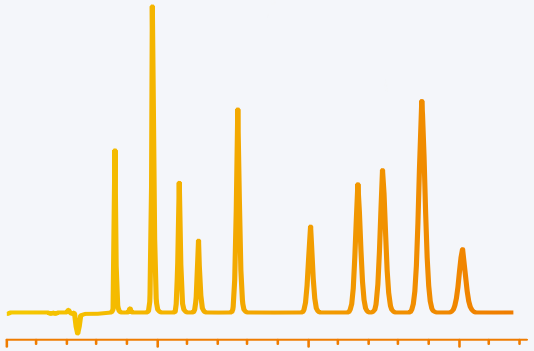
Chromatography

Highest efficiency in HPLC by core-shell technology

NUCLEOSHELL®

Including NEW Phenyl-Hexyl phase



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MACHEREY-NAGEL

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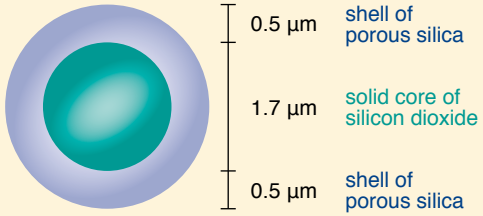
MN Since 1911

Ultrafast separations beyond high pressure driven UHPLC

NUCLEOSHELL® 2.7 µm



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), pore size 90 Å, specific surface 130 m^2/g; lower back pressure enables use on conventional LC systems
- Pressure stability up to 600 bar

NUCLEOSHELL® modifications

The program of surface modifications based on NUCLEOSHELL® silica now comprises the following phases:

- NUCLEOSHELL® RP 18
- NUCLEOSHELL® Phenyl-Hexyl **NEW!**
- NUCLEOSHELL® PFP
- NUCLEOSHELL® HILIC

Several approaches have been made to achieve fast separations without losing chromatographic performance. HPLC columns packed with particles < 2 µm show very high efficiencies (plates/meter) and allow the use of smaller column sizes with the positive side effect of significant solvent saving. However they generate a high back pressure of the mobile phase during column runs which requires specifically designed 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 usually results in peak broadening at higher flow rates (C-term in van Deemter equation). The short diffusion paths in the core-shell particles reduce the dwell time of the analyte molecules in the stationary phase, so that even at high flow velocities of the mobile phase, optimal separation results can be obtained.

The van Deemter plots 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_p [µm]	L [m]	HETP [µm]	Efficiency [plates/m]	L [mm]	N	R_s	Analysis time
NUCLEOSHELL®	**2.7**	**1**	**4**	**250 000**	**100**	**25 000**	**112 %**	**40 %**
NUCLEODUR®	1.8	1	4.5	222 222	100	22 000	105 %	40 %
	3	1	7.5	133 333	150	20 000	100 %	60 %
	5	1	12.5	80 000	250	20 000	100 %	100 %

Benefits of core-shell technology

Short diffusion paths

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

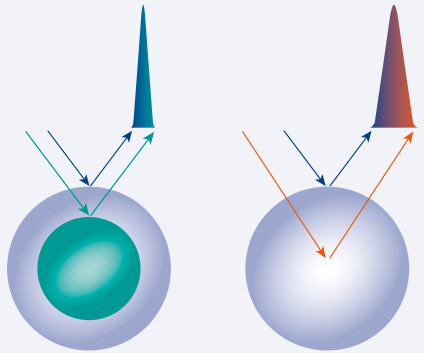
Narrow particle size distribution (d_{90}/d_{10} ~ 1.1)

- Stable packing

High heat transfer

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

Core-shell particles vs. totally porous silica gel



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.

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

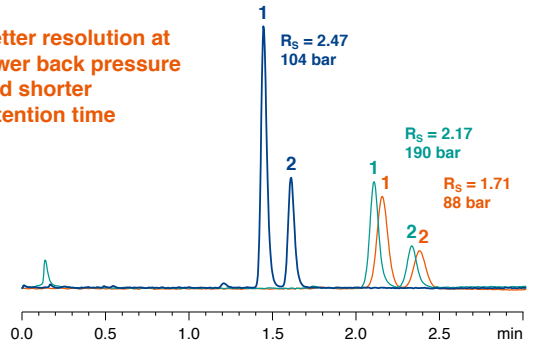
R_s = Resolution
α = Selectivity
k_i' = Retention
N = Theoretical plates $N \propto 1/d_P$
d_P = Particle size

Resolution R_S as function of particle size

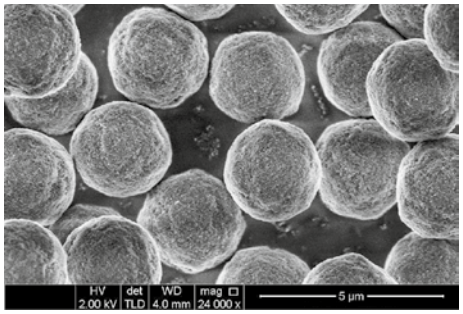
Columns: 50 x 4 mm each
NUCLEOSHELL® RP 18, 2.7 µm
NUCLEODUR® C_{18} Gravity, 3 µm
NUCLEODUR® C_{18} Gravity, 1.8 µm
Eluent: acetonitrile – water (60:40, v/v)
Flow rate: 1 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

Peaks:
1. Naphthalene
2. Ethylbenzene

Better resolution at lower back pressure and shorter retention time



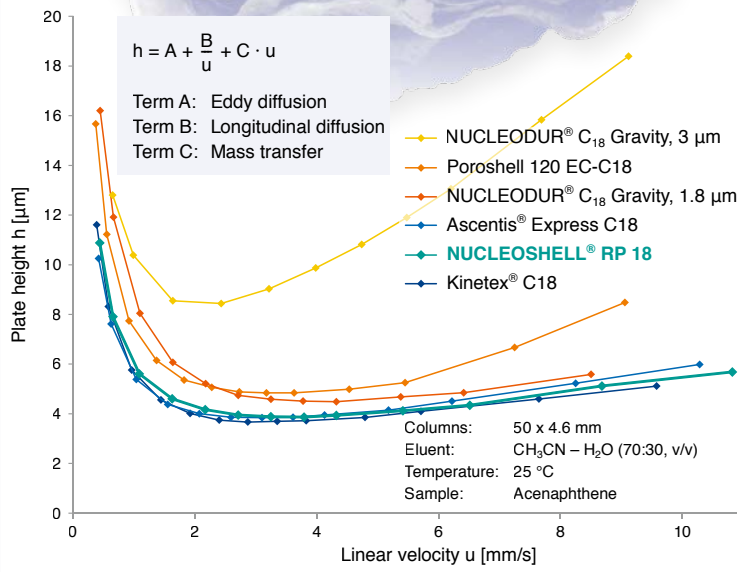
MN Appl. No. 125270



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.

Van Deemter plots



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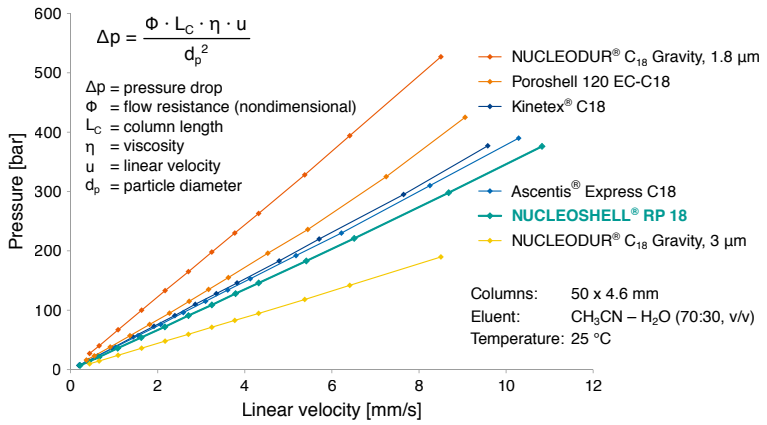
Pressure drop



MN Appl. No. 125510

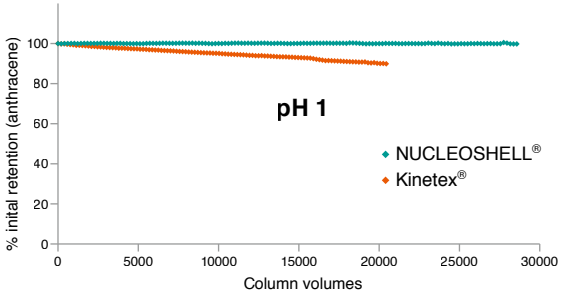
In direct comparison with the "conventional" sub 2 micron phases, NUCLEOSHELL® columns only generate about 60% of the back pressure and can be operated with the majority of conventional HPLC systems. In order to develop the maximum performance of NUCLEOSHELL® columns, we recommend reducing extra column 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.

Features of core-shell silica particles

Stability under acidic and basic conditions

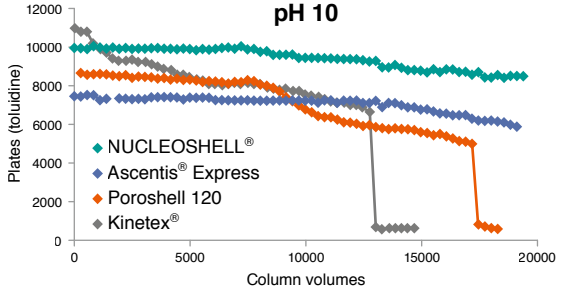
Column: **50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm**
50 x 4.6 mm Kinetex® 2.6 µm C18
Eluent: acetonitrile – 1 % TFA in H_2O, **pH 1** (50:50, v/v)
Flow rate: 1.3 mL/min; temperature 80 °C
Detection: UV, 254 nm

Sample: anthracene



Columns: **50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm**
50 x 4.6 mm Ascentis® Express C18, 2.7 µm
50 x 4.6 mm Poroshell 120 EC-C18
50 x 4.6 mm Kinetex® 2.6 µm C18
Eluent: 20 mmol/L Na borate – 10 mmol/L NaOH – methanol, **pH 10** (21:49:30, v/v))
Flow rate: 1.5 mL/min; temperature 40 °C
Detection: UV, 220 nm

Sample: toluidine



MN Appl. Nos. 125520 / 125530

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

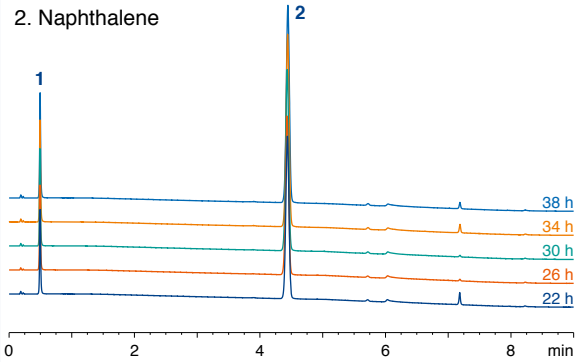
A criterion for the long-term stability of the column at pH extremes is the percentage decrease of initial retention and initial plates, respectively.

