hromatography

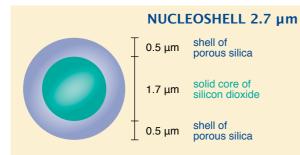
Highest efficiency in HPLC by core-shell technology



MACHEREY-NAGEL

Since 1911

Ultrafast separations beyond high pressure driven UHPLC



Core-shell technology

- Solid core of silicon dioxide, homogeneous shell of porous silica
- Highest efficiency compared to totally porous silica particles
- Particle size 2.7 μm (core 1.7 μm); lower back pressure enables use on conventional LC systems

In the past several approaches have been made to achieve fast separations without losing chromatographic performance. The prevalent strategy is to use very small silica particles, commonly sized smaller than 2 µm. HPLC columns packed with those microparticles show very high efficiencies (plates/meter) and allow the use of smaller column sizes with the positive side effect of significant solvent saving. However the downside of sub 2 micron particle HPLC is the generated high back pressure of the mobile phase during column runs which require specifically designed and often expensive instrumentation (UHPLC suitable solvent delivery and detection equipment).

Core-shell particle technology from MACHEREY-NAGEL is an alternate route to gain highest column efficiency and resolution at almost the same short run time but with much lower back pressure.

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

With conventional fully porous particles the mass transfer between stationary and mobile phase usu-

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

The Van Deemter plots on page 3 demonstrate how efficiency is affected by flow rate. In comparison with fully porous silicas, core-shell particles from various manufacturers maintain the efficiency optimum (max. plates/m) over a long range of increasing linear mobile phase velocity.

Theoretical column efficiency (optimal conditions)

Silica phase	d _ρ [μm]	L [m]	HETP [µm]	Efficiency [plates/m]	L [mm]	N	R_s	Analysis time
NUCLEOSHELL	2.7	1	4	250 000	100	25 000	112%	40 %
NUCLEODUR®	1.8	1	4.5	222 222	100	22 000	105%	40%
	3	1	7.5	133 333	150	20000	100%	60%
	5	1	12.5	80 000	250	20 000	100%	100%

Benefits of core-shell technology

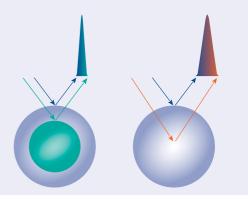
Short diffusion paths

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

Narrow particle size distribution ($d_{90}/d_{10} \sim 1.1$)

- Stable packing
- Column efficiency NUCLEOSHELL ~ 250 000 m⁻¹ (HETP ~ 4 µm)
- Minimized frictional heat

Core-shell particles vs. totally porous silica gel

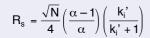


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Core-shell silica

NUCLEOSHELL

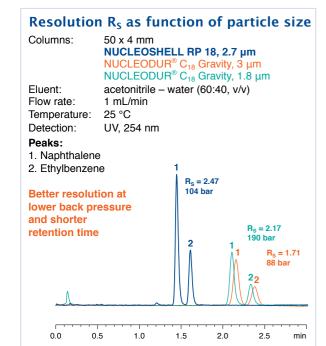
Demands on HPLC separations are constantly increasing with respect to separation efficiency, detection limits, and the time requirements for each analysis. Core-shell technology sets new standards for analyses in research and quality control.

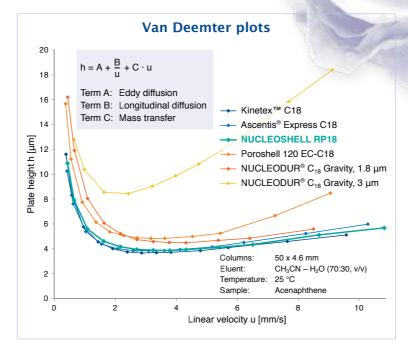


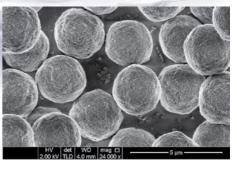
 R_s = Resolution α = Selectivity k_i ' = Retention

