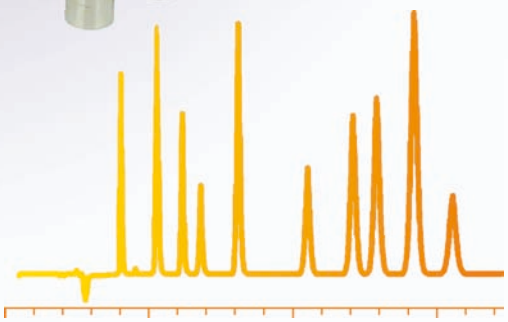
Chromatography

NUCLEODUR® C_{18} HTec

A New Dimension in Preparative HPLC



. . . we Meet your Needs

Ako nás možno kontaktovať:

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MACHEREY-NAGEL

www.mn-net.com

MN Since 1911

NUCLEODUR® C_{18} HTec

- **key features:**
  - reliable and durable standard RP phase for up-scaling to preparative scale, suited for **LC/MS**
  - high loadability and excellent stability
  - outstanding base deactivation
- **technical characteristics:**
  high density octadecyl modification (C_{18})
  pore size 110 Å, particle sizes 5 µm, 7 µm and 10 µm for analytical and preparative separations,
  carbon content 18 %, pH stability 1-11,
  high reproducibility from batch to batch

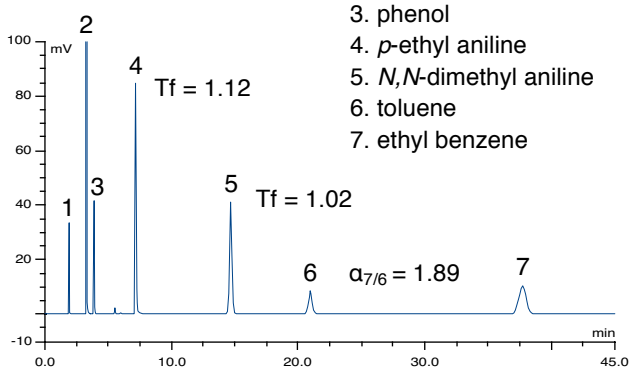
- **recommended application:**
  sophisticated analytical and preparative separations of basic, neutral and acidic pharmaceuticals, derivatised amino acids, pesticides, fat-soluble vitamins, aldehydes, ketones and phenolic compounds
  USP L1

Engelhardt-Test

excellent symmetry and peak shape over the entire polarity range

Column:	NUCLEODUR® 100-5 C_{18} HTec, 250 x 4 mm
Eluent:	methanol / water 49:51 (w/w)
Flow rate:	1.0 ml/min
Temperature:	40 °C
Inj. volume:	5 µl
Pressure:	130 bar
Detection:	UV, 254 nm

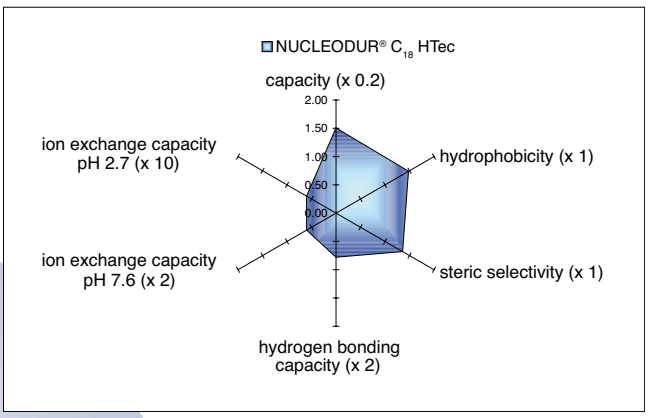
Peaks:
1. uracil
2. anilin
3. phenol
4. *p*-ethyl aniline
5. *N,N*-dimethyl aniline
6. toluene
7. ethyl benzene



MN Appl. No. 123580

Tanaka diagram

outstanding base deactivation and hydrophobic selectivity



MS bleeding test

low bleeding, good MS efficiency

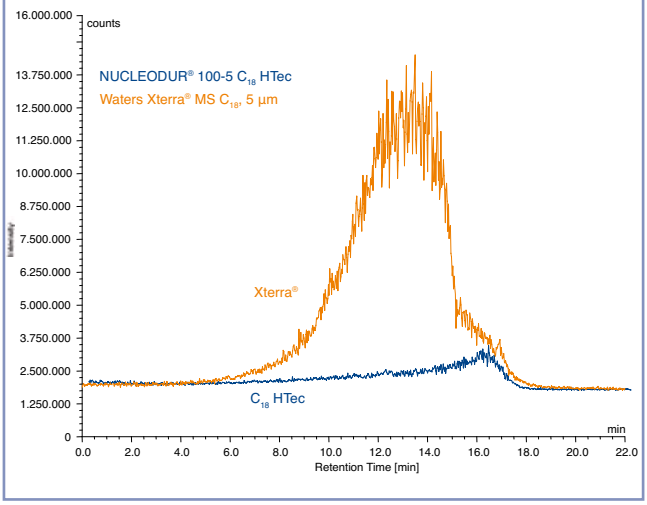
Columns:	NUCLEODUR® 100-5 C_{18} HTec, Waters Xterra® C_{18}, 5 µm (150 x 4.6 mm each)

HPLC conditions:

Eluent A:	water
Eluent B:	acetonitrile
Gradient:	
Start:	10 % B →100 % B in 15 min
	100 % B 5 min
	100 % B → B10 % B in 5 min
Flow rate:	0.26 ml/min
Temperature:	22 °C

MS conditions:

sample temperature:	450 °C
Needle:	3 kV
Cone voltage:	+ 50 V
Detection:	ESI+

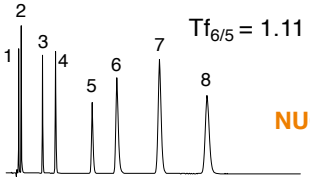


NUCLEODUR® C_{18} HTec: excellent base deactivation

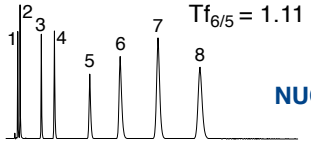
Phase comparison

Columns: 150 x 4.6 mm each; particle size 5 µm
Eluent: methanol / 25 mM KH_2PO_4, pH 7 (75:25, v/v)
Flow rate: 1.3 ml/min
Temperature: 30 °C
Detection: UV, 254 nm