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

Temperature stability

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

Peaks:
1. Phenol
2. Naphthalene



MN Appl. No. 125400

Efficiency test:
Eluent: acetonitrile – water (60:40, v/v)
Flow rate: 0.33 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

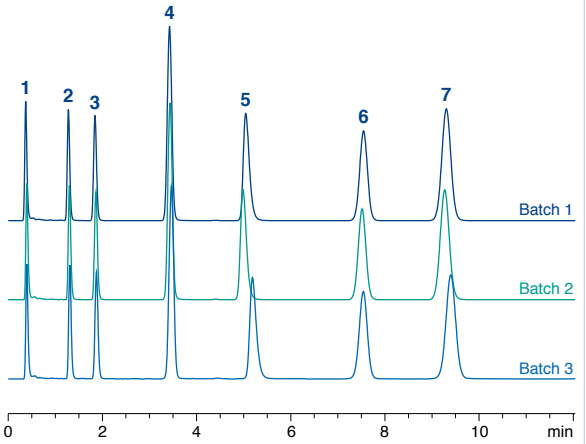
Sample: anthracene

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

Batch-to-batch reproducibility

Column: 50 x 4 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent: methanol – 25 mmol/L KH_2PO_4 pH 7 (70:30, v/v)
Flow rate: 1 mL/min
Temperature: 40 °C
Detection: UV, 254 nm

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



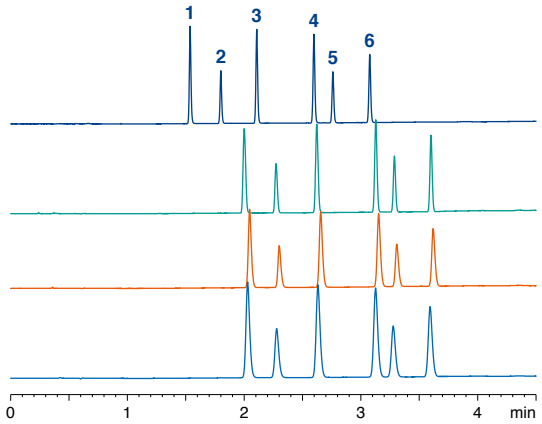
MN Appl. No. 125410

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

Peak capacity

Columns: 100 x 4.6 mm each
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

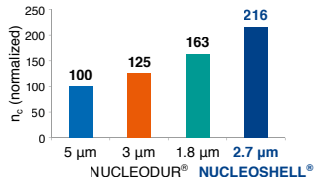
Peaks:
1. Acetophenone
2. Benzoin
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone



MN Appl. No. 125540

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

Peak capacity



$$n_c = 1 + \left(\frac{t_g}{W}\right)$$

n_c: peak capacity
t_g: gradient time
W: peak width (at baseline)

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.

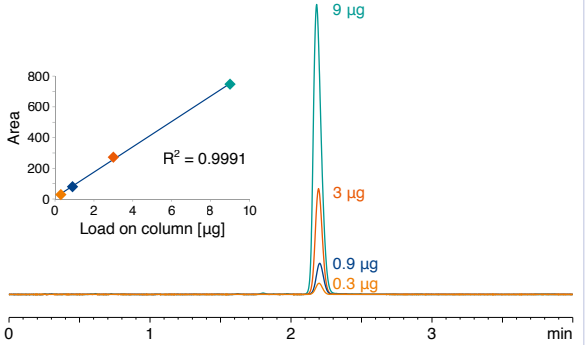
Loading capacity

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

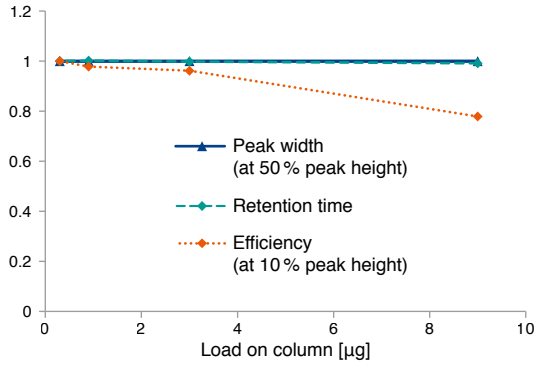
Loading capacity

Column: 50 x 3 mm NUCLEOSHELL® RP 18, 2.7 µm
Eluent: acetonitrile – 25 mmol/L KH_2PO_4, pH 3 (70:30, v/v)
Flow rate: 0.66 mL/min
Temperature: 30 °C
Detection: UV, 285 nm

Peaks:
1. Valerophenone



Normalized column parameters



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, multi-endcapped; 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

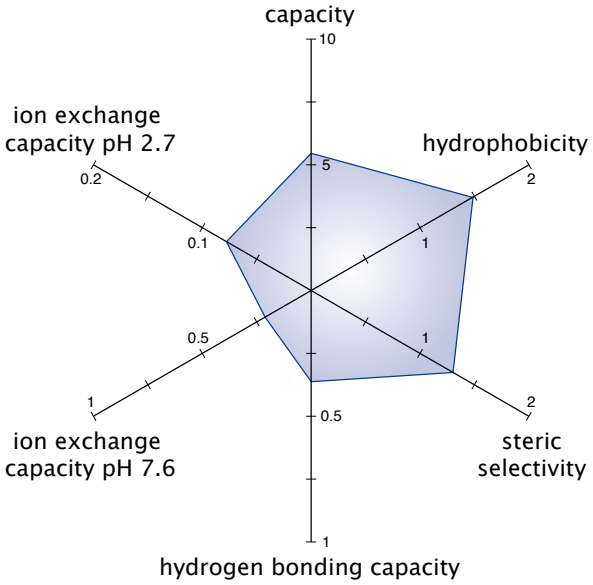
NUCLEOSHELL® RP 18

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)

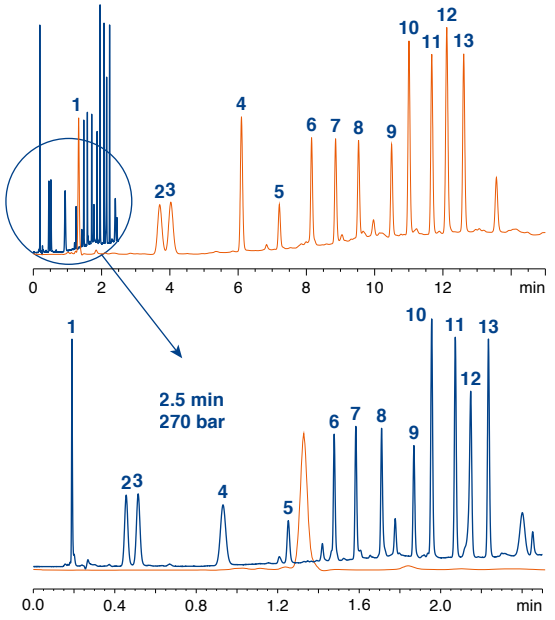
The separation of 13 β-lactam antibiotics illustrates how time of analysis can be shortened to a fractional part by using core-shell particles without loss of resolution at moderate back pressure.

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® C_{18} Gravity, 5 µm
Eluent:	A) acetonitrile; B) 20 mmol/L KH_2PO_4 pH 3.5 **10 % A (0.5 min) → 50 % A in 1.5 min (0.5 min 50 % A)** 10 % A (3 min) → 50 % A in 9 min (3 min 50 % A)
Flow rate:	**2 mL/min,** 1 mL/min
Pressure:	**270 bar,** 110 bar
Temperature:	25 °C
Detection:	UV, 220 nm

Peaks:

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

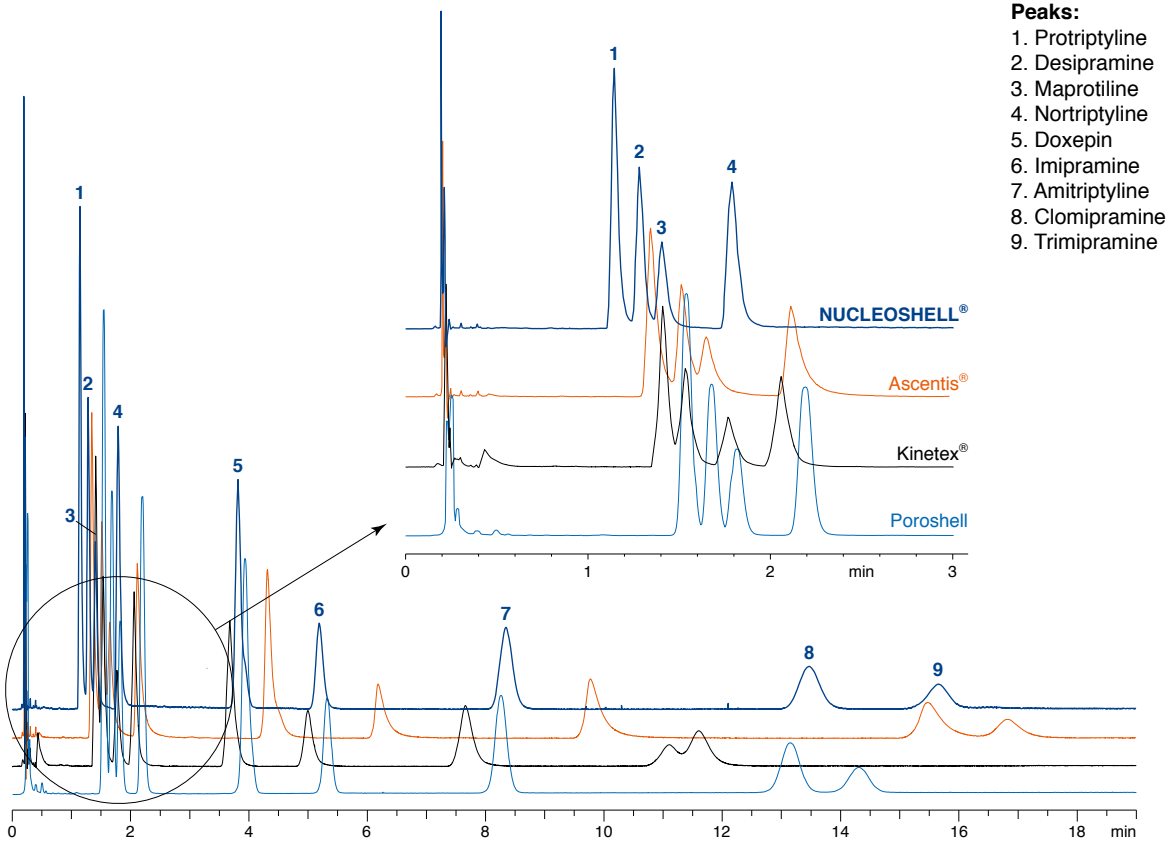


MN Appl. No. 124940

Tricyclic antidepressants · comparison of selectivity and resolution

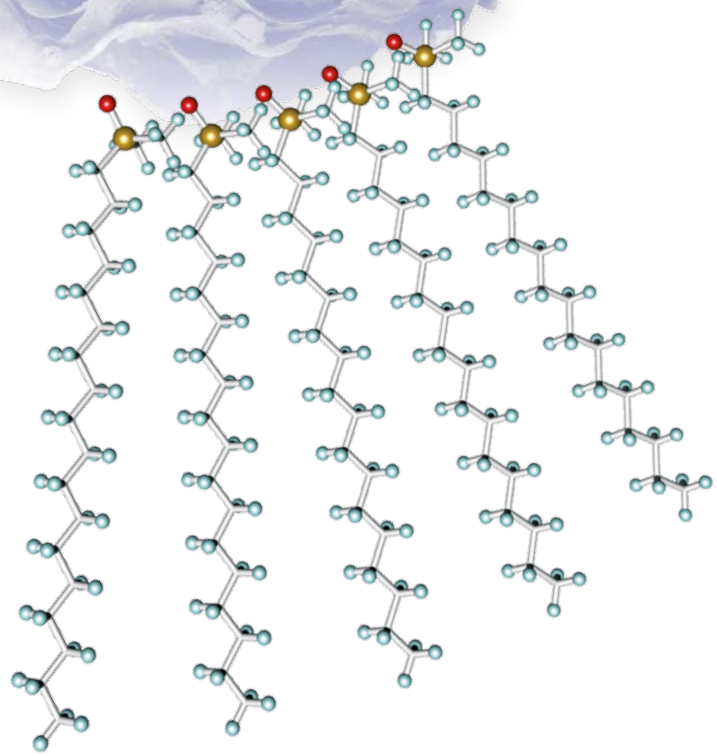
Columns: 50 x 4.6 mm each
NUCLEOSHELL® RP 18, 2.7 µm
Ascentis® Express C18
Kinetex® 2.6 µm C18
Poroshell 120 EC-C18
Eluent: methanol – acetonitrile – 25 mmol/L KH_2PO_4 pH 7 (22.5:22.5:55, v/v/v)
Flow rate: 2 mL/min
Pressure: **224 bar,** 239 bar, 248 bar, 212 bar
Temperature: 40 °C
Detection: UV, 220 nm

	Asymmetry (amitriptyline)	Resolution (8, 9)
NUCLEOSHELL®	**1.12**	**3.35**
Ascentis® Express	2.07	1.91
Kinetex®	1.33	n.a.
Poroshell	1.05	1.95



Peaks:
1. Protriptyline
2. Desipramine
3. Maprotiline
4. Nortriptyline
5. Doxepin
6. Imipramine
7. Amitriptyline
8. Clomipramine
9. Trimipramine

