation of DNA bases

MN Appl. No. 122170

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

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



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

ID	Length → 100 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3 NH_2-RP; particle size 3 µm; eluent in column acetonitrile – water				
Analytical EC columns				
2 mm	760740.20	760741.20		
4.6 mm			760742.46	760739.46
EC guard columns*	4 × 2 mm: 761951.20		4 × 3 mm: 761951.30	
NUCLEODUR® 100-5 NH_2-RP; particle size 5 µm; eluent in column acetonitrile – water				
Analytical EC columns				
2 mm		760730.20		760732.20
3 mm		760730.30		760732.30
4 mm		760730.40		760732.40
4.6 mm		760730.46	760731.46	760732.46
EC guard columns*	4 × 2 mm: 761953.20		4 × 3 mm: 761953.30	
NUCLEODUR® 100-5 NH_2; particle size 5 µm; eluent in column *n*-heptane				
Analytical EC columns				
4 mm		760720.40		760722.40
4.6 mm		760720.46	760721.46	760722.46
EC guard columns*			4 × 3 mm: 761952.30	
EC columns in packs of 1, guard columns in packs of 3.				

Guard column system

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

For details of our column systems see page 258.

NUCLEODUR® columns

NUCLEODUR® SiOH unmodified silica for normal phase · USP L3

Key feature

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

Technical data

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

Recommended application

- Polar and midpolar compounds under normal phase conditions

Eluent in column *n*-heptane

ID	Length → 50 mm	125 mm	150 mm	250 mm
NUCLEODUR® 100-3; particle size 3 µm				
Analytical EC columns				
4.6 mm	760170.46		760172.46	760173.46
EC guard columns*			4 × 3 mm: 761966.30	
NUCLEODUR® 100-5; particle size 5 µm				
Analytical EC columns				
4 mm				760007.40
4.6 mm	760023.46		760012.46	760007.46
EC guard columns*			4 × 3 mm: 761967.30	
Preparative VarioPrep columns				
10 mm	762077.100	762078.100		762007.100
21 mm	762077.210	762078.210		762007.210
40 mm			762075.400	762007.400
VP guard columns *	10 × 8 mm: 762094.80		10 × 16 mm: 762094.160	
	15 × 32 mm: 762330.320			
EC and VarioPrep columns in packs of 1, guard columns see below.				

Guard column systems

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

For details of our column systems see page 258.
Unmodified NUCLEODUR® bulk material in 10 – 50 µm for self-packing of preparative columns see page 264.