Chromatography

MACHEREY-NAGEL

NUCLEODUR® 300 C_{18} ec and C_4 ec

HPLC columns for biochromatography

- Reliable wide pore RP phases for daily routine analysis
- Medium density octadecyl or butyl modification with exhaustive endcapping
- Ideal phases for separation of biomolecules

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NUCLEODUR® 300 C_{18} ec and C_4 ec

Key features

- Reliable wide pore RP phases for daily routine analysis
- Medium density octadecyl or butyl modification with exhaustive endcapping
- Ideal phases for separation of biomolecules

Technical characteristics

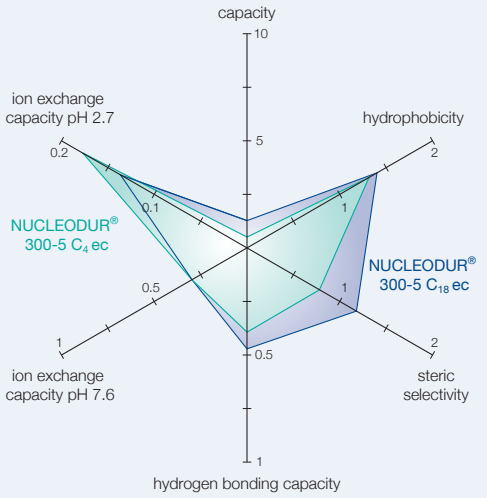
- Pore size 300 Å; particle size 5 µm
- Carbon content 4 % for C_{18}, 2.5 % for C_4
- pH stability 1–9, high reproducibility from lot to lot

Recommended applications

- Biological macromolecules like proteins or peptides

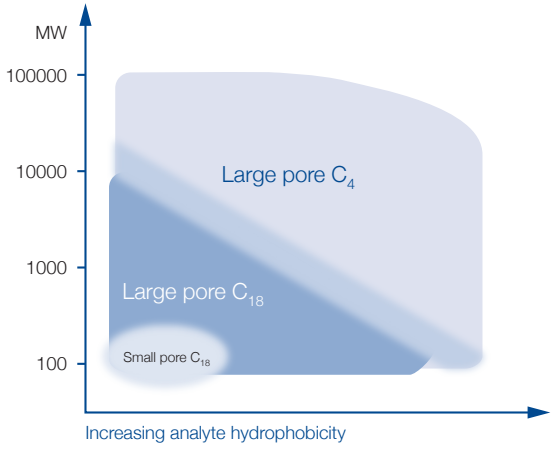
USP L1 (C_{18}) / USP L26 (C_4)

Tanaka plots of NUCLEODUR® wide pore phases



Wide pore silica for biochromatography

Column selection by analyte characteristics

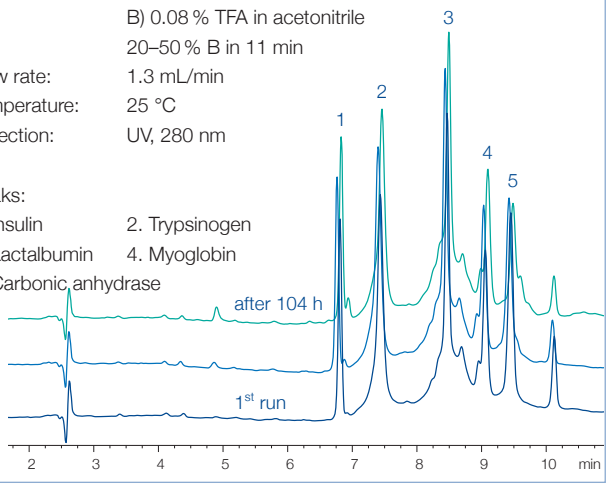


Stability test of NUCLEODUR® 300-5 C_4 ec

MN Appl. No. 126540

Column:	250 x 4.6 mm NUCLEODUR® 300-5 C_4 ec
Eluent:	A) 0.1 % TFA in water
	B) 0.08 % TFA in acetonitrile
	20–50 % B in 11 min
Flow rate:	1.3 mL/min
Temperature:	25 °C
Detection:	UV, 280 nm

Peaks:
1. Insulin
2. Trypsinogen
3. Lactalbumin
4. Myoglobin
5. Carbonic anhydrase



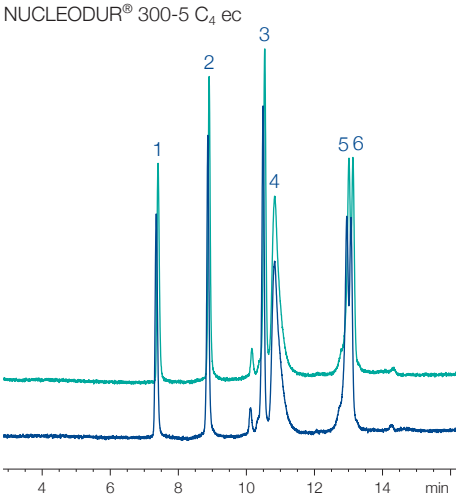
Batch-to-batch reproducibility of NUCLEODUR® 300-5 C_4 ec and NUCLEODUR® 300-5 C_{18} ec