MN Appl. No. 124960



NUCLEOSHELL® RP 18 combines innovative silica technology and excellent surface deactivation, that outperforms conventional C_{18} 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® Phenyl-Hexyl

- **Key features:**
  - Based on core-shell particle technology for fast and efficient HPLC
  - Hydrophobic phase with alternative selectivity in comparison to classical C_{18} modifications
  - Separation principle based on 2 retention mechanisms: π-π interactions and hydrophobic interactions
- **Technical characteristics:**
  Phenyl-Hexyl modification, multi-endcapped; pore size 90 Å, particle size 2.7 µm, carbon content 4.5 %; pH stability 1–10; suitable for **LC/MS**
- **Recommended application:**
  Aromatic and unsaturated compounds, polar compounds like pharmaceuticals, antibiotics

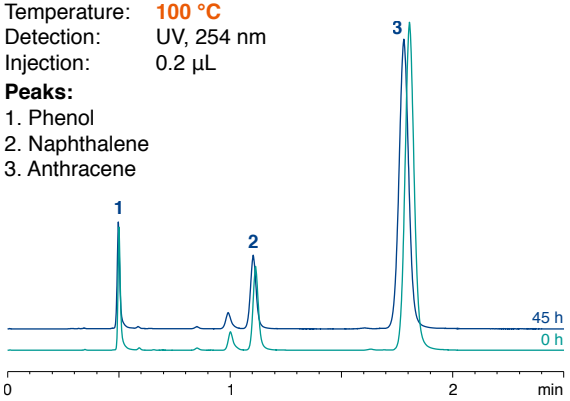
  USP L11

Phenyl-Hexyl modified phases offer an excellent separation efficiency especially for aromatic and unsaturated compounds with electron-withdrawing groups. The combination of hydrophobic and π-π interactions results in an alternative and interesting selectivity profile compared the C_{18} or C_8 modifications. NUCLEOSHELL® Phenyl-Hexyl is based on an unique surface bonding chemistry – therefore it is suitable for LC/MS due to low bleeding characteristics and offers high temperature stability and pH stability from 1 to 10.

Temperature stability of NUCLEOSHELL® Phenyl-Hexyl

Column: 50 x 2 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
Eluent: acetonitrile – 10 mmol/L ammonium formate pH 4 (50:50, v/v)
Flow rate: 0.33 mL/min
Temperature: 100 °C
Detection: UV, 254 nm
Injection: 0.2 µL

Peaks:
1. Phenol
2. Naphthalene
3. Anthracene

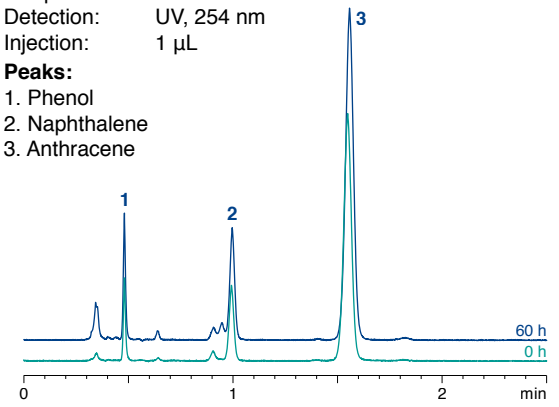


MN Appl. No. 126430

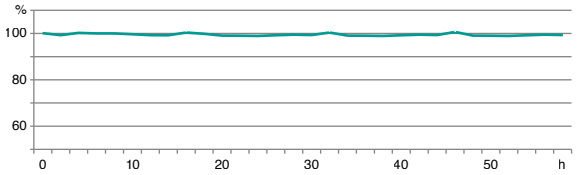
Stability of NUCLEOSHELL® Phenyl-Hexyl at pH 10

Column: 50 x 4 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
Eluent: acetonitrile – 50 mmol/L TEA pH 10 (60:40, v/v); pH of the mixture 10.4
Flow rate: 1 mL/min
Temperature: 40 °C
Detection: UV, 254 nm
Injection: 1 µL

Peaks:
1. Phenol
2. Naphthalene
3. Anthracene



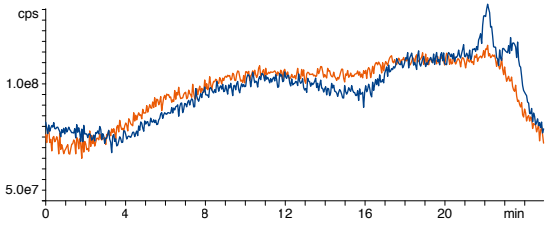
Relative plate numbers



MN Appl. No. 126420

Bleeding characteristics of NUCLEOSHELL® Phenyl-Hexyl

Columns: 50 x 2 mm each
NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
Kinetex® Phenyl-Hexyl
Eluent: A) acetonitrile, B) water; 5–95 % A in 25 min
Flow rate: 0.2 mL/min
Temperature: 25 °C
Detection: MS



MN Appl. No. 126400

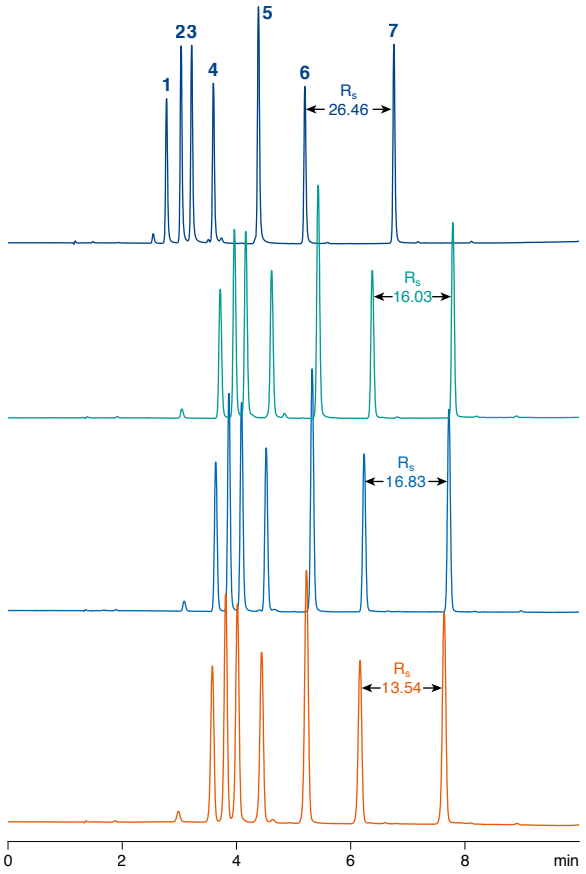
NUCLEOSHELL® Phenyl-Hexyl is a robust phase with an alternative RP selectivity for aromatic and unsaturated analytes compared to classical C_{18} / C_8 phases – it is an additional and useful tool for all chromatographers.

Comparison of Phenyl-Hexyl phases for the separation of sulfonamides

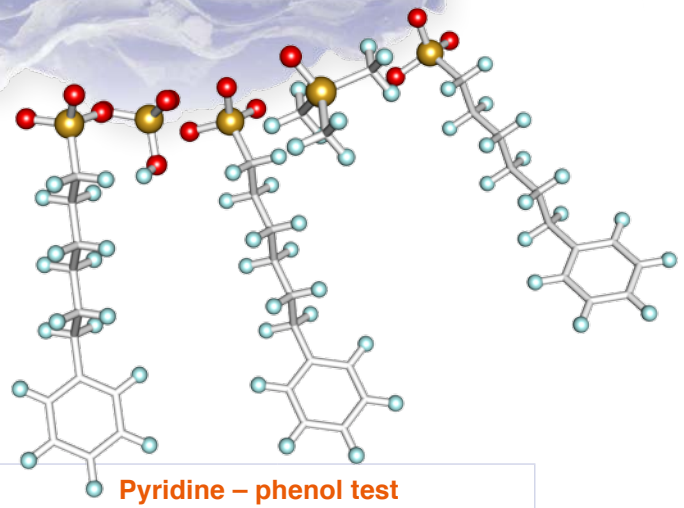
Columns: 150 x 3 mm each
NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
NUCLEODUR® Phenyl-Hexyl, 1.8 µm
NUCLEODUR® Phenyl-Hexyl, 3 µm
NUCLEODUR® Phenyl-Hexyl, 5 µm
Eluent: A) methanol,
B) 0.1 % formic acid in water,
20–80 % A in 10 min
Flow rate: 0.56 mL/min
Temperature: 40 °C
Detection: UV, 254 nm
Injection: 0.5 µL

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

On NUCLEOSHELL® Phenyl-Hexyl the resolution of the last two peaks is higher than on the fully porous 1.8 µm NUCLEODUR® Phenyl-Hexyl.



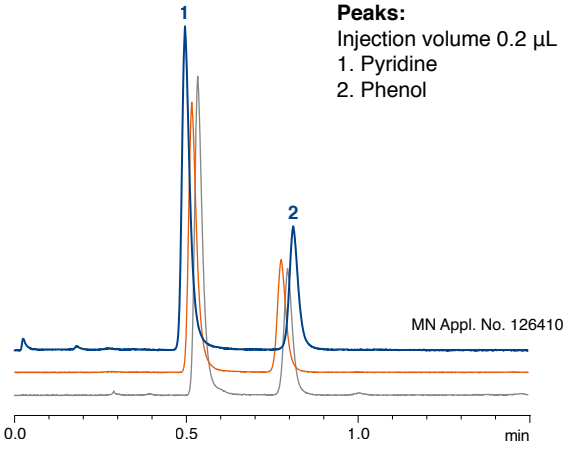
MN Appl. No. 125860



Pyridine – phenol test

Columns: 50 x 2 mm each
NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm
Kinetex® Phenyl-Hexyl
Ascentis® Express Phenyl-Hexyl
Eluent: acetonitrile – water (70:30, v/v)
Flow rate: 0.3 mL/min
Temperature: 40 °C
Detection: UV, 254 nm

Peaks:
Injection volume 0.2 µL
1. Pyridine
2. Phenol



MN Appl. No. 126410

The separation of sulfonamides proves the scalability from fully porous NUCLEODUR® to NUCLEOSHELL® Phenyl-Hexyl. Hereby the core-shell silica exhibits under same conditions identical selectivity, narrower peaks and slightly shorter retention. Thus, method transferability between NUCLEODUR® and NUCLEOSHELL® is guaranteed, either for speeding up your methods or scaling up for preparative requirements. The pyridine-phenol test shows that NUCLEOSHELL® Phenyl-Hexyl provides a symmetrical peak for pyridine and higher resolution in comparison to other core-shell based Phenyl-Hexyl phases, which underlines the excellent base deactivation.

NUCLEOSHELL® PFP

- **Key features:**
  - Based on core-shell particle technology for fast and efficient HPLC
  - 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 interactions
    - π–π interactions
    - hydrophobic interactions
- **Technical characteristics:**
  Pentafluorophenyl propyl modification, multi-endcapped; pore size 90 Å, particle size 2.7 µm, carbon content ~ 3 %; pH stability 1–9; suitable for **LC/MS**
- **Recommended application:**
  Aromatic and unsaturated compounds, phenols, halogenated compounds, isomers, polar compounds like pharmaceuticals, antibiotics; high retention of basic compounds
  USP L43

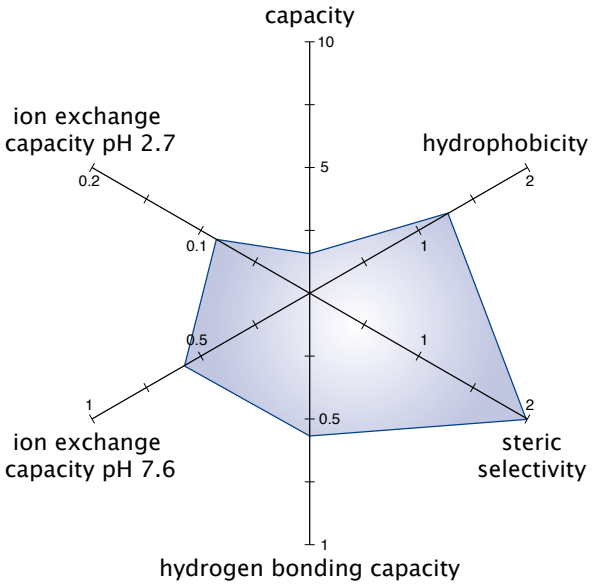
Orthogonality in selectivity

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 F5). Especially the orthogonal selectivity compared to traditional alkyl phases widens the scope in analytical HPLC. Thus NUCLEOSHELL® PFP offers an excellent selectivity especially for highly polar analytes, aromatic and unsaturated compounds, phenols or halogenated hydrocarbons.