N = Theoretical plates N $\propto 1/d_P$

d_□ = Particle size



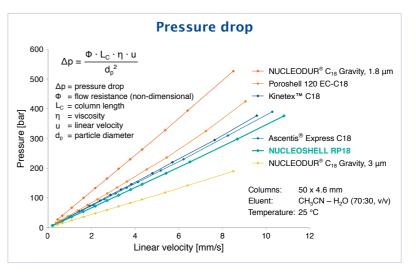




Electron microscopic image of NUCLEOSHELL particles

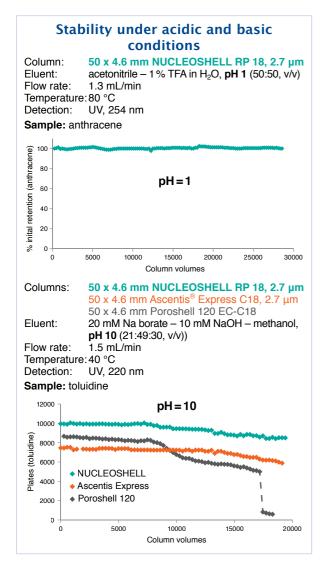
Utilizing a proprietary process of synthesis, NUCLEOSHELL particles exhibit a distinct narrow particle size distribution (d90/d10 ~ 1.1). Columns packed with NUCLEOSHELL core shell particles feature exceptional separation efficiencies with theoretical plate numbers easily comparable to totally porous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

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



MN Appl. No. 125270

Features of core-shell silica particles

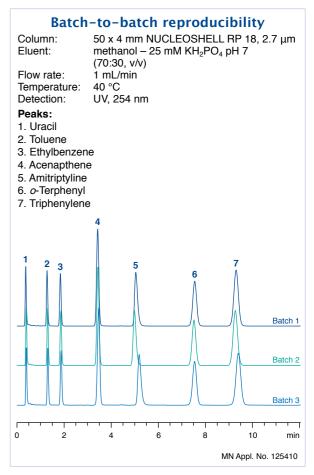


The above figure shows a column stability test of NUCLEOSHELL RP 18 at mobile phase levels pH 1 and pH 10 compared with two competing phases.

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

The column can also be operated at elevated temperatures without loss in retention behavior, efficiency or peak symmetry.

Temperature stability Stability test: 50 x 2 mm NUCLEOSHELL RP 18, 2.7 µm Column: Eluent: A) 10 mM ammonium formate – methanol $(9:1, v/v) + 120 \mu L$ formic acid, ~ pH 4 B) 10 mM ammonium formate – methanol $(1:9. \text{ v/v}) + 120 \text{ µL formic acid.} \sim \text{pH } 4$ 0-100 % B in 7 min 0.5 mL/min Flow rate: Temperature: 100 °C Detection: UV. 220 nm Peaks: 1 Phenol 2. Naphthalene 38 h 34 h 30 h 26 h 22 h min MN Appl. No. 125400 Efficiency test: Eluent: acetonitrile - water (60:40, v/v) Flow rate: 0.33 mL/min Temperature: 25 °C UV, 254 nm Detection: Sample: anthracene HETP [µm] Asymmetry Start (t = 0)5.2 0.98 End (t = 40 h)5.2 1.01



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

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Columns: 100 x 4.6 mm

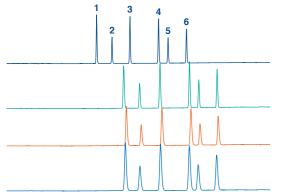
NUCLEOSHELL RP 18, 2.7 μm NUCLEODUR® C_{18} Gravity, 1.8 μm NUCLEODUR® C_{18} Gravity, 3 μm NUCLEODUR® C_{18} Gravity, 5 μm

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

Flow rate: 1.5 mL/min Temperature: 25 °C Detection: UV, 230 nm

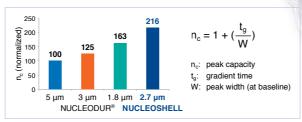
Peaks:





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

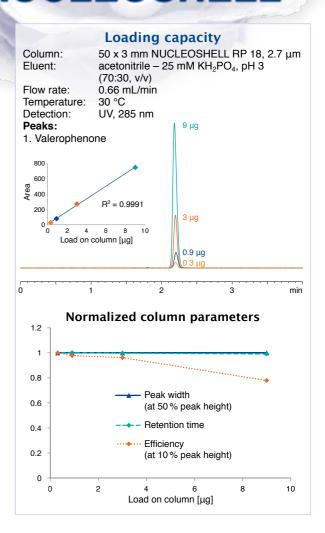
Peak capacity



The peak capacity is a measure of the number of sample analytes that can be separated on HPLC columns per time unit. Narrow peaks increase the peak capacity and efficiency of analytical columns. The example shows, that in comparison with totally porous NUCLEODUR® silica (1.8 µm) NUCLEOSHELL provides 33% higher peak capacity.