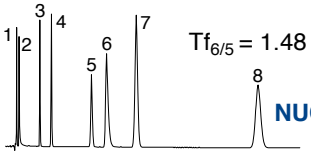
Peaks:
1. 2,7-dihydroxynaphthalene (metal ion content)
2. 2,3-dihydroxynaphthalene (metal ion content)
3. toluene (hydrophobicity, CH_2 group selectivity)
4. ethylbenzene (hydrophobicity, CH_2 group selectivity)
5. acenaphthene (silanol activity)
6. amitriptyline (silanol activity)
7. o-terphenyl (steric selectivity)
8. triphenylene (steric selectivity)



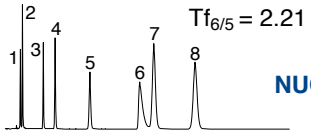
NUCLEODUR® C_{18} HTec



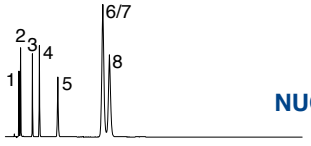
NUCLEODUR® C_{18} Gravity



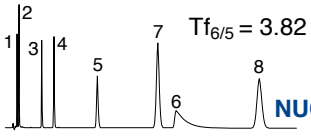
NUCLEODUR® C_{18} Isis



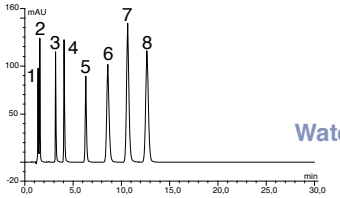
NUCLEODUR® C_{18} Pyramid



NUCLEODUR® Sphinx RP



NUCLEODUR® C_{18} ec



Waters Xterra® MS C_{18}

MN Appl. No. 123770

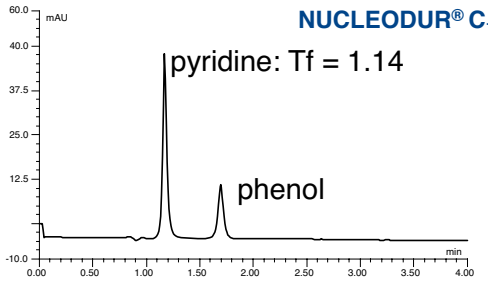
Base deactivation test

Columns: NUCLEODUR® 100-5 C_{18} HTec (125 x 4.6 mm)
Waters Xterra® MS C_{18}, 5 µm (150 x 4.6 mm)
Spherisorb® 80-5 ODS-1 (125 x 4.6 mm)
Eluent: acetonitrile / water (50:50, v/v)
Flow rate: 1.3 ml/min
Temperature: 25 °C
Detection: UV, 254 nm

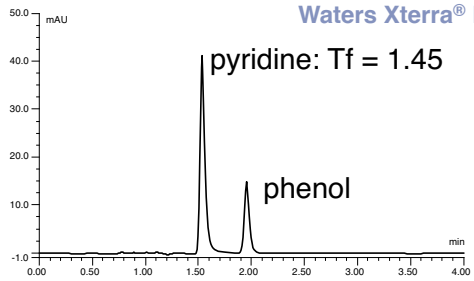
Important note: critical test without buffer, acetonitrile instead of methanol (no masking of pyridine by MeOH protons)

Quality criteria: pyridine elutes prior to phenol, maximal resolution, tailing (Tf) about 1.0

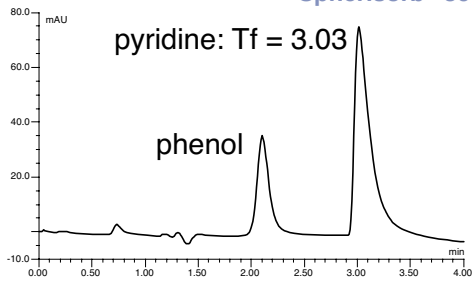
NUCLEODUR® C_{18} HTec



Waters Xterra® MS C_{18}



Spherisorb® 80-5 ODS-1



more applications at
www.mn-net.com/apps

pH Stability & Transferability

NUCLEODUR® C_{18} HTec

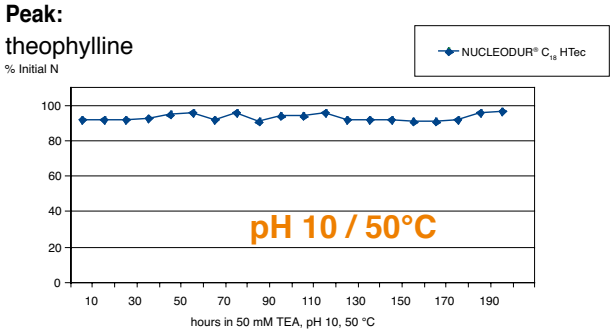
- excellent transferability from analytical to preparative particle sizes
- good peak symmetry and minimal peak broadening
- available in 5, 7 and 10 µm

pH 10 stability test

High pH-values can destroy the silica support resulting in dead volume and loss of theoretical plates.

Column:	NUCLEODUR® 100-5 C_{18} HTec, 150 x 4 mm
Eluent:	methanol / 50 mM triethylamine, pH 10 (25:80)
Flow rate:	1 ml/min
Temperature:	50 °C
Detection:	UV, 254 nm
Inj. volume:	5.0 µl
Injections:	1034

Peak:
theophylline

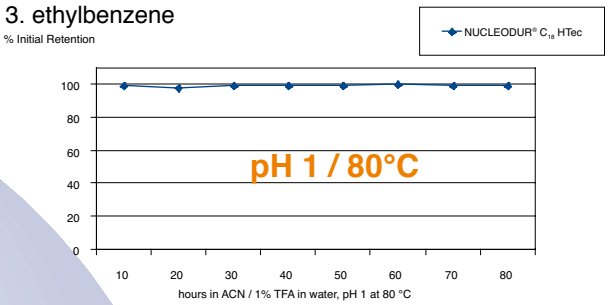


pH 1 stability test

Low pH-values can attack the C_{18}-modification resulting in loss of retention.