Halogen substitutes in molecules result often in an increase of their polarity accompanied by a decrease of typical retention characteristics in RP-HPLC.

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

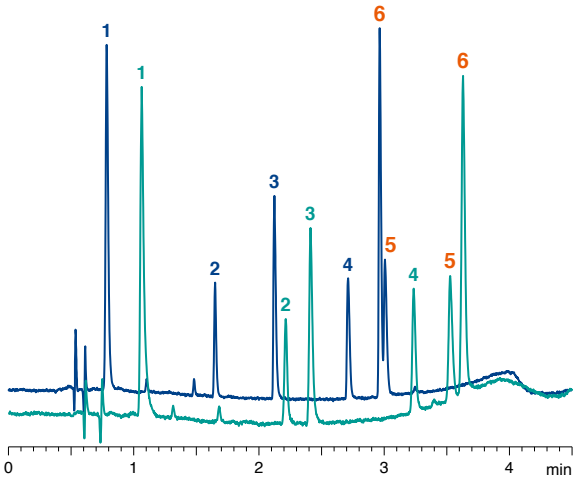
Tanaka plot of NUCLEOSHELL® PFP



β-Blockers · orthogonal selectivity of NUCLEOSHELL® PFP

Columns: 100 x 4.6 mm each
NUCLEOSHELL® RP 18, 2.7 µm
NUCLEOSHELL® PFP, 2.7 µm
Eluents: A) acetonitrile + 0.1 % formic acid;
B) 0.1 % formic acid;
10–35 % A in 2.5 min, 35-50 % A in 2 min
Flow rate: 1.7 mL/min
Temperature: 25 °C
Detection: UV, 280 nm

Peaks:
1. Atenolol
2. Pindolol
3. Metroprolol
4. Labetalol
5. Alprenolol
6. Propranolol



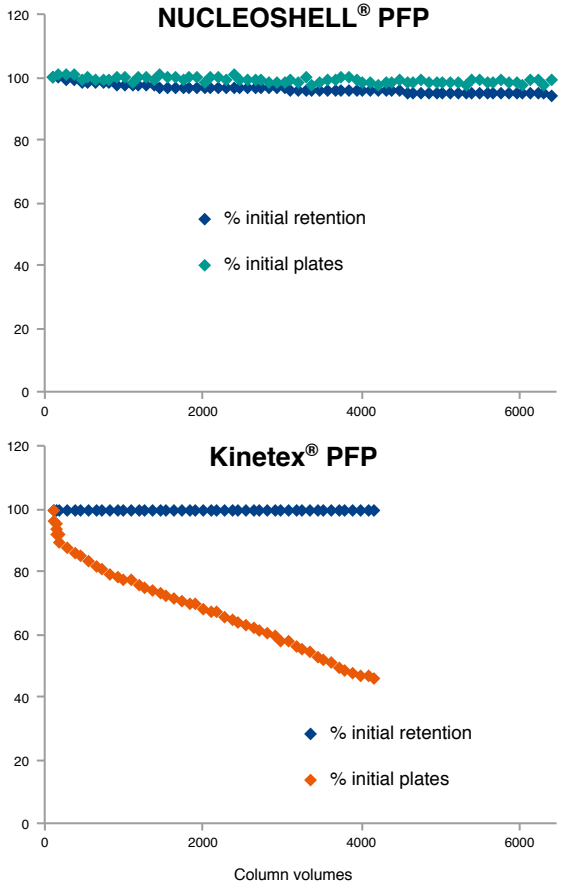
MN Appl. No. 125610

NUCLEOSHELL® PFP combines the benefits of core-shell technology, high stability and orthogonal selectivity. So it is a useful complementary tool for highly efficient separations especially of isomers, halogenated, aromatic and / or polar compounds.

Stability of NUCLEOSHELL® PFP at pH 1

Columns: **100 x 4,6 mm NUCLEOSHELL® PFP, 2.7 µm**
100 x 4.6 mm Kinetex® 2.6 µm PFP
Eluent: acetonitrile – 0.5% TFA pH 1 (50:50, v/v)
Flow rate: 1.3 mL/min
Temperature: 60 °C
Detection: UV, 254 nm

Sample:
Ethylbenzene

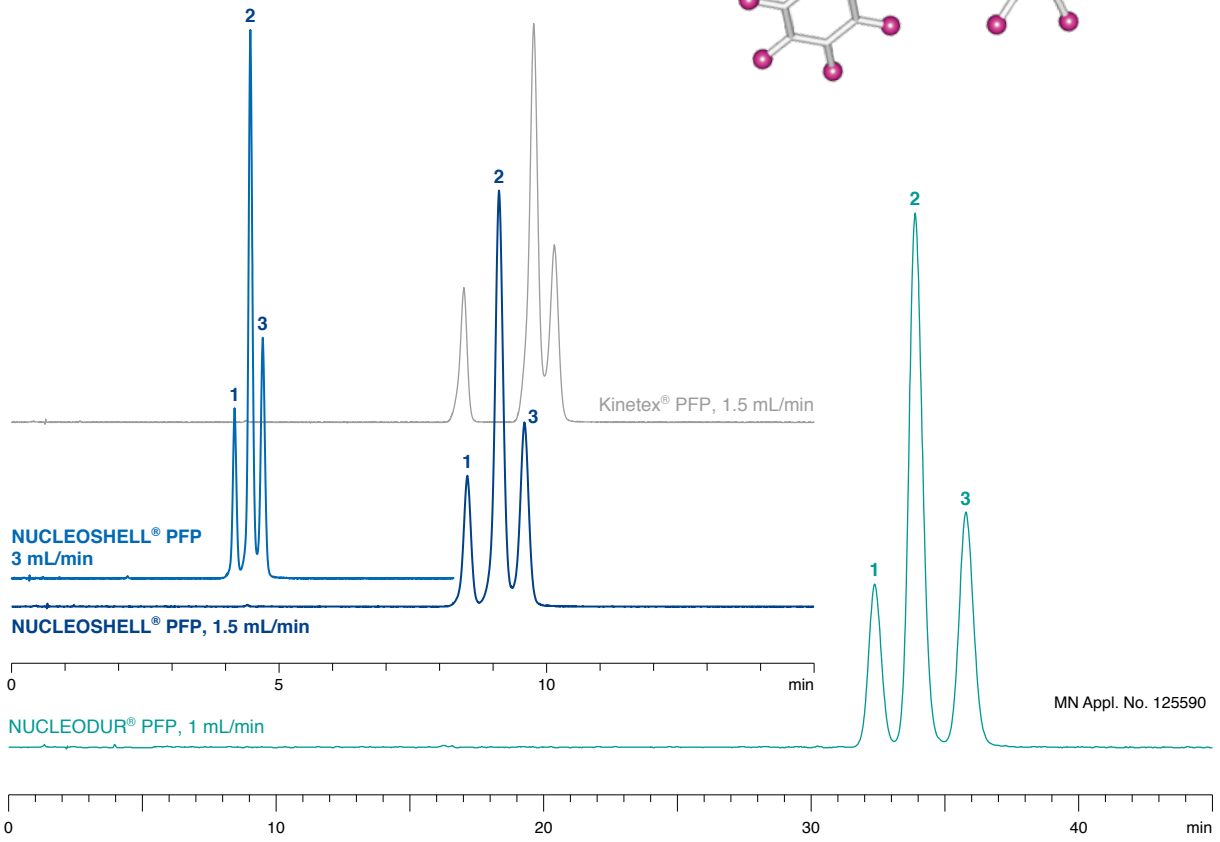


MN Appl. No. 125560

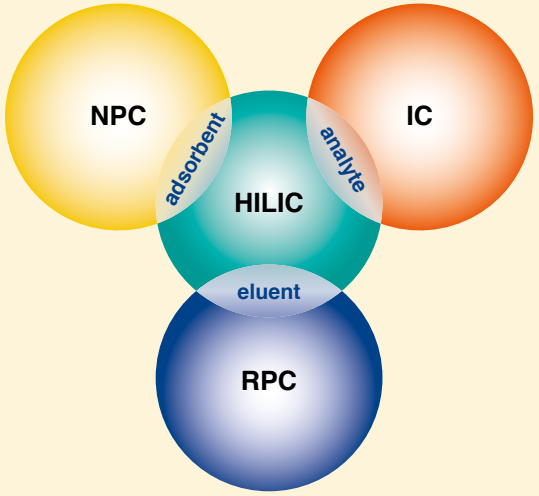
Methylacetophenones

Columns: **100 x 4.6 mm NUCLEOSHELL® PFP, 2.7 µm**
250 x 4 mm NUCLEODUR® PFP, 5 µm
100 x 4.6 mm Kinetex® 2.6 µm PFP
Eluent: methanol – water (35:65, v/v)
Flow rate: **1.5 mL/min, 3 mL/min,** 1 mL/min, 1.5 mL/min
Temperature: 35 °C
Detection: UV, 254 nm

Peaks:
1. *o*-Methylacetophenone
2. *p*-Methylacetophenone
3. *m*-Methylacetophenone



NUCLEOSHELL® HILIC



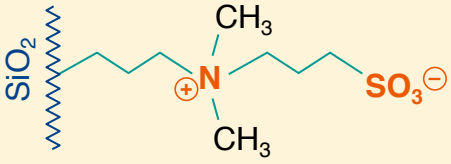
- **Key features:**
  - Based on core-shell particle technology for fast and efficient HPLC
  - Ideal for reproducible and stable chromatography of highly polar analytes
  - Very short column equilibration times
- **Technical characteristics:**
  Ammonium – sulfonic acid modified silica; pore size 90 Å,
  particle size 2.7 µm;
  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 layer between the adsorbent surface and the organic fraction of the mobile phase. The sample molecules become separated in a partition chromatography, in which polar analytes are more strongly retained than neutral, less hydrophilic compounds. Consequently, increasing the aqueous part in the mobile phase will diminish retention of the polar sample constituents. In this way HILIC behaves inverse to classical RP chromatography. The particular retention profile of HILIC enables the chromatography of very polar and often small molecules, which won't show any retention on C_8 or C_{18} reversed phases.

Ultra-fast separations at moderate back pressure

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

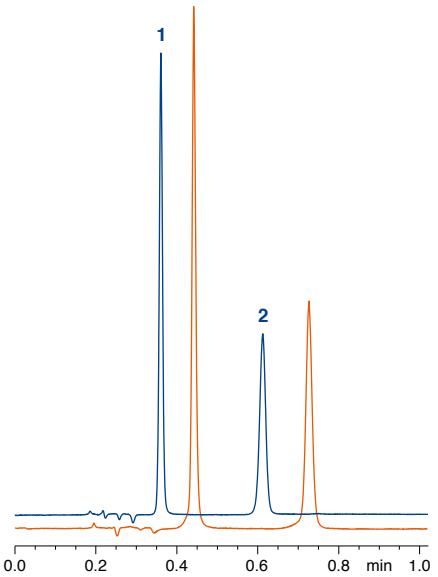


Good separation of polar compounds like the physiologically important substances creatine and creatinine can be achieved on NUCLEOSHELL® HILIC as well as on NUCLEODUR® HILIC, 1.8 µm at similar retention, but much lower back pressure.