Loading capacity

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



NUCLEOSHELL RP 18

Key features:

- Based on core-shell particle technology for fast and efficient HPLC
- Suitable for LC/MS and HPLC at pH extremes (pH 1-11)
- Superior base deactivation, ideal for method development

Technical characteristics:

Octadecyl modification, multiendcapped; pore size 90 Å, particle size 2.7 µm, carbon content 7.5%

Recommended application:

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

USP L1

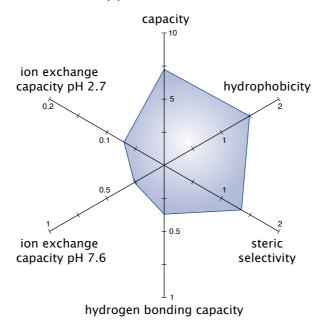
NUCLEOSHELL RP 18

NUCLEOSHELL RP 18 is based on core-shell particle technology silica. A unique derivatization process generates a homogeneous surface with a high density of bonded silanes (carbon content ~7.5%). The following thorough endcapping suppresses any unwanted polar interactions between the silica surface and the sample, which makes NUCLEOSHELL RP 18 particularly suitable for the separation of basic and other ionizable analytes.

The extremely reduced silanol activity of the phase can be demonstrated by applying basic analytes, such as tricyclic antidepressants. The chromatogram on page 7 shows a sharp elution profile (superior resolution!) of these highly polar compounds with an excellent asymmetry value for amitriptyline of 1.12.

Tanaka plot of NUCLEOSHELL RP 18

The diagram below underlines the distinct hydrophobic characteristics and the low silanol activity of the stationary phase.



Parameters of the Tanaka diagram

Capacity = k'(pentylbenzene) Hydrophobicity = α (pentylbenzene, butylbenzene) Steric selectivity = α (triphenylene, o-terphenyl) Hydrogen bonding capacity (silanol capacity) = α (caffeine, phenol)

Ion exchange capacity at 2 different pH values (2.7 and 7.6) = α (benzylamine, phenol)

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

13 β-lactam antibiotics in less than 3 min

Columns: 50 x 4 mm NUCLEOSHELL RP 18, 2.7 µm 150 x 4 mm NUCLEODUR® C₁₈ Gravity, 5 µm

Eluent: A) acetonitrile; B) 20 mM KH₂PO₄ pH 3.5

10 % A (0.5 min) → 50 % A in 1.5 min

(0.5 min 50 % A)

10 % A (3 min) → 50 % A in 9 min

(3 min 50 % A)

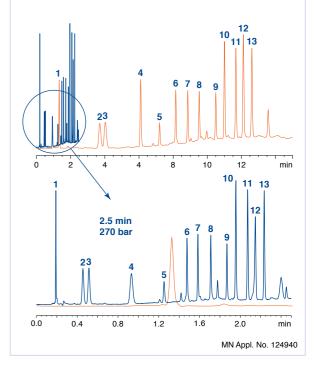
Flow rate: 2 mL/min, 1 mL/min
Pressure: 270 bar, 110 bar

Temperature: 25 °C Detection: UV, 220 nm

Peaks:

1. Amoxicillin6. Cefamandole11. Cloxacillin2. Ampicillin7. Cephalothin12. Nafcillin3. Cephalexin8. Piperacillin13. Dicloxacillin

4. Cefotaxime 9. Penicillin V 5. Cefoxitin 10. Oxacillin



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Tricyclic antidepressants · comparison of selectivity and resolution

Columns: 50 x 4.6 mm each

NUCLEOSHELL RP 18, 2.7 µm

Ascentis® Express C18
Kinetex™ 2.6 µm C18
Poroshell 120 EC-C18

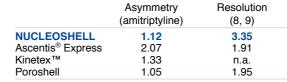
Eluent: methanol – acetonitrile – 25 mM KH₂PO₄ pH 7