MN Appl. Nos. 126551 / 126552

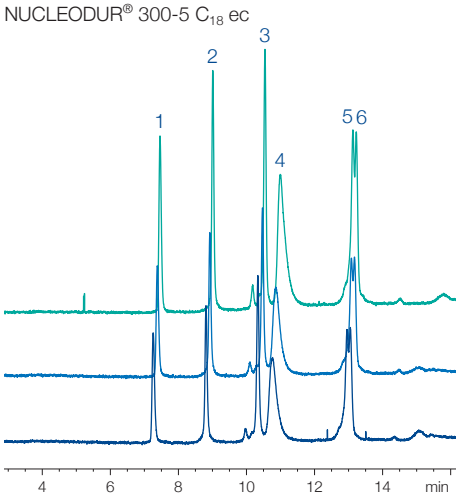
Columns:	250 x 4 mm
Eluent:	A) 0.1 % TFA in water
	B) 0.08 % TFA in acetonitrilel
	20–60 % B in 15 min
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV, 280 nm

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

NUCLEODUR® 300-5 C_4 ec



NUCLEODUR® 300-5 C_{18} ec



NUCLEODUR® 300 C_{18} ec and C_4 ec

Column comparison for protein separation

MN Appl. No. 126570

Columns: 250 x 4.6 mm NUCLEODUR® 300-5 C_{18} ec
250 x 4.6 mm NUCLEOSIL® 300-5 C_{18}
250 x 4.6 mm Jupiter® C_{18}, 5 µm

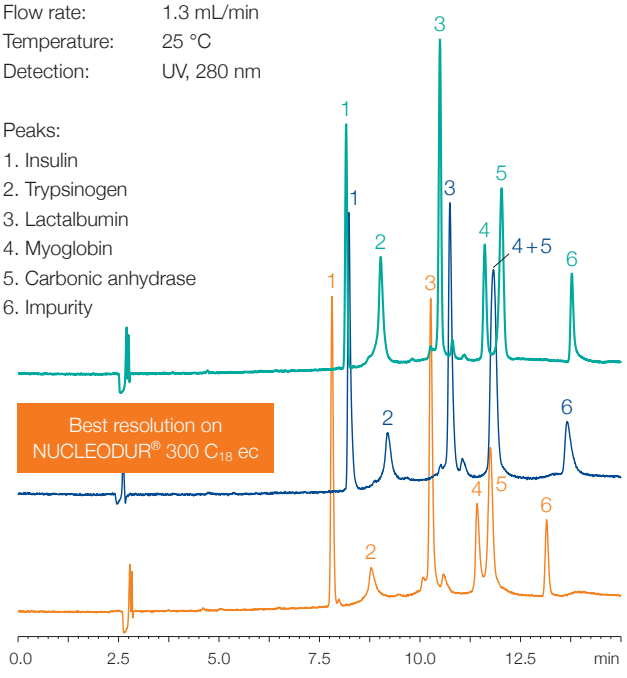
Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
20–65 % B in 15 min (2 min 65 % B)

Flow rate: 1.3 mL/min

Temperature: 25 °C

Detection: UV, 280 nm

Peaks:
1. Insulin
2. Trypsinogen
3. Lactalbumin
4. Myoglobin
5. Carbonic anhydrase
6. Impurity



Comparison of narrow and wide pore NUCLEODUR® for the separation of proteins

MN Appl. No. 126590

Columns: 250 x 4,6 mm NUCLEODUR® 300-5 C_{18} ec
250 x 4,6 mm NUCLEODUR® C_{18} Gravity, 5 µm

Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
20–65 % B in 15 min
(3 min 65 % B)