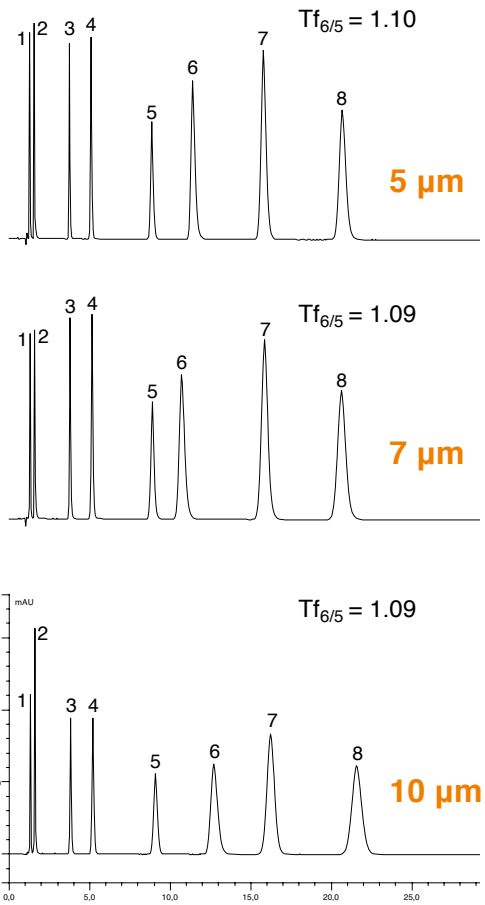
Column:	NUCLEODUR® 100-5 C_{18} HTec, 150 x 4 mm
Eluent:	acetonitrile / 1 % TFA in water, pH 1 (50:50, v/v)
Flow rate:	1 ml/min
Temperature:	80 °C
Detection:	UV, 254 nm
Inj. volume:	5.0 µl
Injections:	693

Peaks:
1. pyridine
2. toluol
3. ethylbenzene



Transferability

Column:	NUCLEODUR® 100-5 C_{18} HTec 150 x 4.6 mm each
Eluent:	methanol / 25 mM KH_2PO_4, pH 7 (75:25)
Flow rate:	1.3 ml/min
Temperature:	30 °C
Detection:	UV, 254 nm



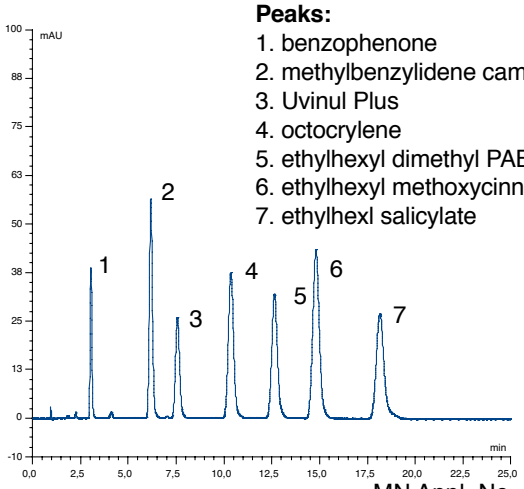
Peaks:
1. 2,7-dihydroxynaphthalene (metal ion content)
2. 2,3-dihydroxynaphthalene (metal ion content)
3. toluene (hydrophobicity, CH_2 group selectivity)
4. ethylbenzene (hydrophobicity, CH_2 group selectivity)
5. acenaphthene (silanol activity)
6. amitriptyline (silanol activity)
7. o-terphenyl (steric selectivity)
8. triphenylene (steric selectivity)

Analytical application examples for NUCLEODUR® C_{18} HTec

Sunscreen ingredients

Column: NUCLEODUR® 100-5 C_{18} HTec, 125 x 4 mm
Eluent: methanol / 100 mM ammonium acetate, pH 4.5 (80:20, v/v)
Flow rate: 0.9 ml/min
Temperature: 35 °C
Inj. volume: 12 µl
Detection: UV, 275 nm

Peaks:
1. benzophenone
2. methylbenzylidene camphor
3. Uvinul Plus
4. octocrylene
5. ethylhexyl dimethyl PABA
6. ethylhexyl methoxycinnamate
7. ethylhexl salicylate

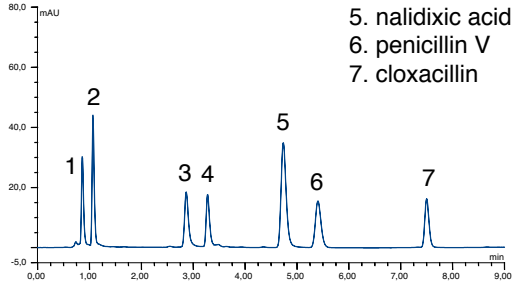


MN Appl. No. 123640

Antibiotics

Column: NUCLEODUR® 100-5 C_{18} HTec, 125 x 4 mm
Eluents: A: acetonitrile B: 0.05 % TFA
Gradient: 70 % B for 1 min
70 % B → 60 % B in 0.5 min
60 % B for 3.5 min
60 % B → 50 % B in 1 min
50 % B → 37.5 % B in 4 min
Flow rate: 0.9 ml/min
Temperature: 25 °C
Inj. volume: 10 µl
Detection: UV, 254 nm
Concentration: 300 µg/ml

Peaks:
1. amoxicillin
2. enrofloxacin
3. cinoxacin
4. oxolinic acid
5. nalidixic acid
6. penicillin V
7. cloxacillin

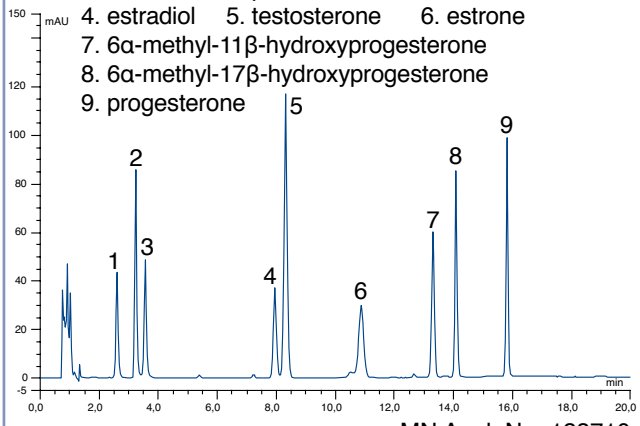


MN Appl. No. 123760

Steroids

Column: NUCLEODUR® 100-5 C_{18} HTec, 125 x 4 mm
Eluents A: acetonitrile B: water
Gradient: 70 % B → 60 % B in 5 min
60 % B for 5 min
60 % B → 35 % B in 5 min
35 % B for 5 min
Flow rate: 1.0 ml/min
Temperature: 35 °C
Inj. volume: 10 µl
Detection: UV, 254 nm

Peaks:
1. estriol 2. prednisolone 3. cortisone
4. estradiol 5. testosterone 6. estrone
7. 6α-methyl-11β-hydroxyprogesterone
8. 6α-methyl-17β-hydroxyprogesterone
9. progesterone