(22.5:22.5:55, v/v/v)

Flow rate: 2 mL/min

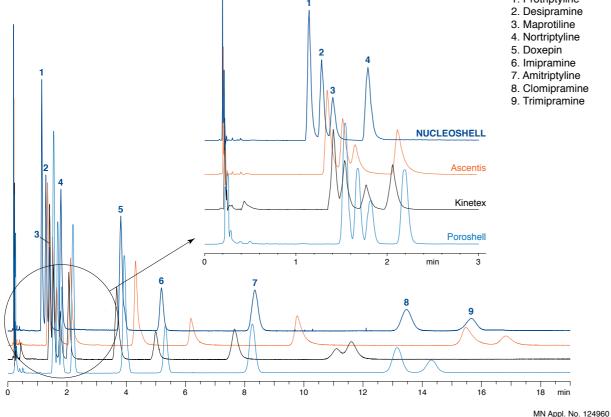
Pressure: 224 bar, 239 bar, 248 bar, 212 bar

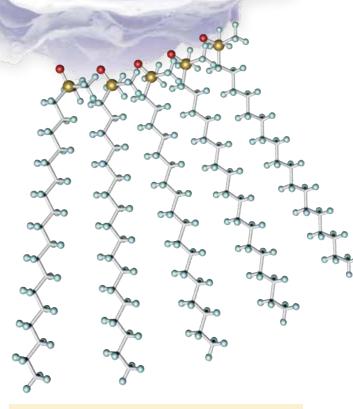
Temperature: 40 °C Detection: UV, 220 nm



Peaks:

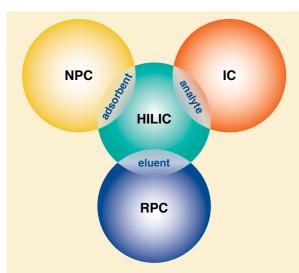
Protriptyline





NUCLEOSHELL RP 18 combines innovative silica technology and excellent surface deactivation, that outperforms conventional C₁₈ silicas in terms of efficiency, resolution and speed. Due to the applied core-shell particle design the back pressure at elevated flow rates remains at a moderate level and permits the use of existing HPLC equipment in many cases. NUCLEOSHELL RP 18 with extended pH stability, low bleed characteristics in LC/MS applications and overall robustness is an ideal tool for method development and routine analysis in modern HPLC.

NUCLEOSHELL HILIC



Key features:

- Based on core-shell particle technology for fast and efficient HPLC
- Ideal for reproducible and stable chromatography of highly polar analytes
- · Very short column equilibration times
- Technical characteristics:

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

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

NUCLEOSHELL HILIC

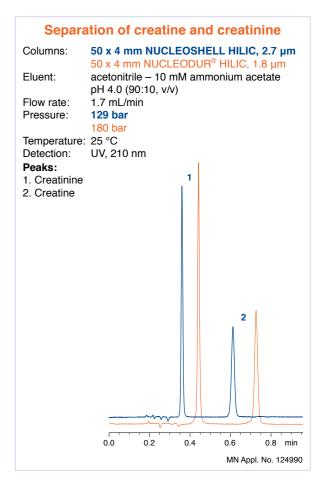
Hydrophilic interaction chromatography (HILIC) is a separation technique using polar stationary phases and organic-aqueous mobile phases. A minimum water content of at least 2% is indispensable to provide a permanent water laver between the adsorbent surface and the organic fraction of the mobile phase. The sample molecules become separated in a partition chromatography, in which polar analytes are more strongly retained than neutral, less hydrophilic compounds. Consequently, increasing the aqueous part in the mobile phase will diminish retention of the polar sample constituents. In this way HILIC behaves inverse to classical RP chromatography. The particular retention profile of HILIC enables the chromatography of very polar and often small molecules, which won't show any retention on C8 or C18 reversed phases.