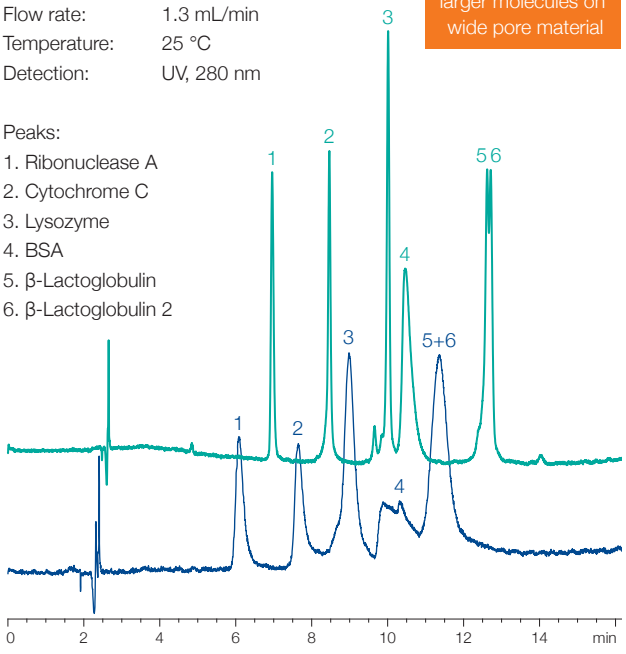
Flow rate: 1.3 mL/min

Temperature: 25 °C

Detection: UV, 280 nm

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

Sharper peaks of larger molecules on wide pore material



Separation of cytochrome C of different origins on NUCLEODUR® 300 RP column

MN Appl. No. 126560

Column: 250 x 4.6 mm
NUCLEODUR® 300-5 C_{18} ec

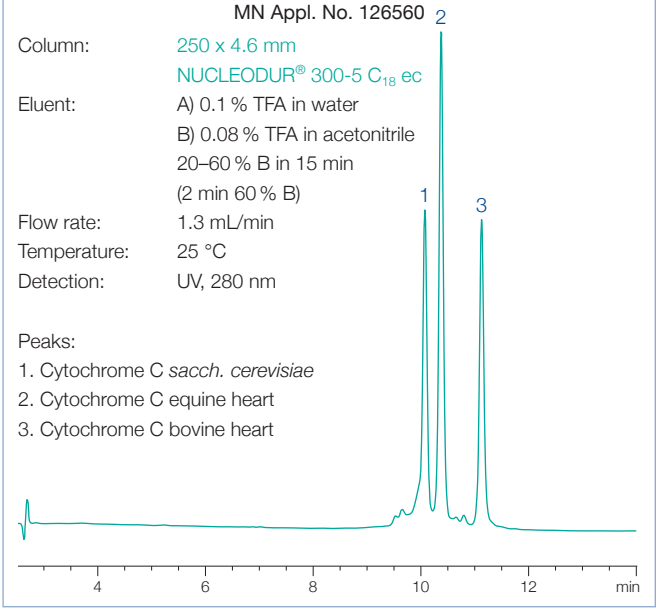
Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
20–60 % B in 15 min
(2 min 60 % B)

Flow rate: 1.3 mL/min

Temperature: 25 °C

Detection: UV, 280 nm

Peaks:
1. Cytochrome C *sacch. cerevisiae*
2. Cytochrome C equine heart
3. Cytochrome C bovine heart



Protein test

MN Appl. No. 126580

Column: 250 x 4.6 mm
NUCLEODUR® 300-5 C_{18} ec

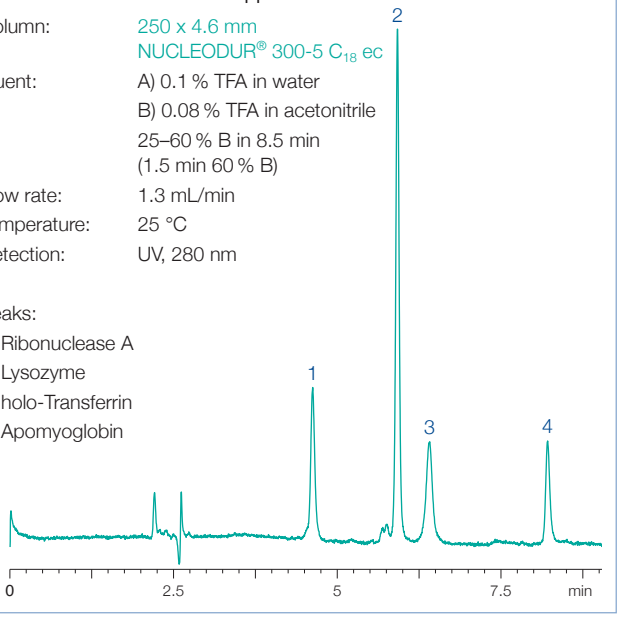
Eluent: A) 0.1 % TFA in water
B) 0.08 % TFA in acetonitrile
25–60 % B in 8.5 min
(1.5 min 60 % B)