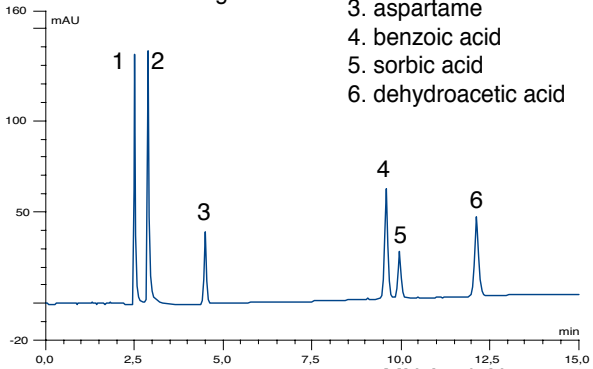


MN Appl. No. 123710

Sweeteners

Column: NUCLEODUR® 100-5 C_{18} HTec, 125 x 4.6 mm
Eluents: A: acetonitrile B: 25 mM KH_2PO_4, pH 3.5
Gradient: 15 % A for 2.5 min
15 % A → 25 % A in 9.5 min
25 % A for 3 min
Flow rate: 1.3 ml/min
Temperature: 40 °C
Inj. volume: 5 µl
Detection: UV, 220 nm
Concentration: 0.1 mg/ml each

Peaks:
1. acesulfame
2. saccharin
3. aspartame
4. benzoic acid
5. sorbic acid
6. dehydroacetic acid



MN Appl. No. 123750

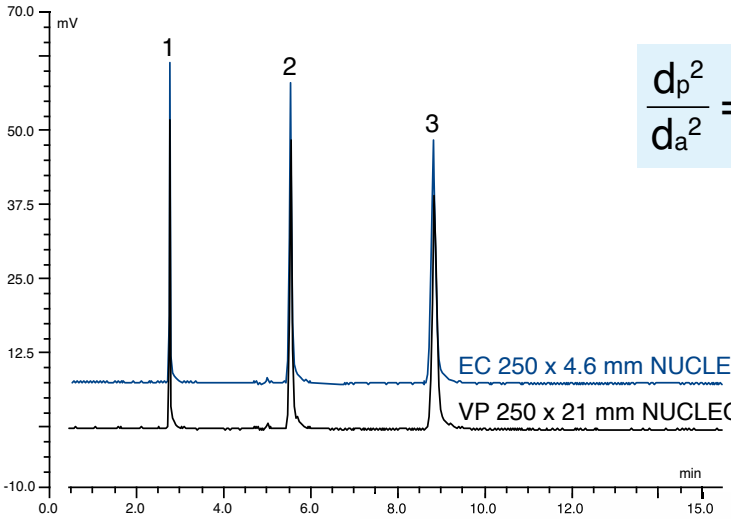
more applications at
www.mn-net.com/apps

Up-Scaling

NUCLEODUR® C_{18} HTec . . . ideal for up-scaling

Identical peak shapes and retention while up-scaling from analytical to preparative column dimension.

$$\frac{d_p^2}{d_a^2} = \frac{u_p}{u_a} = \frac{(21\,mm)^2}{(4{,}6\,mm)^2} \approx \frac{27\ ml/min}{1{,}3\ ml/min}$$



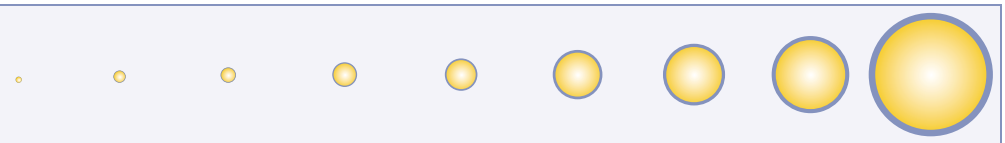
Columns: EC 250 x 4.6 mm NUCLEODUR® 100-5 C_{18} HTec
VP 250 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
Eluent: acetonitrile / water (80:20, v/v)
Flow rate: 1.3 ml/min
27.0 ml/min
Temperature: 22 °C
Detection: UV, 254 nm
Pressure: 84 bar
109 bar

Peaks:
1. phenol
2. naphthalene
3. anthracene
(1 mg/ml each)

Inj. volume: 3 µl
60 µl

MN Appl. No. 123780

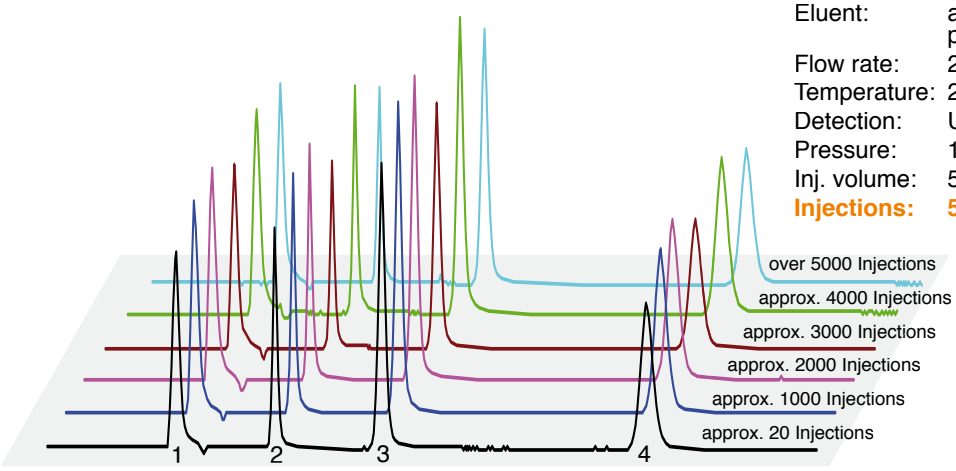
Scale up factors and parameters for common MN column dimensions



ID x length [mm]	4 x 250	8 x 250	10 x 250	16 x 250	21 x 250	32 x 250	40 x 250	50 x 250	80 x 250
Linear scale-up factor	1	4	6,25	16	27,6	64	100	161,3	400
Typical sample mass* [mg]	0,02 – 2	0,08 – 8	0,13 – 13	0,3 – 35	0,6 – 60	1,3 – 130	2 – 210	3 – 350	10 – 850
Typical flow rate [ml/min]	0,5 – 1,5	2 – 6	3 – 9	8 – 24	14 – 40	32 – 96	50 – 150	80 – 250	200 – 600

* For RP material; the maximum amounts given here always depend on the separation problem and on the sample composition. In some cases half of the amount given can cause drastic overload, in other cases the maximum amounts can be even higher still giving acceptable separations.

