

RP-HPLC with highly aqueous mobile phases

The efforts to neutralize unwanted silanol activity often results in well base-deactivated RP phases with high carbon load, but a limited scope of selectivity beyond non-polar interactions. Polar compounds like carboxylic acids or drug metabolites show only weak retention on densely bonded RP columns due to distinct hydrophobic properties but low polar interactions. Very polar analytes require highly aqueous mobile phases for solubility and retention. Conventional reversed phase columns often display stability problems in eluent systems with high percentage of water (> 95 %) as evidenced by a sudden decrease of retention time and overall poor reproducibility. This phenomenon is described as phase collapse caused by the mobile phase expelled from the pores due to the fact, that hydrophobic RP phases are incompletely wetted with the mobile phase [3].

Different approaches can be used to increase column stability with highly aqueous mobile phase systems. The most promising concepts are incorporating a polar group in the hydrophobic alkyl chain, or using hydrophilic endcapping procedures to improve the wettability of the reversed phase modification. NUCLEODUR® PolarTec may be taken as an example for the embedded polar group strategy, in which a C₁₈ silane with a polar function is successfully linked to the silica surface.

Key features

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

Technical data

- Special C₁₈ phase; polar endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm (7 and 10 µm particles for preparative purposes on request); carbon content 14 %; pH stability 1–9

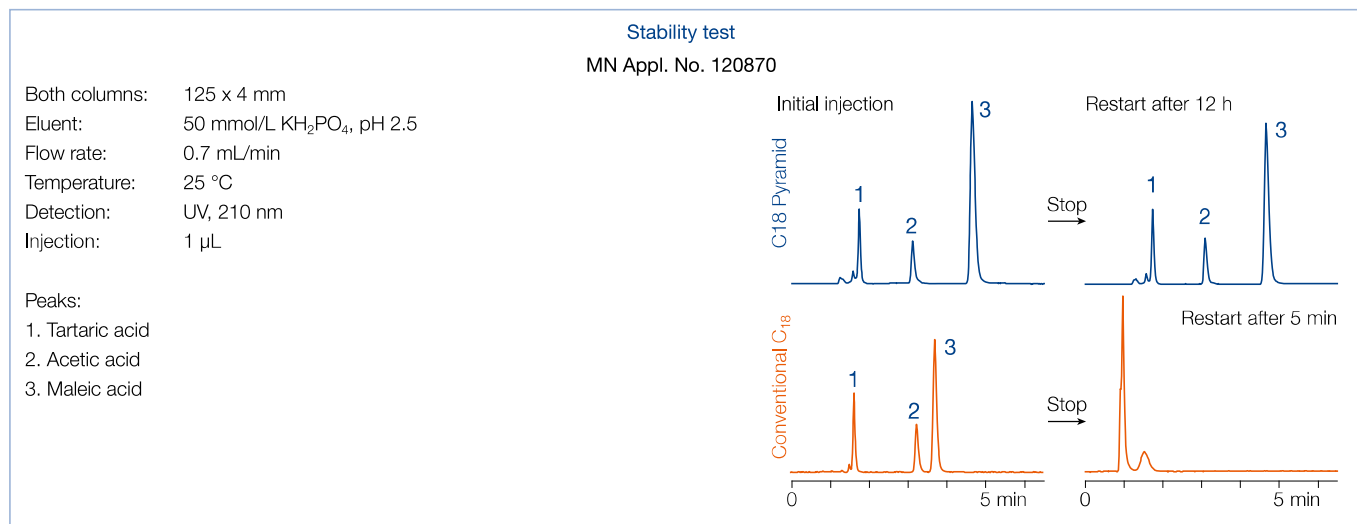
Recommended applications

- USP listing L1
- Analgesics, penicillin antibiotics, nucleic acid bases, water-soluble vitamins, complexing agents, organic acids

Stability features

NUCLEODUR® C18 Pyramid is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. The lower figure shows the retention behavior of tartaric, acetic and maleic acid under purely aqueous conditions on NUCLEODUR® C18 Pyramid in comparison with a conventionally bonded C₁₈ phase.

It can be shown that the retention times for NUCLEODUR® C18 Pyramid remain nearly unchanged between initial injection and restart after the flow has been stopped for 12 h, whilst the performance of the conventional RP column already totally collapsed after 5 min.



NUCLEODUR® C18 Pyramid

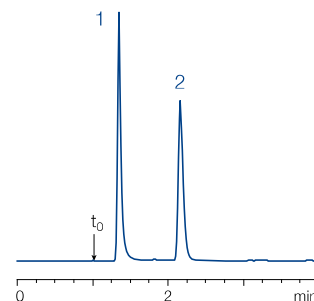
Retention characteristics

Separation of very polar compounds

MN Appl. No. 119170

Column: 125 x 4 mm NUCLEODUR® C18 Pyramid, 5 µm
 Eluent: 0.2 % H₃PO₄
 Flow rate: 1.0 mL/min
 Temperature: 22 °C
 Detection: UV, 202 nm
 Injection: 2 µL

Peaks:
 1. Formic acid
 2. Acetic acid



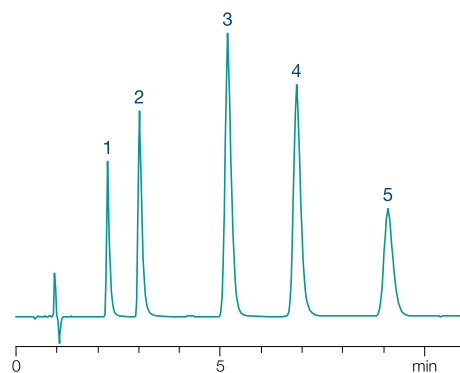
The polar surface exhibits retention characteristics different from conventional C₁₈ phases. Application 119170 shows improved retention behavior of very polar short chain organic acids, which are insufficiently retained on RP columns with predominantly hydrophobic surface properties. In addition to the exceptional polar selectivity NUCLEODUR® C18 Pyramid also provides adequate hydrophobic retention (application No. 119190 at ChromaAppDB.mn-net.com). The perceptible increase in polarity has no impact on the retention behavior of ionizable analytes. Even with the strongly basic compounds of the tricyclic antidepressant drug test mixture, no unwanted interactions or a so-called lack in base deactivation are observed in application 119200.

Tricyclic antidepressants

MN Appl. No. 119200

Column: 125 x 4 mm NUCLEODUR® C18 Pyramid, 5 µm
 Eluent: methanol – 20 mM NH₄H₂PO₄, pH 6.95 (70:30, v/v)
 Temperature: 40 °C
 Injection volume: 5 µL
 Detection: UV, 254 nm
 Injection volume: 2 µL

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



Ordering information

NUCLEODUR® C18 Pyramid

Analytical EC columns NUCLEODUR® C18 Pyramid (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760202.46	761917.30
250	4	5	760202.40	761917.30
150	4.6	5	760203.46	761917.30
125	4	5	760201.40	761917.30
150	4.6	3	760261.46	761916.30
125	3	3	760260.30	761916.30
100	4.6	3	760264.46	761916.30
50	2	3	760263.20	761916.20
100	2	1.8	760273.20	761915.20
50	2	1.8	760272.20	761915.20

* Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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