Ultra-fast separations at moderate back pressure

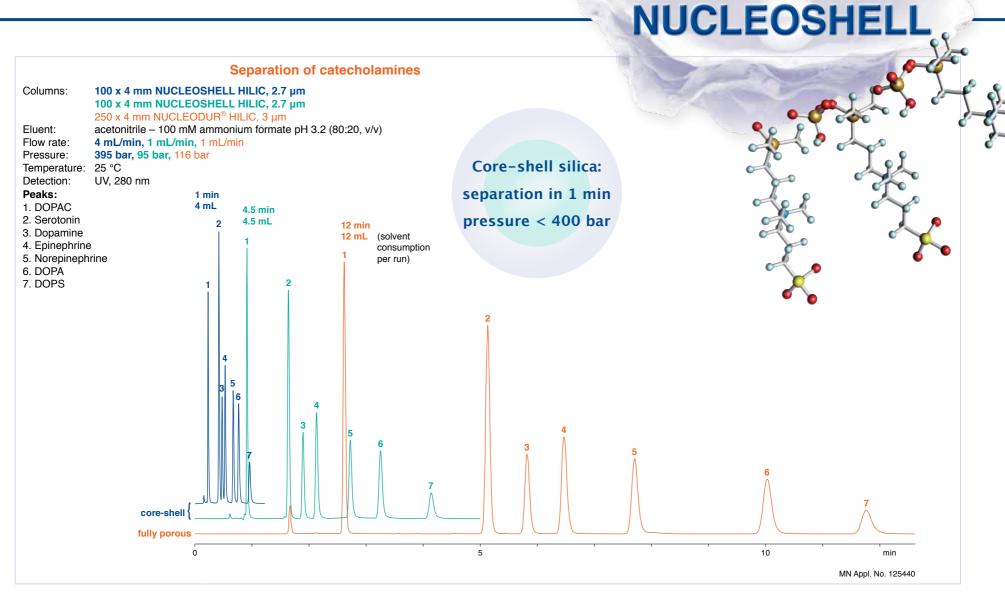
NUCLEOSHELL HILIC is a core-shell technology based stationary phase with a covalently bonded 3-N,N-dimethylaminopropane sulfonic acid ligand (pat. pend.). The betaine character of the strong ion-exchanger results in full charge balancing and facilitates fast equilibration times.

$$O_{0}^{N}$$
 O_{0}^{N}
 O_{0

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



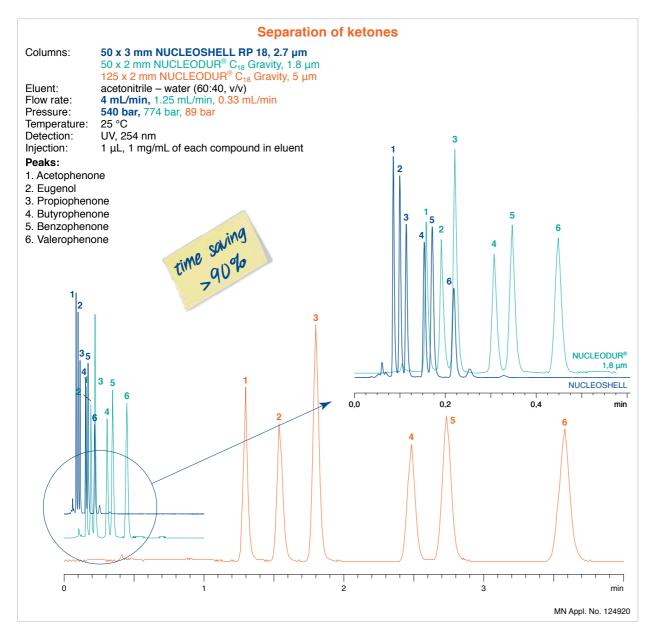
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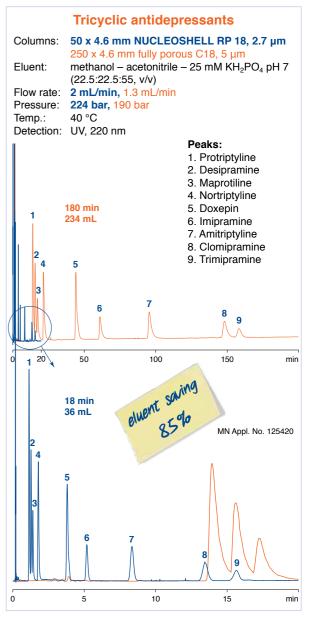


The chromatograms show the method transfer from a fully porous 3 μ m HILIC phase to 2.7 μ m core-shell silica with equal selectivity features. Run time has been cut down to 1 min. Column back pressure remains modest < 400 bar, while solvent demand is reduced to less than 35%.