NUCLEODUR® C_{18} HTec . . . exceptional column life time under critical conditions



Column:	VP 100 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
Eluent:	acetonitrile / formic acid in water, pH 3.0 (10:90, v/v)
Flow rate:	28 ml/min
Temperature:	22 °C
Detection:	UV, 254 nm
Pressure:	137 bar
Inj. volume:	50 µl
Injections:	**5146**

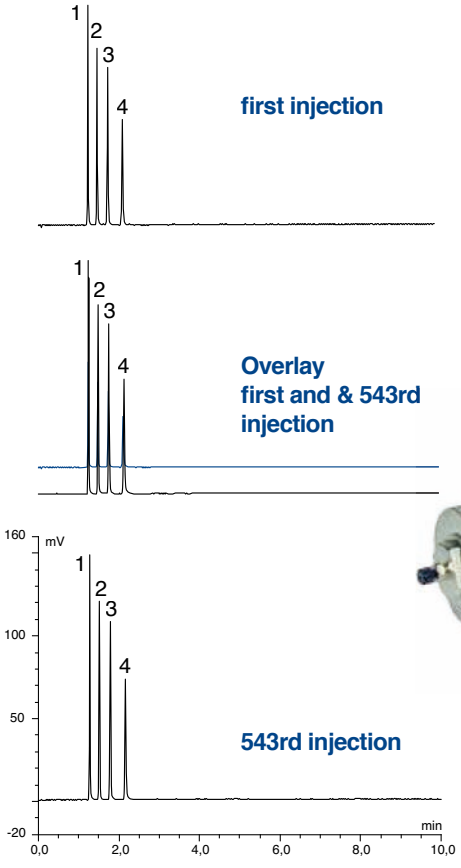
Peaks:
1. xanthine (62.5 mg/ml)
2. theobromine (12.5 mg/ml)
3. theophylline (12.5 mg/ml)
4. caffeine (12.5 mg/ml)

Sample dissolved in **DMSO**.

NUCLEODUR® C_{18} HTec shows reproducible retention times and excellent peak shapes even after 5000 injections.

MN Appl. No. 123790

at high flow rates



Column:	VP 100 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
Eluent:	acetonitrile / water (80:20, v/v)
Flow rate:	**60 ml/min**
Temperature:	22 °C
Detection:	UV, 254 nm
Pressure:	**275 bar**
Inj. volume:	50 µl

Peaks:
1. acetophenone
2. propanone
3. butyrophenone
4. valerophenone

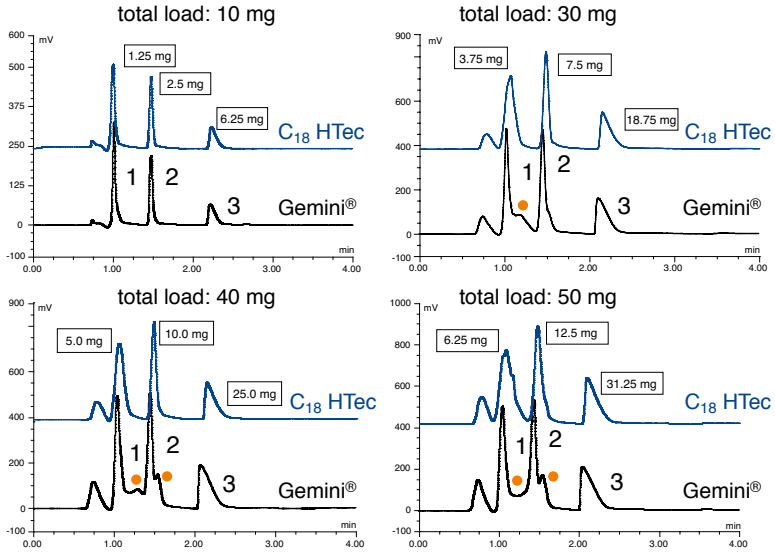
NUCLEODUR® C_{18} HTec shows reproducible retention times and excellent peak shapes even during operations at very high flow rates.

MN Appl. No. 123800

Loadability 1

NUCLEODUR® C_{18} HTec
highest loadability under acidic and basic conditions

acidic conditions pH 3.0



Columns: VP 100 x 21 mm NUCLEODUR® 100-5 C_{18} HTec, 5 µm
AXIA™ 100 X 21,2 mm Gemini® 5 µm C18 110 Å
Eluent: acetonitrile / formic acid in water, pH 3.0, (30:70 v/v)
Flow rate: 28 ml/min
Temperature: 22 °C
Detection: UV, 254 nm
Pressure: 124 bar

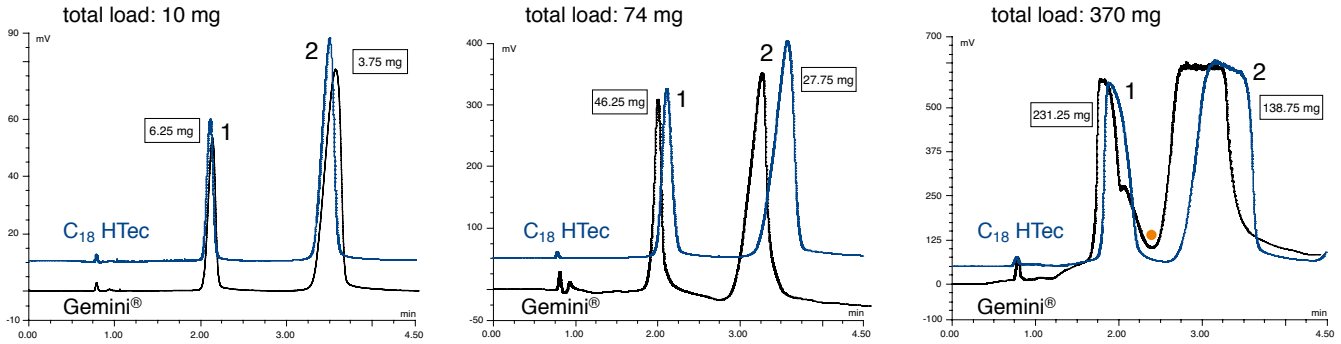
Peaks:
1. 4-acetamido phenol (paracetamol)
2. 2-acetamido phenol
3. acetylsalicylic acid

Sample dissolved in **DMSO**.