Separation of creatine and creatinine

Columns:	**50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
	50 x 4 mm NUCLEODUR® HILIC, 1.8 µm
Eluent:	acetonitrile – 10 mmol/L ammonium acetate pH 4.0 (90:10, v/v)
Flow rate:	1.7 mL/min
Pressure:	**129 bar**
	180 bar
Temperature:	25 °C
Detection:	UV, 210 nm

Peaks:
1. Creatinine
2. Creatine



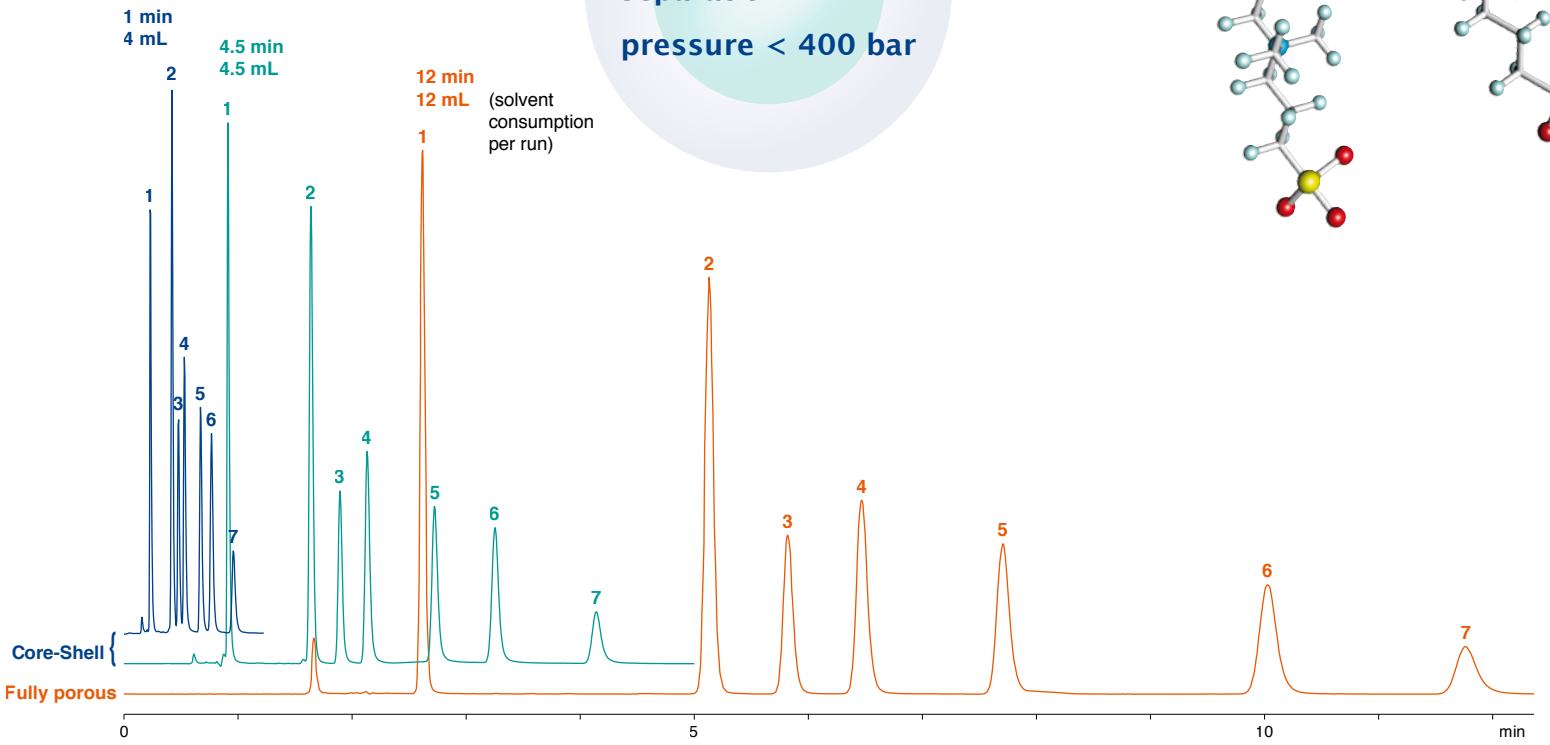
MN Appl. No. 124990

Separation of catecholamines

Columns: **100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm
250 x 4 mm NUCLEODUR® HILIC, 3 µm
Eluent: acetonitrile – 100 mmol/L ammonium formate pH 3.2 (80:20, v/v)
Flow rate: **4 mL/min, 1 mL/min,** 1 mL/min
Pressure: **395 bar, 95 bar,** 116 bar
Temperature: 25 °C
Detection: UV, 280 nm

Peaks:
1. DOPAC
2. Serotonin
3. Dopamine
4. Epinephrine
5. Norepinephrine
6. DOPA
7. DOPS

Core-shell silica: separation in 1 min pressure < 400 bar



MN Appl. No. 125440

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

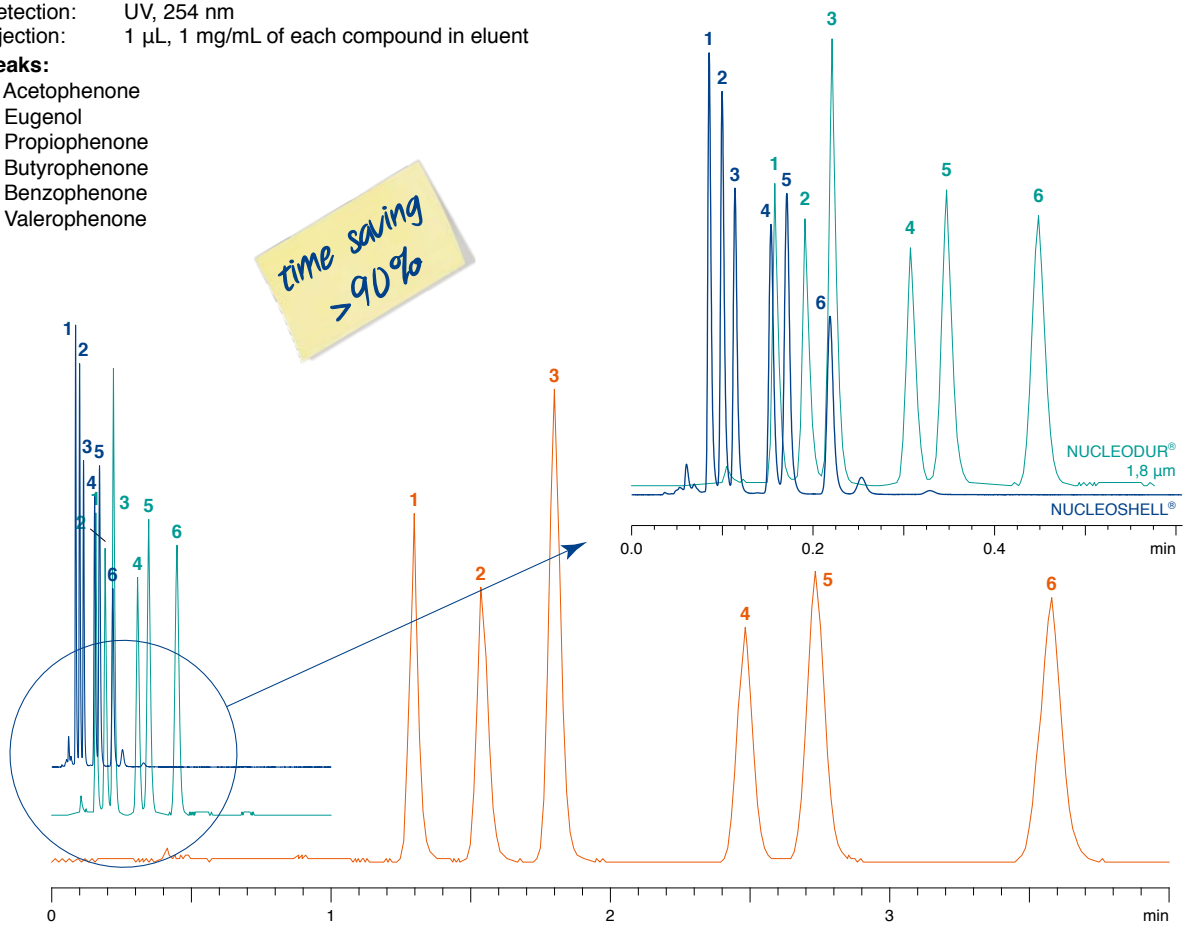
NUCLEOSHELL® HILIC provides stable and reproducible chromatography, comprising all the benefits of a state-of-the-art core-shell silica.

Applications

Separation of ketones

Columns: **50 x 3 mm NUCLEOSHELL® RP 18, 2.7 µm**
50 x 2 mm NUCLEODUR® C_{18} Gravity, 1.8 µm
125 x 2 mm NUCLEODUR® C_{18} Gravity, 5 µm
Eluent: acetonitrile – water (60:40, v/v)
Flow rate: **4 mL/min,** 1.25 mL/min, 0.33 mL/min
Pressure: **540 bar,** 774 bar, 89 bar
Temperature: 25 °C
Detection: UV, 254 nm
Injection: 1 µL, 1 mg/mL of each compound in eluent

Peaks:
1. Acetophenone
2. Eugenol
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone

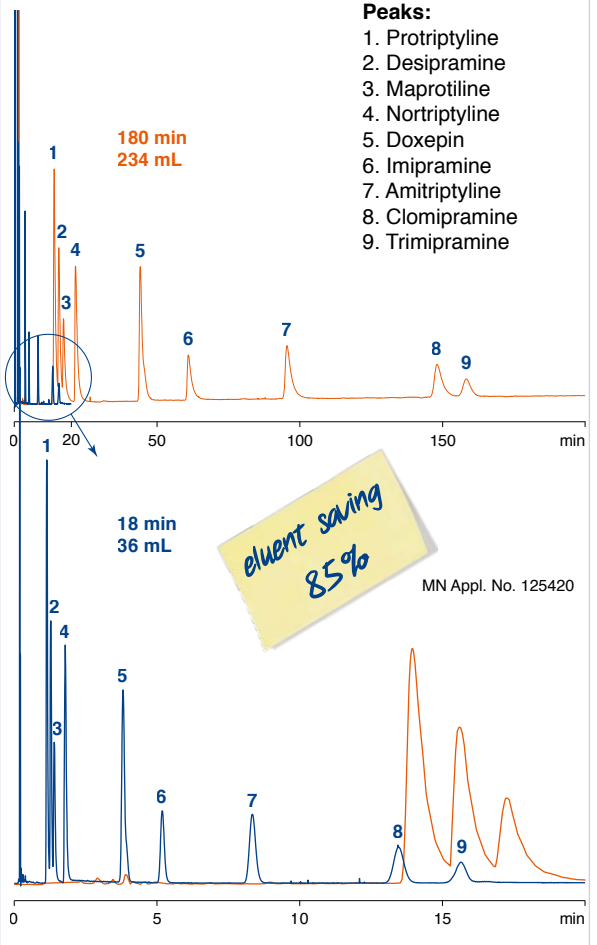


MN Appl. No. 124920

Tricyclic antidepressants

Columns: **50 x 4.6 mm NUCLEOSHELL® RP 18, 2.7 µm**
250 x 4.6 mm fully porous C18, 5 µm
Eluent: methanol – acetonitrile – 25 mmol/L KH_2PO_4 pH 7 (22.5:22.5:55, v/v)
Flow rate: **2 mL/min,** 1.3 mL/min
Pressure: **224 bar,** 190 bar
Temp.: 40 °C
Detection: UV, 220 nm

Peaks:
1. Protriptyline
2. Desipramine
3. Maprotiline
4. Nortriptyline
5. Doxepin
6. Imipramine
7. Amitriptyline
8. Clomipramine
9. Trimipramine



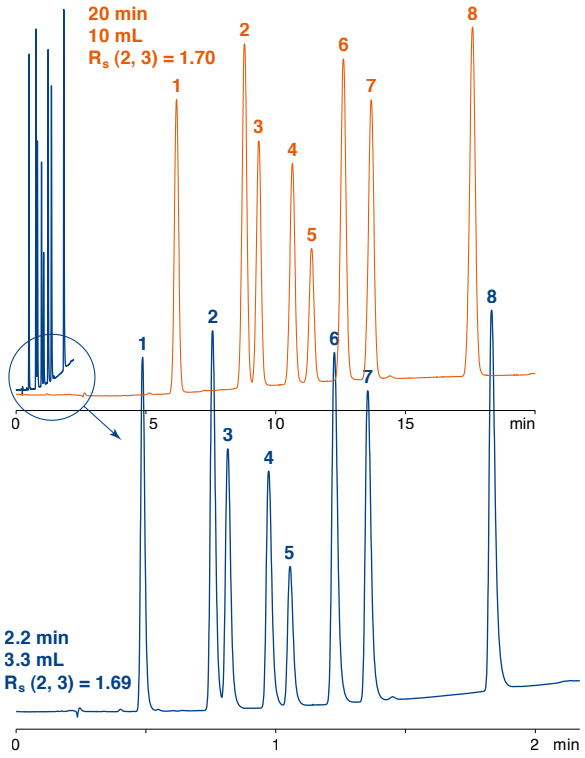
MN Appl. No. 125420

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 mmol/L KH_2PO_4 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:

