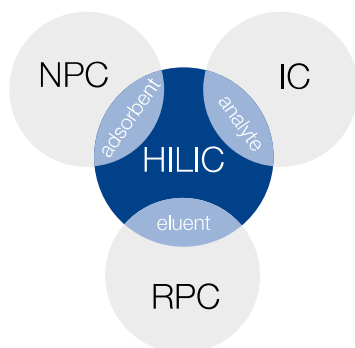


Hydrophilic interaction chromatography



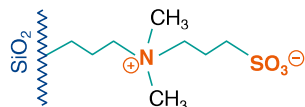
Especially for polar compounds reversed phase HPLC – the most common analytical method – is often limited. Here, hydrophilic stationary phases provide an additional tool for the separation of polar analytes in HPLC.

The expression HILIC (Hydrophilic Interaction Chromatography) was firstly published by Andrew Alpert in 1990 – since then it took quite some efforts to develop robust and reproducible hydrophilic HPLC phases for HILIC chromatography [4].

HILIC combines the characteristics of the 3 major methods in liquid chromatography reversed phase (RPC), normal phase (NPC) and ion chromatography (IC):

- Stationary phases (adsorbents) are mostly polar modifications of silica or polymers (SiOH, NH₂, Diol, (zwitter) ions, ...) – like in NPC.
- Mobile phases (eluent) are mixtures of aqueous buffer systems and organic modifiers like acetonitrile or methanol – like in RPC.
- Fields of application include quite polar compounds as well as organic and inorganic ions – like in IC.

Summarized: “HILIC is NP chromatography of polar and ionic compounds under RP conditions.”



NUCLEODUR® HILIC is a special zwitterionic modified stationary phase based on ultra-spherical NUCLEODUR® particles. The betaine character of the ammonium-sulfonic acid ligands results in total charge equalization and in an overall neutrally but highly polar surface.

Retention characteristic

Commonly HILIC is described as partition chromatography or liquid-liquid extraction system between mobile and stationary phases. Versus a water-poor mobile phase a water-rich layer on the surface of the polar stationary phase is formed. Thus, a distribution of the analytes between these two layers will occur. Furthermore, HILIC includes weak electrostatic mechanisms as well as hydrogen donor interactions between neutral polar molecules under high organic elution conditions. This distinguishes HILIC from ion exchange chromatography - main principle for HILIC separation is based on compound's polarity and degree of solvation.

Stability features

Due to an advanced and unique surface modification procedure NUCLEODUR® HILIC columns provide short equilibration times. After just 20 min equilibration the 2nd injection already shows stable and reproducible results.

Key features

- Ideal for reproducible and stable chromatography of highly polar analytes
- Suitable for analytical and preparative applications
- Very short column conditioning period

Technical data

- Zwitterionic ammonium-sulfonic acid phase; not endcapped
- Pore size 110 Å; particle sizes 1.8 µm, 3 µm and 5 µm; carbon content 7 %; pH stability 2–8.5

Recommended applications

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

Beyond this, NUCLEODUR® HILIC columns are characterized by an outstanding column life time - even after nearly 800 runs the columns show no loss of its pristine performance - peak shape and retention are still immaculate. Due to its high loading capacity NUCLEODUR® HILIC is suitable for (semi-)preparative applications.

Good to know

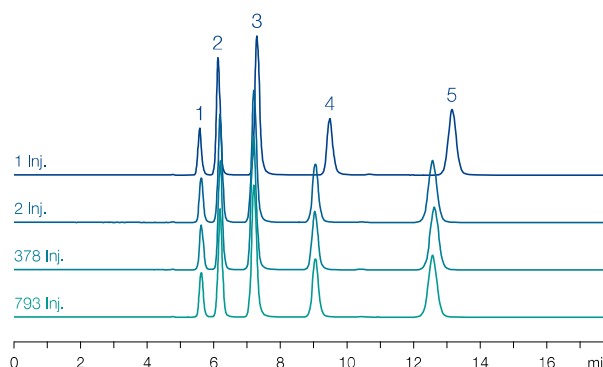
NUCLEODUR® HILIC is a patented phase modification (pat. number DE102009006007 (B4))

Stability and equilibration

MN Appl. No. 123100

Column: 250 x 4 mm NUCLEODUR® HILIC, 5 µm
Eluent: CH₃CN – 5 mmol/L ammonium acetate (80:20, v/v)
Flow rate: 0.6 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

Peaks:
1. Thymine
2. Uracil
3. Adenine
4. Cytosine
5. Guanosine



Overall NUCLEODUR® HILIC provides excellent chromatographic features and is hereby the perfect choice for separation of polar or charged compounds which can be shown in application 122920.

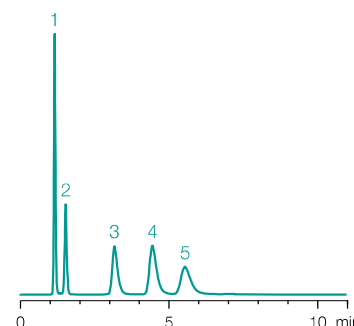
Separation of adenosine and phosphates

MN Appl. No. 122920

Column: 125 x 4 mm NUCLEODUR® HILIC, 5 µm
Eluent: acetonitrile – 100 mM ammonium acetate, pH 5.3 (70:30, v/v)
Flow rate: 1.3 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

Peaks:
1. Adenosine
2. cAMP
3. AMP

4. ADP
5. ATP



Ordering information

NUCLEODUR® HILIC

Analytical EC columns NUCLEODUR® HILIC (pack of 1)

Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*
250	4.6	5	760550.46	761962.30
150	2	5	760553.20	761962.20
250	4.6	3	760530.46	761961.30
250	3	3	760530.30	761961.30
150	4.6	3	760533.46	761961.30
125	4.6	3	760531.46	761961.30
125	2	3	760531.20	761961.20
100	3	3	760534.30	761961.30
100	2	1.8	760526.20	761960.20
50	2	1.8	760523.20	761960.20

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

For more products
and information
Or visit www.mn-net.com