- In comparison NUCLEODUR® C_{18} HTec shows no overloading or peak overlapping even at high sample concentrations.

MN Appl. No. 123890

basic conditions pH 7.7



Columns: VP 100 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
AXIA™ 100 X 21.2 mm Gemini® 5 µm C18 110 Å
Eluent: methanol / 25 mM NH_4HCO_3, pH 7.7 (60:40, v/v)
Flow rate: 28 ml/min
Temperature: 22 °C
Detection: UV, 254 nm
Pressure: 161 bar

Peaks:
1. hexobarbital
2. thiamylal

Sample dissolved in **DMSO**.

- Competitor's column provides no baseline separation even at medium load.

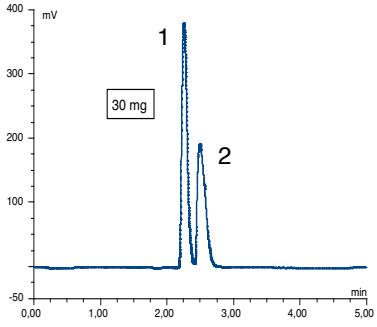
AXIA™ and Gemini® are trademarks of Phenomenex

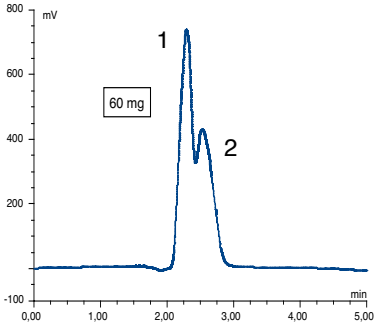
MN Appl. No. 123900

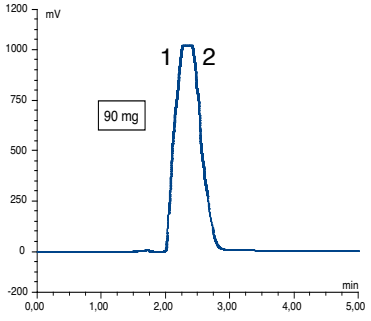
- **no overloading effects even at maximum sample concentration**
- **no overlapping effects or intermixture of compounds**
- **high yields and efficiency**

NUCLEODUR® C_{18} HTec
optimal loadability by variation of pH

acidic conditions pH 3.75





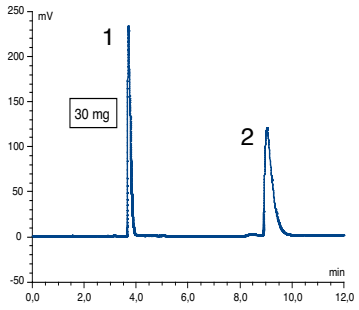


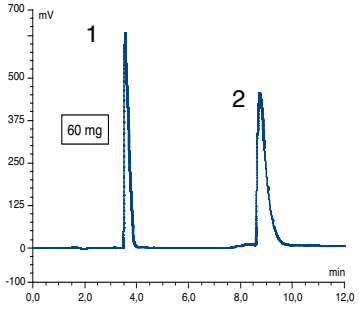
Column: VP 250 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
Eluent: acetonitrile / acetic acid (75:25, v/v)
pH: 3.75
Flow rate: 27 ml/min
Temperature: 22 °C
Detection: UV, 254 nm

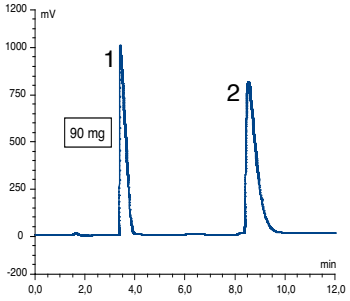
Peaks:
1. lidocaine
2. amitriptyline

- **limited loadability under acidic conditions**

basic conditions pH 10.0







Column: VP 250 x 21 mm NUCLEODUR® 100-5 C_{18} HTec
Eluent: acetonitrile / NH_4HCO_3 in water (75:25, v/v)
pH: 10.0
Flow rate: 27 ml/min
Temperature: 22 °C
Detection: UV, 254 nm

Peaks:
1. lidocaine
2. amitriptyline

- **significantly higher loadability by optimisation of pH at 10**
- **at 90 mg limit of solubility is reached**
- **long term stability at pH 10 guaranteed!***

*(see page 4)

- **limited loadability at acidic conditions (pH 3.75)**
- **at pH 10 even 90 mg sample don't result in overloading effects**
- **highest yields and efficiency**

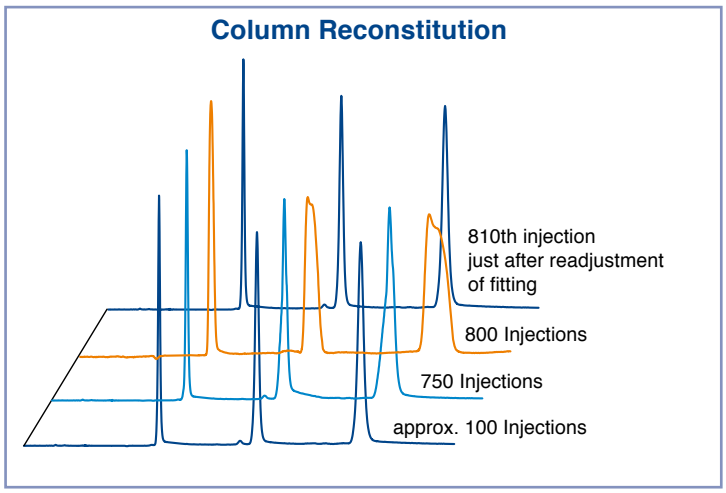
The VarioPrep System

VarioPrep columns

- column system for preparative HPLC manufactured from stainless steel with adjustable end fittings (suitable for frequent use of back-flushing techniques)
- allows compensation of a dead volume, which could result at the column inlet after some time of operation at demanding conditions, without need for opening the column
- supplied with all NUCLEODUR® and NUCLEOSIL® spherical silicas

The VarioPrep principle

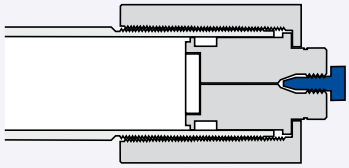
Based on our special packing procedure VarioPrep columns are produced with highest packing quality and bed density (**A**). Due to intensively chemical and/or mechanical exposure of the column adsorbent, shrinking of the column bed can occur (**B;** orange gap). In this even unlikely case readjustment of the VarioPrep column fitting (**C**; turning the nut at column inlet clockwise) will eliminate the emerged dead volume (**D**). The performance of the VarioPrep column is completely reconstituted and column lifetime is significantly extended.