Flow rate: 1.3 mL/min

Temperature: 25 °C

Detection: UV, 280 nm

Peaks:
1. Ribonuclease A
2. Lysozyme
3. holo-Transferrin
4. Apomyoglobin



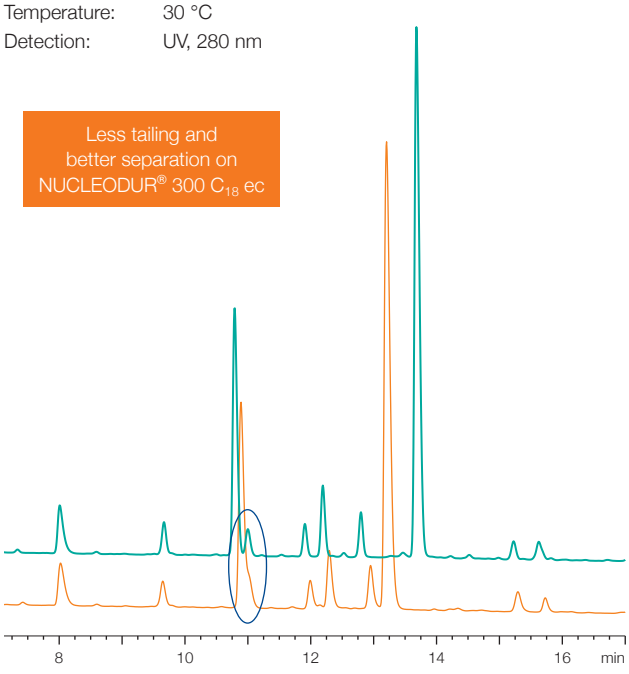
NUCLEODUR® 300 C_{18} ec and C_4 ec

Tryptic digest of cytochrome C

MN Appl. No. 126600

Columns:	250 x 4.6 mm NUCLEODUR® 300-5 C_{18} ec
	250 x 4.6 mm Jupiter® C_{18}, 5 µm
Eluent:	A) 0.1 % TFA in water
	B) 0.08 % TFA in acetonitrile
	5–40 % B in 15 min (1 min 40 % B)
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	UV, 280 nm

Less tailing and better separation on NUCLEODUR® 300 C_{18} ec

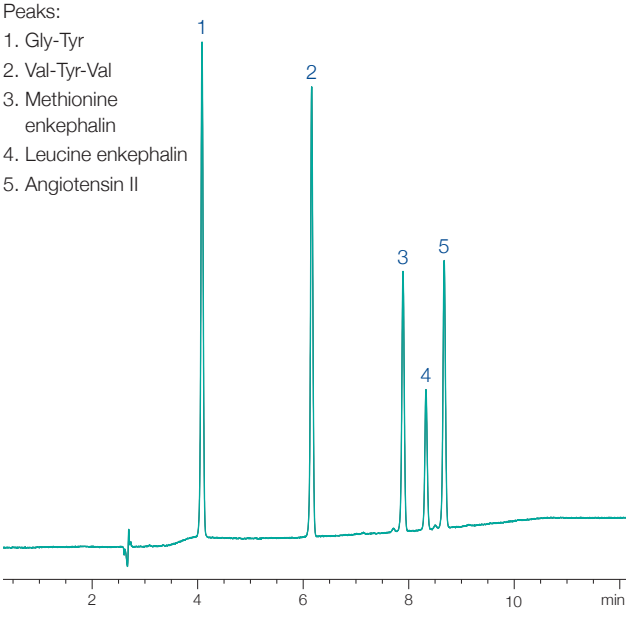


Peptide test

MN Appl. No. 126610

Columns:	250 x 4.6 mm NUCLEODUR® 300-5 C_{18} ec
Eluent:	A) 0.1 % TFA in water
	B) 0.08 % TFA in acetonitrile
	10–40 % B in 7.5 min (2 min 40 % B)
Flow rate:	1.3 mL/min
Temperature:	25 °C
Detection:	UV, 280 nm

Peaks:
1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine enkephalin
4. Leucine enkephalin
5. Angiotensin II



Ordering information

NUCLEODUR® 300-5 C_{18} ec

Octadecyl modification, endcapped, pore size 300 Å, particle size 5 µm; eluent in column acetonitrile – water

Length	100 mm	125 mm	150 mm	250 mm	EC guard columns*
2 mm ID	760183.20	760184.20	760185.20	760186.20	761988.20
3 mm ID	760183.30	760184.30	760185.30	760186.30	761988.30
4 mm ID	760183.40	760184.40	760185.40	760186.40	761988.30
4.6 mm ID	760183.46	760184.46	760185.46	760186.46	761988.30

NUCLEODUR® 300-5 C_4 ec

Butyl modification, endcapped, pore size 300 Å, particle size 5 µm; eluent in column acetonitrile – water

Length	100 mm	125 mm	150 mm	250 mm	EC guard columns*
2 mm ID	760193.20	760194.20	760195.20	760196.20	761989.20
3 mm ID	760193.30	760194.30	760195.30	760196.30	761989.30
4 mm ID	760193.40	760194.40	760195.40	760196.40	761989.30
4.6 mm ID	760193.46	760194.46	760195.46	760196.46	761989.30

* EC guard columns require the Column Protection System guard column holder 718966

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