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

Applications





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90% to to 66% time saing saing

Acidic pharmaceuticals

Columns: 50 x 4 mm NUCLEOSHELL RP 18, 2.7 µm

150 x 4 mm fully porous C18, 5 μm

Eluent: A) acetonitrile, B) 25 mM KH₂PO₄ pH 7,

25–40 % A in 2.2 min, 25–40 % A in 20 min

Flow rate: 1.5 mL/min. 0.5 mL/min

Pressure: 219 bar, 92 bar

Temp.: 20 °C Detection: UV, 215 nm

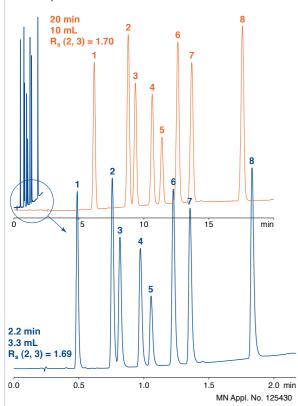
Peaks:

Ketoprofen
 Fenoprop
 Fenoprofen
 Flurbiprofen

5. Ibuprofen

7. Carprofen 8. Diclofenac

9. Meclofenamic acid





Columns: 50 x 3 mm NUCLEOSHELL RP 18, 2.7 µm 125 x 3 mm NUCLEODUR® C₁₈ Gravity, 3 µm

Eluent: A) acetonitrile, B) water

30–80 % A in 1 min (0.4 min 80 % A)

30–80 % A in 5 min (2 min 80 % A)

Flow rate: 2 mL/min

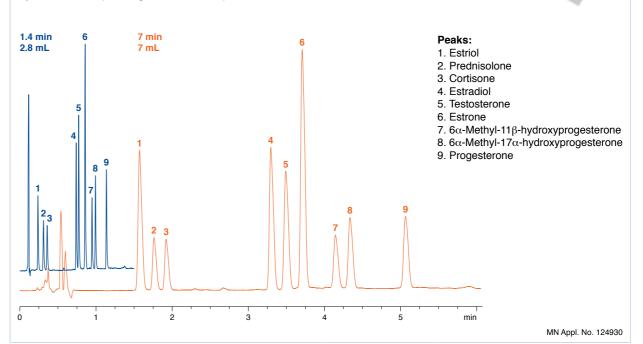
1 mL/min Pressure: 350 bar

280 bar

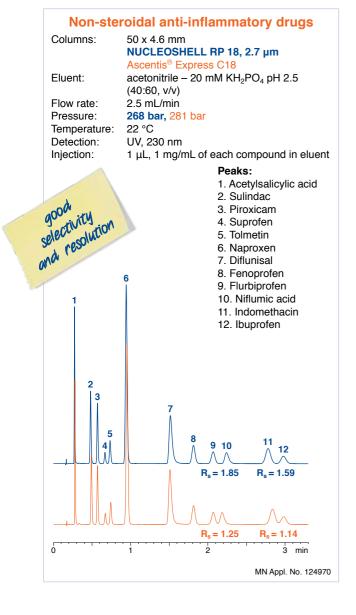
Temperature: 25 °C

Detection: UV, 240 nm

Injection: 1 µL, 1 mg/mL of each compound in eluent



Applications





Column: 100 x 4 mm NUCLEOSHELL HILIC, 2.7 µm

Eluent: A) acetonitrile – 100 mM ammonium acetate

A) acetonitrile – 100 mM ammonium acetate pH 3.2 (90:10, v/v), B) water;

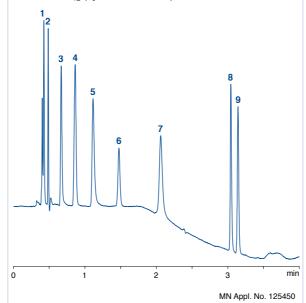
4 % B (1 min) → 20 % B in 1.6 min

(0.7 min 20 % B)