1. Ketoprofen
2. Fenoprop
3. Fenoprofen
4. Flurbiprofen
5. Ibuprofen
7. Carprofen
8. Diclofenac
9. Meclofenamic acid

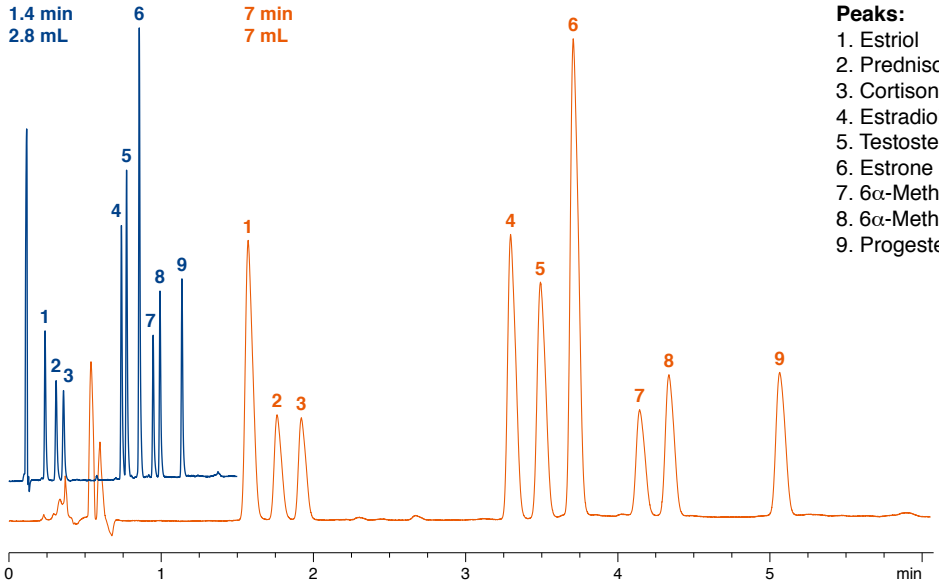


MN Appl. No. 125430

Separation of steroids

Columns: **50 x 3 mm NUCLEOSHELL® RP 18, 2.7 µm**
125 x 3 mm NUCLEODUR® C_{18} 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

up to
90% time saving
66% solvent saving



Peaks:

1. Estriol
2. Prednisolone
3. Cortisone
4. Estradiol
5. Testosterone
6. Estrone
7. 6α-Methyl-11β-hydroxyprogesterone
8. 6α-Methyl-17α-hydroxyprogesterone
9. Progesterone

MN Appl. No. 124930

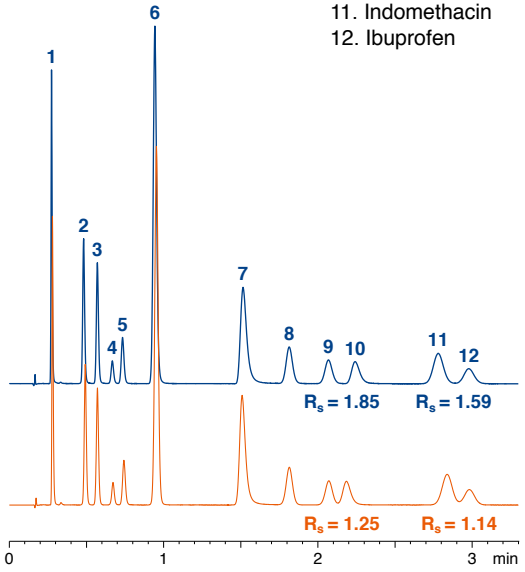
Applications

Non-steroidal anti-inflammatory drugs

Columns: 50 x 4.6 mm each
NUCLEOSHELL® RP 18, 2.7 µm
Ascentis® Express C18
Eluent: acetonitrile – 20 mmol/L KH_2PO_4 pH 2.5 (40:60, v/v)
Flow rate: 2.5 mL/min
Pressure: **268 bar,** 281 bar
Temperature: 22 °C
Detection: UV, 230 nm
Injection: 1 µL, 1 mg/mL of each compound in eluent

Peaks:
1. Acetylsalicylic acid
2. Sulindac
3. Piroxicam
4. Suprofen
5. Tolmetin
6. Naproxen
7. Diflunisal
8. Fenoprofen
9. Flurbiprofen
10. Niflumic acid
11. Indomethacin
12. Ibuprofen

good selectivity and resolution

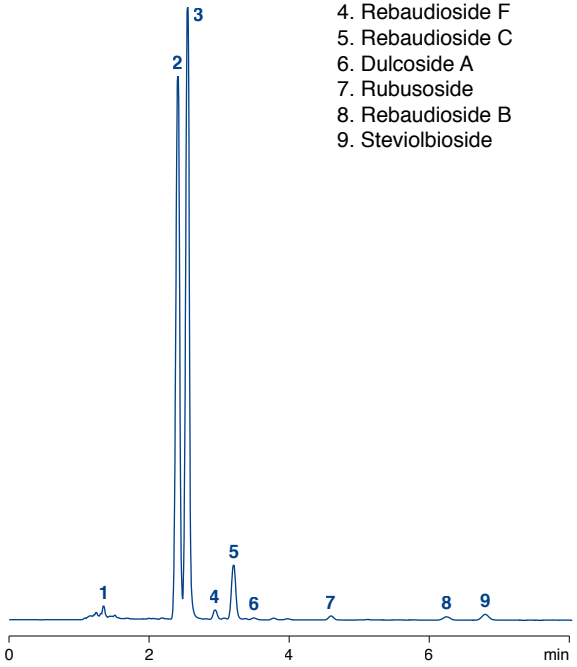


MN Appl. No. 124970

Steviol glycosides

Columns: **150 x 4.6 mm**
NUCLEOSHELL® RP 18, 2.7 µm
Eluent: acetonitrile – 10 mmol/L NaH_2PO_4 pH 2.6 (32:68, v/v)
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV, 210 nm
Injection: 5 µL

Peaks:
1. Rebaudioside D
2. Rebaudioside A
3. Stevioside
4. Rebaudioside F
5. Rebaudioside C
6. Dulcoside A
7. Rubusoside
8. Rebaudioside B
9. Steviolbioside

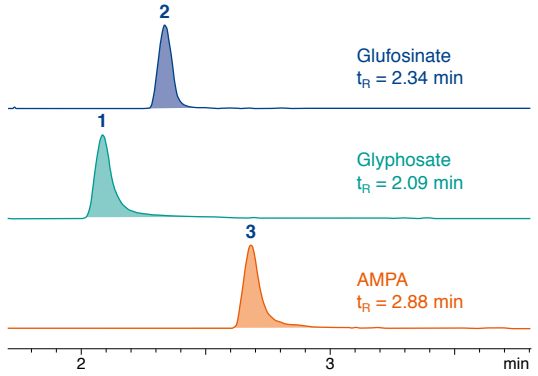


MN Appl. No. 125621

Phosphonic acid herbicides

Columns: **100 x 2 mm**
NUCLEOSHELL® RP 18, 2.7 µm
Eluent: A) acetonitrile,
B) 50 mmol/L ammonium acetate;
5–50 % A in 3.7 min,
50–95 % A in 0.6 min (2 min 95 % A),
95–5 % A in 0.5 min (2 min 5 % A)
Flow rate: 0.5 mL/min
Temperature: 30 °C
Detection: MS
Injection: 5 µL

Peaks:
1. Glyphosate (167 ng/mL)
2. Glufosinate (16.7 ng/mL)
3. AMPA (167 ng/mL)



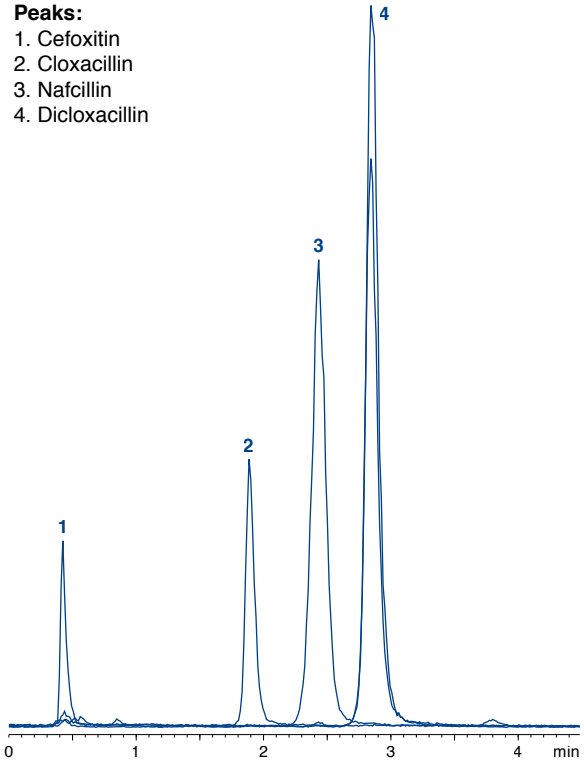
Courtesy of KUDZU SCIENCE, Illkirch, France

MN Appl. No. 126110

β-Lactam antibiotics

Column: **50 x 2 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm**
Eluent: methanol – 10 mmol/L ammonium formate, pH 3 (50:50, v/v)
Flow rate: 0.45 mL/min
Temperature: 40 °C
Detection: MS
Injection: 1 µL

Peaks:
1. Cefoxitin
2. Cloxacillin
3. Nafcillin
4. Dicloxacillin

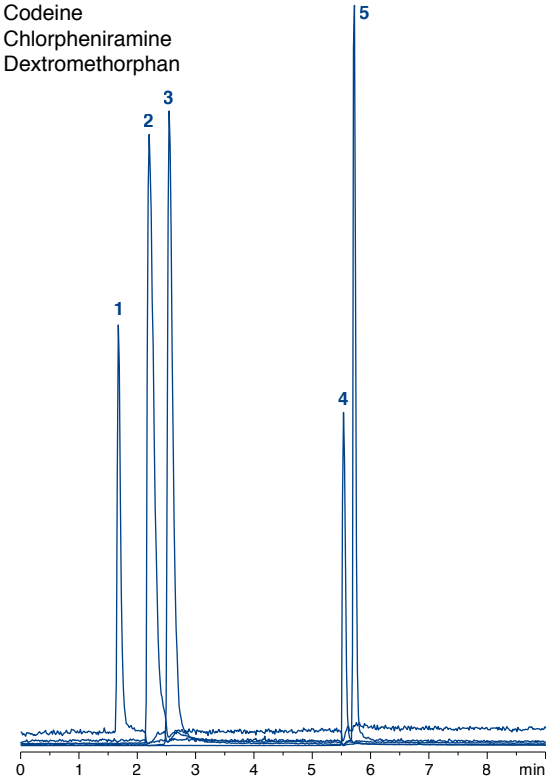


MN Appl. No. 126030

Antihistamines

Column: **100 x 3 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm**
Eluent: A) methanol,
B) 10 mmol/L ammonium formate, pH 2.8;
17.5 % A (2.5 min) → 65 % A in 1.5 min → 75 % A in 1.5 min (4.5 min 75 % A)
Flow rate: 0.6 mL/min
Temperature: 40 °C
Detection: MS
Injection: 0.5 µL

Peaks:
1. 4-Acetaminophenol
2. Pseudoephedrine
3. Codeine
4. Chlorpheniramine
5. Dextromethorphan