Reconstitution of VarioPrep column performance:

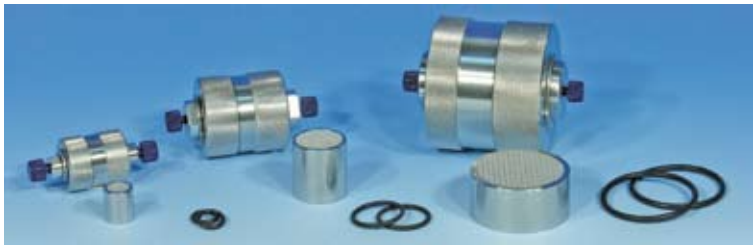
- **sligth peak broadening and deformation after 800 injections under strongly demanding conditions (pH 11; 50°C; sample in DMSO)**
- **readjustment of the column fitting restores the column performance and prolongs column lifetime noticeably**

Available standard dimensions of VarioPrep columns with axially adjustable end fittings

ID [mm]	Length [mm]										end fitting design
	10*	15*	20*	30*	50	100	125	150	250	500	
8	x				x	x	x	x	x		
10					x	x	x	x	x		
16			x		x	x	x	x	x		
21					x	x	x	x	x		
32		x				x		x	x		
40					x	x	x	x	x	x	
50				x		x		x	x		
80									x	x	

* 10 x 8, 20 x 16, 15 x 32 and 30 x 50 mm ID columns are used as guard columns and require the adequate holders.
Details see on page 13.

VarioPrep Guard Columns



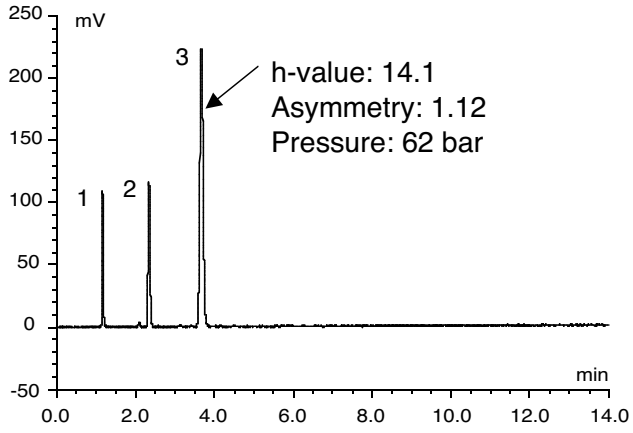
VP 8/10	VP 20/16	VP 15/32	VP 30/50
for 8 and 10 mm ID columns	for 16 and 21 mm ID columns	for 32 and 40 mm ID columns	for ≥ 50 mm ID columns

The new guard column system for (semi-) preparative HPLC

- easy handling and cartridge exchange
- robust hardware
- cost-efficient cartridges
- minimally invasive / no disturbance of the separation efficiency of main column
- low back pressure

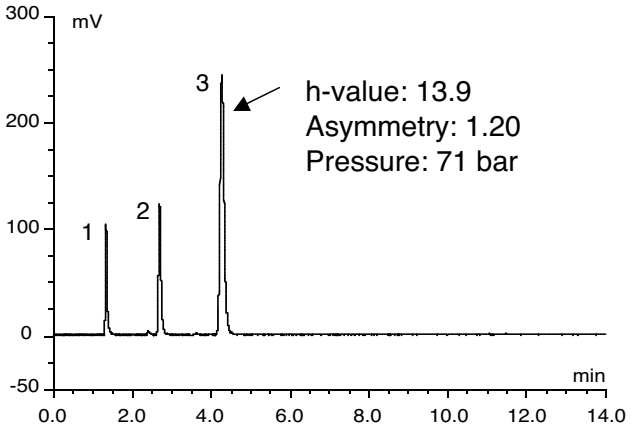
Performance of preparative column when used with VarioPrep guard column

VP 125/16 NUCLEODUR® 100-5 C_{18} HTec



Eluent: acetonitrile / water (80:20, v/v)
Flow rate: 16 ml/min
Temperature: 22 °C

VP 125/16 NUCLEODUR® C_{18} 100-5 HTec
VP 20/16 NUCLEODUR® C_{18} HTec (guard column)



Peaks:
1. phenol
2. naphthaline
3. anthracene

Using VarioPrep guard columns provides ideal protection of your main column – symmetry, pressure and retention stay almost constant.

Technical data:

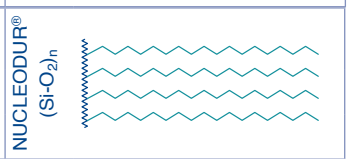
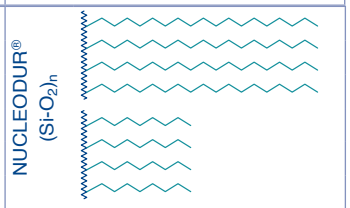
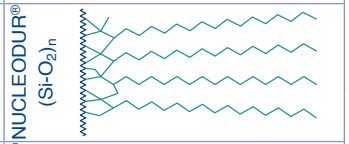
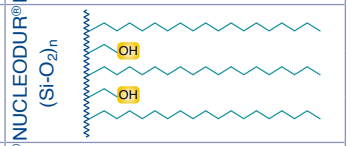
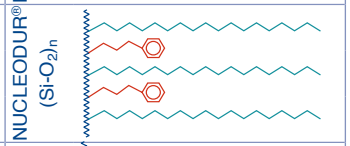
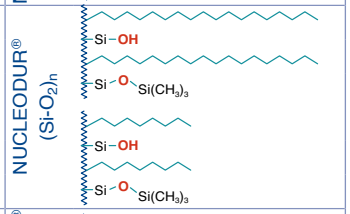
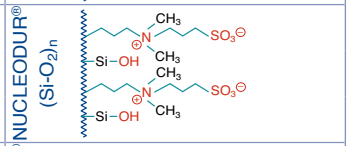
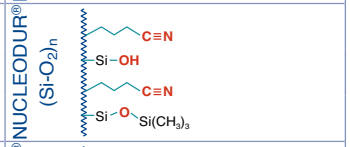
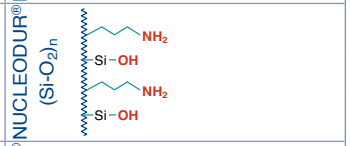
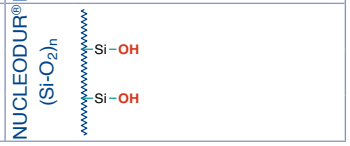
- 1/16" thread
- free rotary plunger fittings – low O-ring abrasion
- stainless steel

Guard cartridge	Recommended for column ID	Prefered capillary ID	Typical flow rate
VP 10/8	8 and 10 mm ID	0.17 and 25 mm	1 – 12 ml/min
VP 20/16	16 and 21 mm ID	0.17, 0.25 and 0.5 mm	2 – 32 ml/min
VP 15/32	32 and 40 mm ID	0.25, 0.5 and 1.0 mm	5 – 150 ml/min
VP 30/50	≥ 50 mm ID	0.5 and 1.0 mm	20 – 250 ml/min

The cartridge system is designed for pressures up to 400 bar.