Flow rate: 2 mL/min
Pressure: 218 bar
Temperature: 25 °C
Detection: UV, 260 nm

Peaks:

- 1. PABA (p-aminobenzoic acid)
- 2. Nicotinamide
- 3. Vitamin B₆ (pyridoxine)
- 4. Riboflavin
- 5. Nicotinic acid
- 6. Vitamin C (ascorbic acid)
- 7. Vitamin B₁ (thiamine)
- 8. Folic acid
- 9. Vitamin B₁₂ (cyanocobalamine)



Anions and cations

Column: 100 x 4 mm NUCLEOSHELL HILIC, 2.7 µm

Eluent: A) 30 mM ammonium formate pH 3,

B) acetonitrile

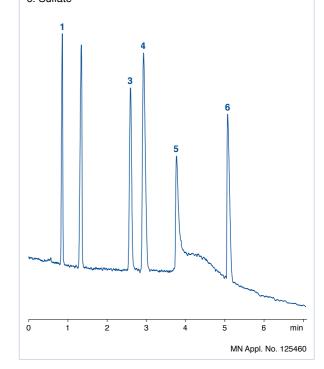
 $80 \% B (3 min) \rightarrow 20 \% B in 7 min$

Flow rate: 1.5 mL/min
Pressure: 200 bar
Temperature: 40 °C

Detection: CAD (Nebulizer: 35 °C)

Peaks: 1. Nitrate

- 2. Chloride
- 3. Potassium
- 4. Sodium
- 5. Phosphate
- 6. Sulfate



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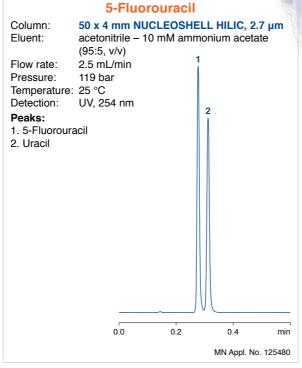
Metformin Columns: 50 x 4 mm NUCLEOSHELL HILIC, 2.7 μm 50 x 4 mm NUCLEODUR® HILIC, 1.8 μm Eluent: acetonitrile - 10 mM ammonium acetate pH 3.2 (75:25, v/v) Flow rate: 3 mL/min 1.5 mL/min 202 bar Pressure: 167 bar Temperature: 25 °C Detection: UV, 218 nm Peaks: 1. Dicyandiamide 2. Metformin

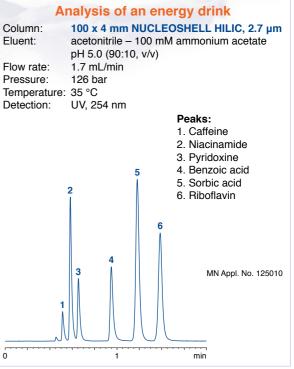
NUCLEOSHELL

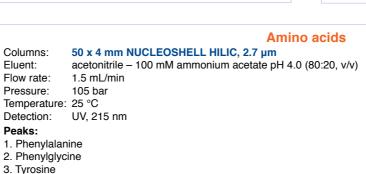
NUCLEODUR®

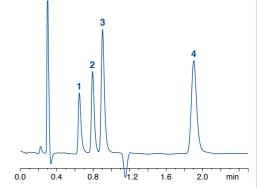
MN Appl. No. 125470

1.0









MN Appl. No. 125000

4. Histamine

0.0

0.5

Packed columns · Ordering information

EC standard columns for analytical HPLC



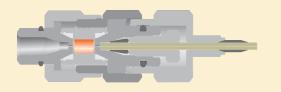
 Analytical column system made of stainless steel

M 8 outer threads on both ends

Combination of sealing element and very fine-meshed stainless steel screen, PTFE ring and fitting adapter

Column heads SW 12, with inner threads M8 x 0.75 and UNF 10-32 (= 1/16" fitting)

As screw-on guard column system we recommend the Column Protection System used with EC guard column cartridges with 4 mm length (see next page).