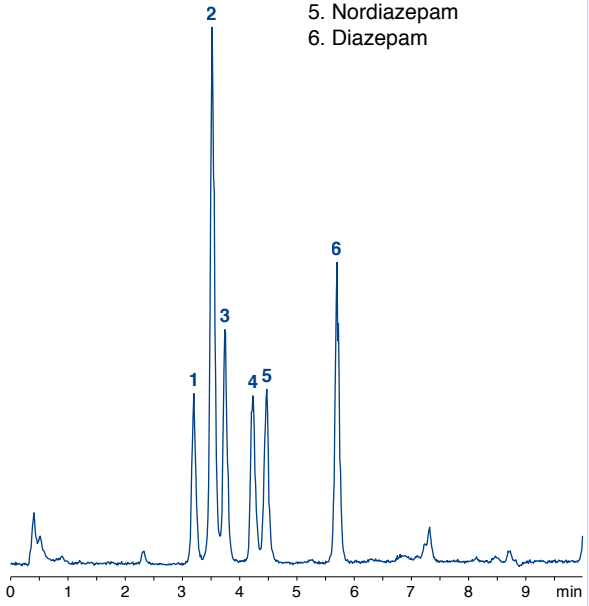


MN Appl. No. 125950

Benzodiazepines

Column: **50 x 2 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm**
Eluent: A) acetonitrile,
B) 20 mmol/L ammonium formate, pH 6.4;
25–55 % A in 10 min
Flow rate: 0.33 mL/min
Temperature: 25 °C
Detection: MS
Injection: 2.5 µL

Peaks:
1. Oxazepam
2. Chlordiazepoxide
3. Alprazolam
4. Trazodone
5. Nordiazepam
6. Diazepam



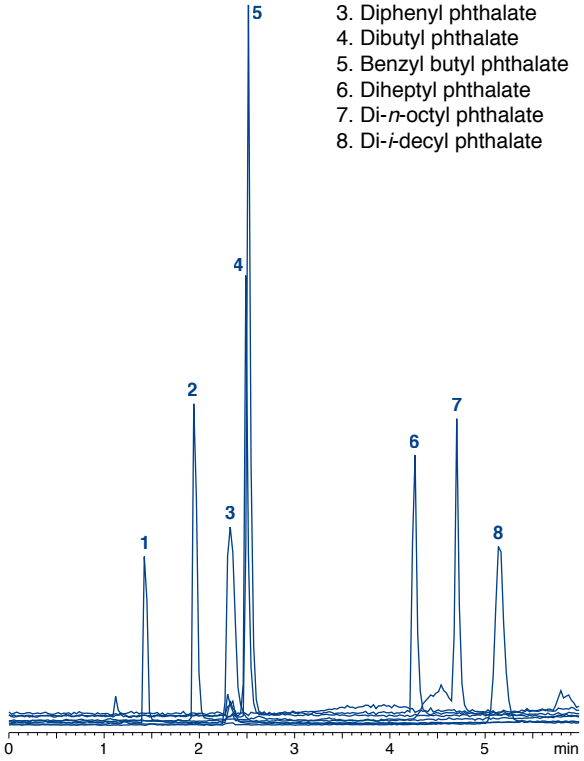
MN Appl. No. 126140

Applications

Phthalates

Column: **100 x 3 mm NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm**
Eluent: A) acetonitrile – methanol (50:50, v/v), B) 0.1 % formic acid in water; 75 –98 % A in 3.5 min (2.5 min 98 % A)
Flow rate: 0.75 mL/min
Temperature: 20 °C
Detection: MS
Injection: 0.5 µL

Peaks:
1. Dimethyl phthalate
2. Dipropyl phthalate
3. Diphenyl phthalate
4. Dibutyl phthalate
5. Benzyl butyl phthalate
6. Diheptyl phthalate
7. Di-*n*-octyl phthalate
8. Di-*i*-decyl phthalate



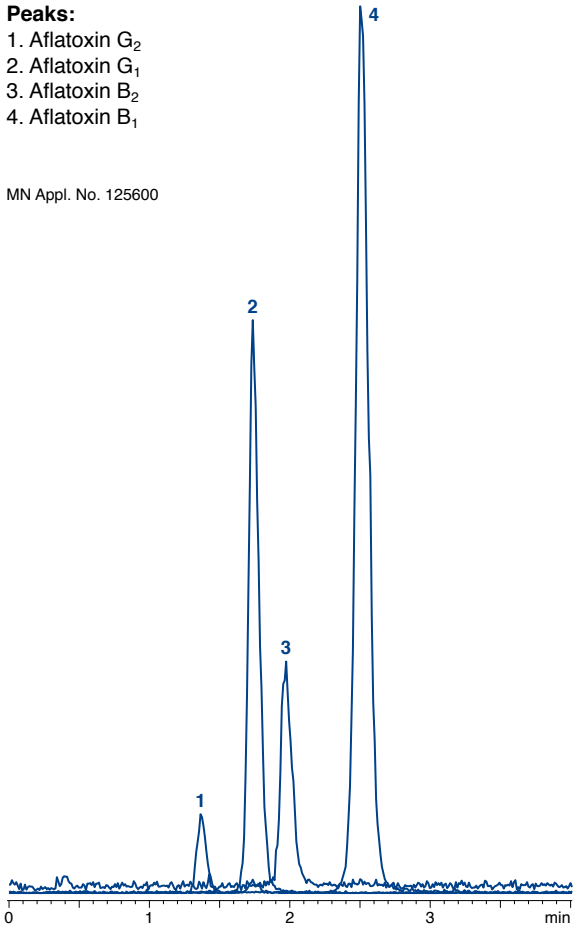
MN Appl. No. 126070

Aflatoxins

Column: **50 x 2 mm NUCLEOSHELL® PFP, 2.7 µm**
Eluent: methanol – 10 mmol/L ammonium acetate (45:55, v/v)
Flow rate: 0.33 mL/min
Temperature: 25 °C
Detection: MS
Injection: 0.1 ng each

Peaks:
1. Aflatoxin G_2
2. Aflatoxin G_1
3. Aflatoxin B_2
4. Aflatoxin B_1

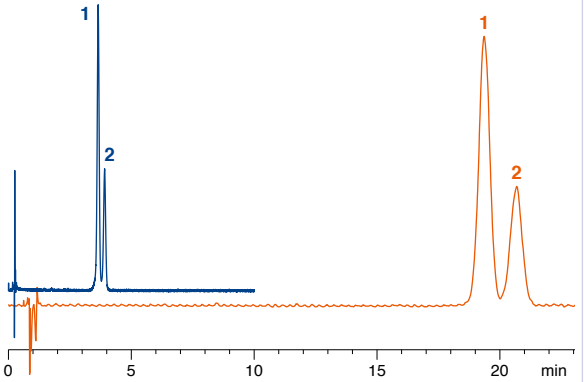
MN Appl. No. 125600



Beta- and dexamethasone

Columns: **50 x 4 mm NUCLEOSHELL® PFP, 2.7 µm**
100 x 4.6 mm NUCLEODUR® PFP, 5 µm
Eluent: acetonitrile – water (20:80, v/v)
Flow rate: **1.5 mL/min**
1.3 mL/min
Temperature: 30 °C
Detection: UV, 260 nm

Peaks:
1. Betamethasone
2. Dexamethasone

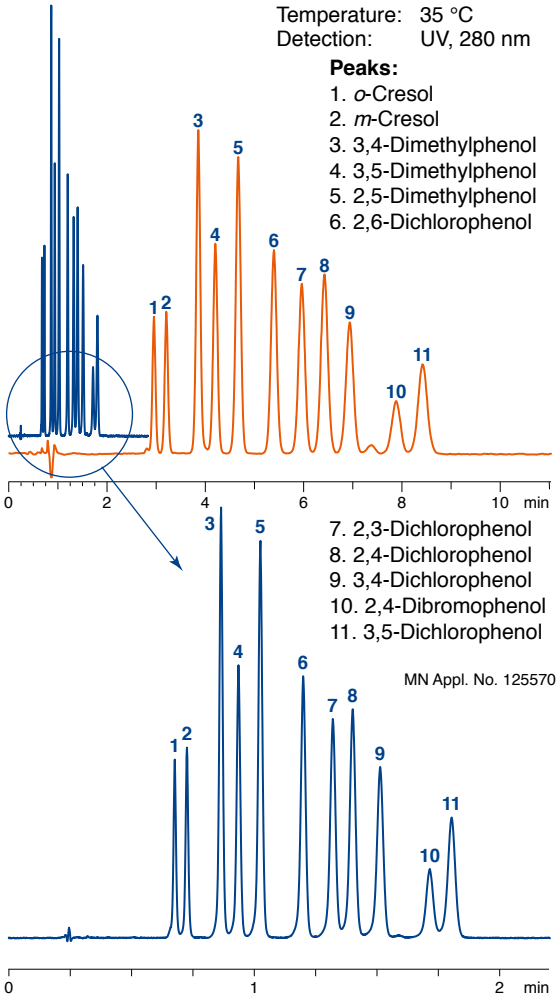


MN Appl. No. 125580

Phenols

Columns: **100 x 4.6 mm NUCLEOSHELL® PFP, 2.7 µm**
100 x 4.6 mm NUCLEODUR® PFP, 5 µm
Eluent: acetonitrile + 0.1 % formic acid – 0.1 % formic acid (35:65, v/v)
Flow rate: **4** / **1.3** mL/min
Temperature: 35 °C
Detection: UV, 280 nm

Peaks:
1. *o*-Cresol
2. *m*-Cresol
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-Dichlorophenol

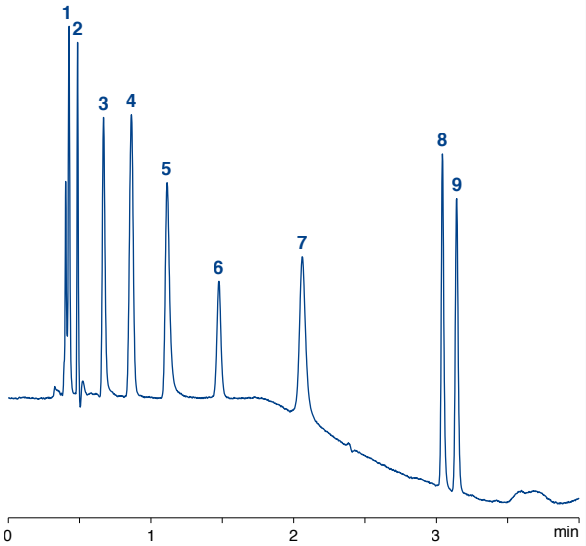


MN Appl. No. 125570

Water-soluble vitamins

Column: **100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: A) acetonitrile – 100 mmol/L 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 B_6 (pyridoxine)
4. Riboflavin
5. Nicotinic acid
6. Vitamin C (ascorbic acid)
7. Vitamin B_1 (thiamine)
8. Folic acid
9. Vitamin B_{12} (cyanocobalamine)

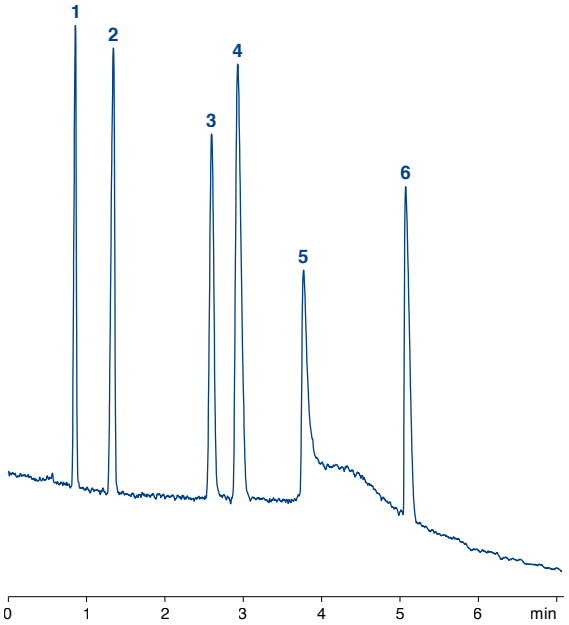


MN Appl. No. 125450

Anions and cations

Column: **100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: A) 30 mmol/L ammonium formate pH 3, B) acetonitrile
80 % B (3 min) → 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