Further NUCLEODUR® Phases

MACHEREY-NAGEL offers all NUCLEODUR® phases as VarioPrep-columns in preparative dimensions.

Phase	Specification	Characteristics*			Stability	Structure
C18 HTec	octadecyl phase, with high capacity, high density coating multi-endcapping 18 % C · USP **L1**	A			pH stability 1 - 11, suitable for LC/MS	
		B				
		C				
C18 / C8 Gravity	octadecyl phase, high density coating multi-endcapping C_{18} Gravity: 18 % C · USP **L1** C_8 Gravity: 11 % C · USP **L7**	A	C_{18}		pH stability 1 - 11, suitable for LC/MS	
			C_8			
		B	C_{18}			
			C_8			
		C	C_{18}			
			C_8			
C18 Isis	octadecyl phase with specially crosslinked surface modification endcapping 20 % C · USP **L1**	A			pH stability 1 - 10, suitable for LC/MS	
		B				
		C				
C18 Pyramid	C_{18} modification with polar endcapping 14 % C · USP **L1**	A			stabel against 100 % aqueous eluents pH stability 1 - 9, suitable for LC/MS	
		B				
		C				
Sphinx RP	bifunctional RP phase, propylpheny and C_{18} ligands; endcapping 15 % C; USP **L1** and **L11**	A			pH stability 1 - 10, suitable for LC/MS	
		B				
		C				
C18 ec C8 ec	octadecyl / octyl phase, medium density coating endcapping C_{18} ec: 17.5 % C · USP **L1** C_8 ec: 10.5 % C · USP **L7**	A	C_{18}		pH stability 1 – 9	
			C_8			
		B	C_{18}			
			C_8			
		C	C_{18}			
			C_8			
HILIC	zwitterionic ammonium sulfonic acid modification 7 % C	A			pH stability 2 – 8,5, suitable for LC/MS	
		B				
		C		–		
CN / CN-RP	cyano (nitrile) phase for NP and RP separations 7 % C · USP **L10**	A			pH stability 1 – 8, stable towards highly aqueous mobile phases	
		B				
		C		–		
NH_2 / NH_2-RP	amino phase for NP and RP separations 2.5 % C · USP **L8**	A			pH stability 1 – 8, stable towards highly aqueous mobile phases suitable for LC/MS	
		B				
		C		–		
SiOH	unmodified high purity silica USP **L3**	A		–	pH stability 2 – 8	
		B		n.a.		
		C		–		

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

Ordering Informations

NUCLEODUR® C_{18} HTec

Lenght →	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	guard columns#
analytical columns (5 µm)							
2 mm ID	760311.20			760314.20		760316.20	761110.30
3 mm ID	760311.30			760314.30		760316.30	761110.30
4 mm ID	760311.40			760314.40		760316.40	761110.40
4.6 mm ID	760311.46	760312.46	760313.46	760314.46	760315.46	760316.46	761110.40

requires EC adaptor REF 721359

Lenght →	50 mm	100 mm	125 mm	150 mm	250 mm	guard columns*
preparative columns (5 µm)						
10 mm ID	762551.100		762554.100		762556.100	762594.80
21 mm ID	762551.210	762553.210	762554.210		762556.210	762593.160
32 mm ID		762553.320		762555.320	762556.320	762592.320
40 mm ID				762555.400	762556.400	762592.320
50 mm ID		762553.500		762555.500	762556.500	762595.500

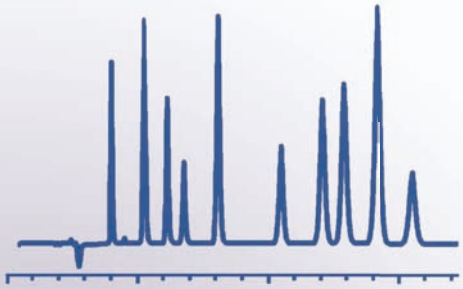
Lenght →	50 mm	100 mm	125 mm	150 mm	250 mm	guard columns*
preparative columns (7 µm)						
10 mm ID	762561.100		762564.100		762566.100	762594.80
21 mm ID	762561.210	762563.210	762564.210		762566.210	762593.160
32 mm ID		762563.320		762565.320	762566.320	762592.320
40 mm ID				762565.400	762566.400	762592.320
50 mm ID		762563.500		762565.500	762566.500	762595.500

Lenght →	50 mm	100 mm	125 mm	150 mm	250 mm	guard columns*
preparative columns (10 µm)						
10 mm ID	762571.100		762574.100		762576.100	762594.80
21 mm ID	762571.210	762573.210	762574.210		762576.210	762593.160
32 mm ID		762573.320		762575.320	762576.320	762592.320
40 mm ID				762575.400	762576.400	762592.320
50 mm ID		762573.500		762575.500	762576.500	762595.500

Lenght →	10 mm	15 mm	20 mm	30 mm	Pack of	Holder
***preparative guard columns for NUCLEODUR® C_{18} HTec and holders**						
8 mm ID	762591.80				1	718251
16 mm ID			762593.160		1	718250
32 mm ID		762592.320			1	718253
50 mm ID				762594.500	1	718254

	Pack of 100 g	Pack of 1 kg
NUCLEODUR® C_{18} HTec bulk packings		
NUCLEODUR® 100-5 C_{18} HTec	713830.0100	713830.1
NUCLEODUR® 100-7 C_{18} HTec	713831.0100	713831.1
NUCLEODUR® 100-10 C_{18} HTec	713832.0100	713832.1

For all questions about Chromatography: Tel.: +49 2421 969 175
e-mail: tech-chroma@mn-net.com



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