NUCLEOSHELL RP 18

Pore size 90 Å; octadecyl modification, multi-endcapped, 7.5 % C; eluent in column CH₃CN - H₂O

Length →	50 mm	100 mm	150 mm	EC guard columns*	
NUCLEOS	particle size 2.7 µm				
EC analytical columns					
2 mm ID	763132.20	763134.20	763136.20	4 x 2 mm: 763138.20	
3 mm ID	763132.30	763134.30	763136.30	4 x 3 mm: 763138.30	
4 mm ID	763132.40	763134.40	763136.40	4 x 3 mm: 763138.30	
4.6 mm ID	763132.46	763134.46	763136.46	4 x 3 mm: 763138.30	
EC columns in packs of 1, guard columns in packs of 3					

NUCLEOSHELL HILIC

Pore size 90 Å; ammonium - sulfonic acid modification, endcapped, 1.3 % C; eluent in column CH₃CN - H₂O

Length →	50 mm	100 mm	150 mm	EC guard columns*	
NUCLEOS	particle size 2.7 µm				
EC analytical columns					
2 mm ID	763332.20	763334.20	763336.20	4 x 2 mm: 763338.20	
3 mm ID	763332.30	763334.30	763336.30	4 x 3 mm: 763338.30	
4 mm ID	763332.40	763334.40	763336.40	4 x 3 mm: 763338.30	
4.6 mm ID	763332.46	763334.46	763336.46	4 x 3 mm: 763338.30	
EC columns in packs of 1, guard columns in packs of 3					

^{*} EC guard columns require the Column Protection System Cartridge Holder (see right)



Column Protection System

Innovative and universal screw-on guard column holder system

Suitable for all analytical HPLC columns with 1/16" fittings



- Cartridges filled with specified NUCLEOSHELL, NUCLEODUR®, and NUCLEOSIL® HPLC adsorbents
- Ideal protection for your analytical main column → significant increase in column lifetime
- Minimized void volume → suitable also for ultra fast HPLC
- Special ferrules → pressure stability up to 1034 bar (15 000 psi)
- Visual contamination check → in-time changing of the guard column
- Guard column length 4 mm, ID 2 mm (for main columns with 2 mm ID) or ID 3 mm (for main columns with 3, 4 and 4.6 mm ID)
- UNIVERSAL RP guard columns available for all HPLC columns under RP conditions

Content of the Column Protection System



Replacement parts for the Column Protection System · Ordering information

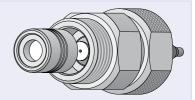
Description	Pack of	REF
Ferrules	5	718967
Replacement connector including O-ring	1	718968
Capillary tubes, nuts and metal ferrules	3	718969
Wrench (size 12 and 14 mm)	1	718970
EC 4/2 UNIVERSAL RP guard column (for main columns with 2 mm ID)	3	728777.20
EC 4/3 UNIVERSAL RP guard column (for main columns with 3, 4 and 4.6 mm ID)	3	728777.30

Visual Contamination Check

The cartridge is assembled with a white filter membrane.

A discoloration of the filter membrane usually indicates that the cartridge should be replaced.

In case of colorless contaminants, a rising back pressure and/or loss of chromatographic performance advise to change the guard column.



Trademarks: NUCLEODUR® (MACHEREY-NAGEL GmbH & Co. KG, Germany), Ascentis® (Sigma-Aldrich Co., USA), Kinetex™ (Phenomenex Inc., USA), Poroshell (Agilent Technologies Inc., USA) Image credits: © by-studio / Jaeeho (Fotolia.com)





GC



TLC



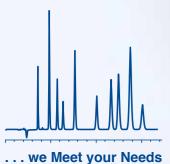
SPE & Flash



Syringe Filters



Vials



local distributor

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CHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany Switzerland: France:

EN 500 9001: 2008 and international: Tel.: +49 24 21 969-0 Fax: +49 24 21 969-199 E-mail: info@mn-net.com

MACHEREY-NAGEL AG Tel.: +41 62 388 55 00 Fax: +41 62 388 55 05

E-mail: sales-ch@mn-net.com

MACHEREY-NAGEL EURL +33 388 68 22 68 +33 388 51 76 88

E-mail: sales-fr@mn-net.com

USA: **MACHEREY-NAGEL Inc.**

Tel.: +1 484 821 0984 Fax: +1 484 821 1272 E-mail: sales-us@mn-net.com