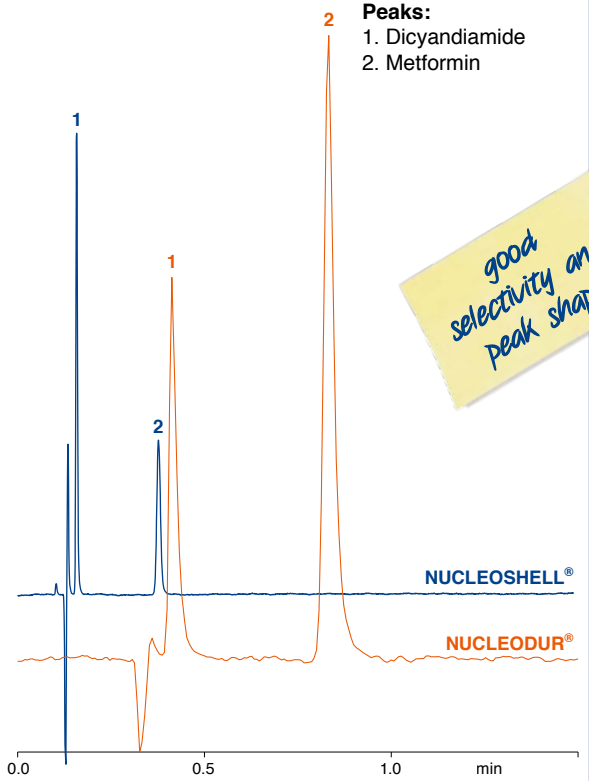
MN Appl. No. 125460

Applications

Metformin

Columns: **50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
50 x 4 mm NUCLEODUR® HILIC, 1.8 µm
Eluent: acetonitrile – 10 mmol/L ammonium acetate pH 3.2 (75:25, v/v)
Flow rate: **3 mL/min**
1.5 mL/min
Pressure: **202 bar**
167 bar
Temperature: 25 °C
Detection: UV, 218 nm

Peaks:
1. Dicyandiamide
2. Metformin



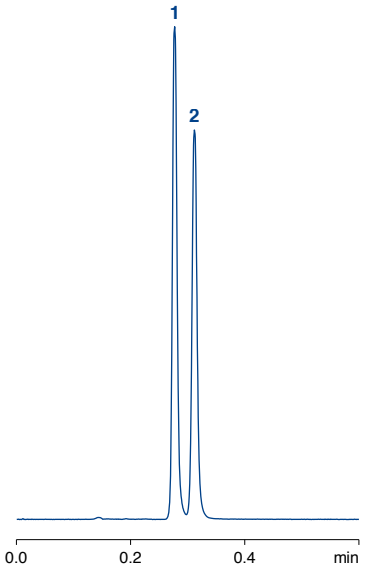
good selectivity and peak shape

MN Appl. No. 125470

5-Fluorouracil

Column: **50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: acetonitrile – 10 mmol/L ammonium acetate (95:5, v/v)
Flow rate: 2.5 mL/min
Pressure: 119 bar
Temperature: 25 °C
Detection: UV, 254 nm

Peaks:
1. 5-Fluorouracil
2. Uracil

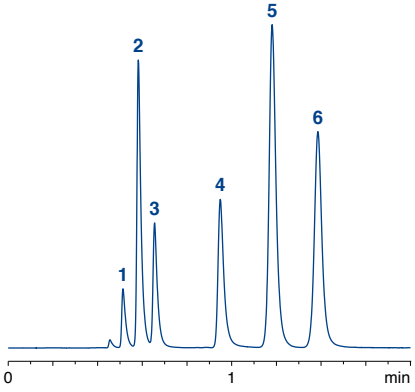


MN Appl. No. 125480

Analysis of an energy drink

Column: **100 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: acetonitrile – 100 mmol/L ammonium acetate pH 5.0 (90:10, v/v)
Flow rate: 1.7 mL/min
Pressure: 126 bar
Temperature: 35 °C
Detection: UV, 254 nm

Peaks:
1. Caffeine
2. Niacinamide
3. Pyridoxine
4. Benzoic acid
5. Sorbic acid
6. Riboflavin

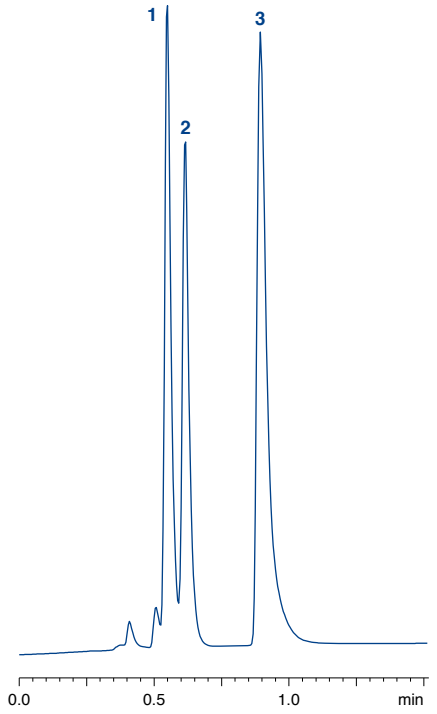


MN Appl. No. 125010

Acrylamide and analogs

Column: **50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: acetonitrile – 0.1% formic acid in water (98:2, v/v)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV, 200 nm
Injection: 1 µL, 1 mg/mL of each compound in eluent

Peaks:
1. Acrylamide
2. Methacrylamide
3. Methacrylic acid

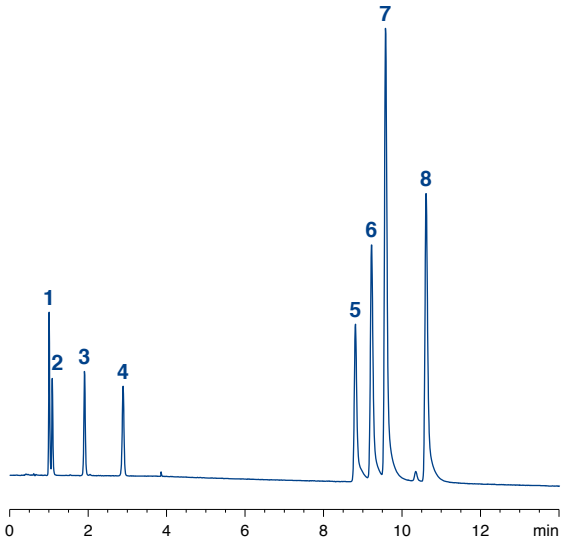


MN Appl. No. 125160

Nucleotides

Column: **50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluents: A) acetonitrile; B) 100 mmol/L ammonium acetate pH 5.35; 87.5–60 % A in 18 min
Flow rate: 2.2 mL/min
Temperature: 30 °C
Detection: UV, 259 nm
Injection: 1 µL, 1 mg/mL of each compound in eluent

Peaks:
1. Uridine
2. Adenosine
3. Cytidine
4. Cyclic adenosine monophosphate
5. Uridine monophosphate
6. Adenosine monophosphate
7. Inosine monophosphate
8. Cytidine monophosphate

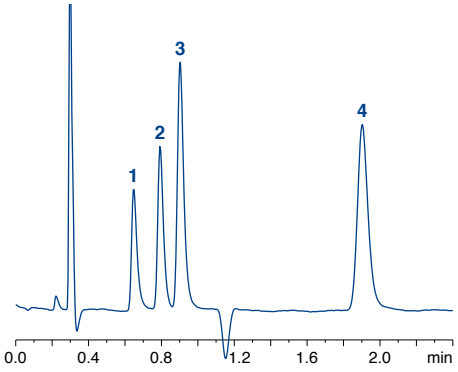


MN Appl. No. 125200

Amino acids

Column: **50 x 4 mm NUCLEOSHELL® HILIC, 2.7 µm**
Eluent: acetonitrile – 100 mmol/L ammonium acetate pH 4.0 (80:20, v/v)
Flow rate: 1.5 mL/min
Pressure: 105 bar
Temperature: 25 °C
Detection: UV, 215 nm

Peaks:
1. Phenylalanine
2. Phenylglycine
3. Tyrosine
4. Histamine



MN Appl. No. 125000

Packed columns · Ordering information

EC standard columns for analytical HPLC

- Analytical column system made of stainless steel

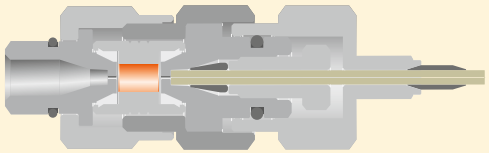
  M 8 outer threads on both ends

  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)

  EC column hardware guarantees pressure stability of 1200 bar – hence EC columns are suitable for U-HPLC applications (ultra fast HPLC) and all modern HPLC systems.

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



EC analytical columns

All phases: pore size 90 Å, particle size 2.7 µm; eluent in column CH_3CN – H_2O

Length →	50 mm	100 mm	150 mm	EC guard columns*
NUCLEOSHELL® RP 18, 2.7 µm			octadecyl modification, multi-endcapped, 7.5 % C	
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
NUCLEOSHELL® Phenyl-Hexyl, 2.7 µm			phenyl-hexyl modification, multi-endcapped, 4.5 % C	
2 mm ID	763732.20	763734.20	763736.20	4 x 2 mm: 763738.20
3 mm ID	763732.30	763734.30	763736.30	4 x 3 mm: 763738.30
4 mm ID	763732.40	763734.40	763736.40	4 x 3 mm: 763738.30
4.6 mm ID	763732.46	763734.46	763736.46	4 x 3 mm: 763738.30
NUCLEOSHELL® PFP, 2.7 µm			pentafluorophenyl modification, multi-endcapped, ~3 % C	
2 mm ID	763532.20	763534.20	763536.20	4 x 2 mm: 763538.20
3 mm ID	763532.30	763534.30	763536.30	4 x 3 mm: 763538.30
4 mm ID	763532.40	763534.40	763536.40	4 x 3 mm: 763538.30
4.6 mm ID	763532.46	763534.46	763536.46	4 x 3 mm: 763538.30
NUCLEOSHELL® HILIC, 2.7 µm			ammonium – sulfonic acid modification, 1.3 % C	
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 guard columns require the Column Protection System Cartridge Holder REF 718966 (see right).
EC columns in packs of 1, guard columns in packs of 3

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

Description	REF
Column Protection System	718966
Details	**Content**
Cartridge Holder	1
Replacement capillaries (0.12 mm ID)	2
Ferrules	3
Wrenches	2
Manual	1

Replacement parts for the Column Protection System · Ordering information

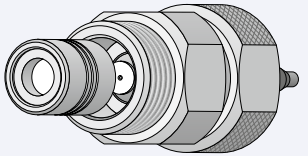
Description	Pack of	REF
Ferrules	5	718967
Replacement connector including O-ring	1	718968
Stainless steel capillaries 0.12 mm ID, nuts and metal ferrules	3	718969
Stainless steel capillaries 0.18 mm ID (for higher flow rates), nuts and metal ferrules	3	718971
Wrench (size 12 and 14 mm)	1	718970
EC 4/2 UNIVERSAL RP guard column (for main columns with 2 mm ID)	3	728777.20
EC 4/2 UNIVERSAL RP guard column (for main columns with 2 mm ID), value pack	9	728778.20
EC 4/3 UNIVERSAL RP guard column (for main columns with 3, 4 and 4.6 mm ID)	3	728777.30
EC 4/3 UNIVERSAL RP guard column (for main columns with 3, 4 and 4.6 mm ID), value pack	9	728778.30

Visual Contamination Check

The cartridge is fitted with a special filter membrane.

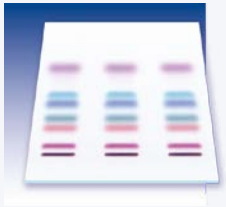
If the silver membrane is contaminated (bright or dark discoloration), it is advisable to replace the cartridge.

If the contaminants are colorless, replace the cartridge as soon as the pressure rises or the chromatographic performance decreases.



HPLC

GC

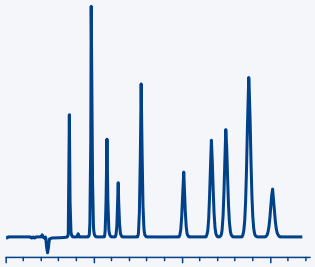


TLC

SPE and Flash

Syringe filters

Vials and caps



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