

NUCLEODUR® HILIC - A useful complement to conventional C18 HPLC phases

Key Features:

- ideal for reproducible and stable chromatography of highly polar analytes
- suitable for analytical and preparative applications as well as LC-MS
- very short column conditioning period

Technical Characteristics:

Ammonium – Sulphonic acid modified silica: pore size 110 Å, available particle sizes 3 µm and 5 µm; carbon content 7 %; pH stability 2 – 8.5

Recommended application: hydrophilic compounds such as [organic polar acids](#) and bases, polar natural compounds, nucleosides, [oligonucleotides](#), amino acids, peptides, [water soluble vitamins](#)

NUCLEODUR® HILIC

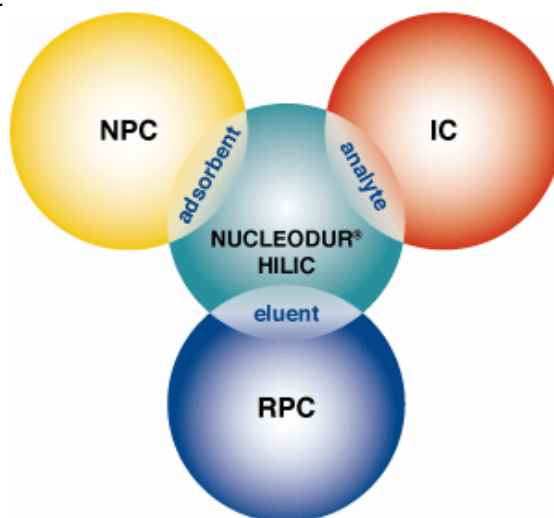
Separation science is always looking for new and effective strategies to accomplish the tasks of modern analytics. Especially for polar compounds reversed phase HPLC – the most common analytical method – is often limited. Here, hydrophilic stationary phases provide an additional tool for the separation of polar analytes in HPLC. The expression HILIC (Hydrophilic Interaction Liquid 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 [A. Alpert, J. Chromatography 499 (1990), 177–196].

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, Amino, Diol, (zwitter) ions, ...) – like in **NPC**

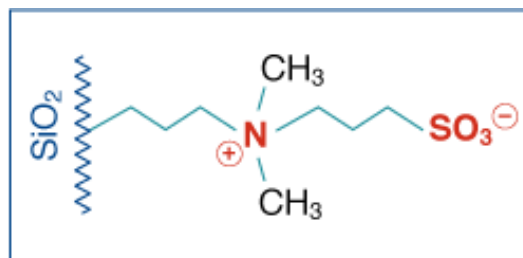
mobile phases (eluent) are mixtures of aqueous buffer systems and organic modifier like acetonitrile or methanol - like in **RPC**

fields of application include quite polar compounds as well as organic and inorganic ions – like in **IC**



"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 equalisation and in an overall neutrally charged but highly polar surface.



Retention characteristic

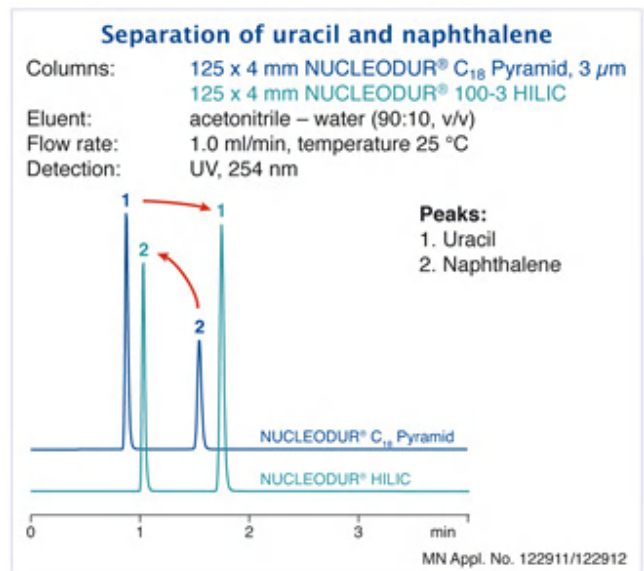
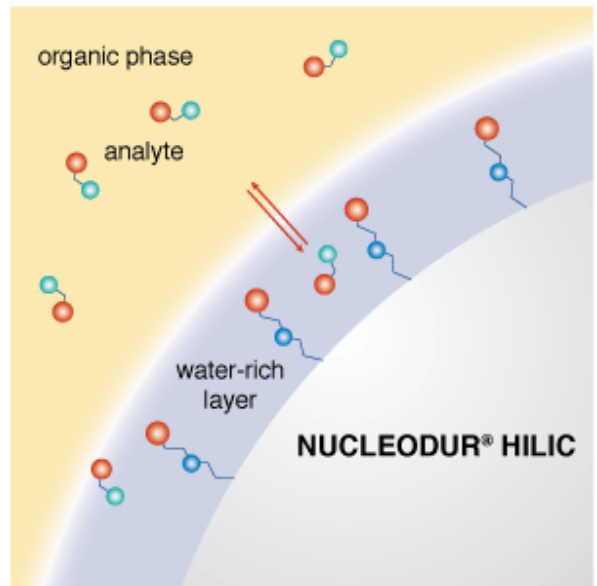
Commonly HILIC is described as partition chromatography or liquid/liquid extraction system between the mobile and stationary phase. Versus a water-poor layer of mobile phase a water-rich layer on the surface of the polar stationary phase is formed. Thus, a distribution of the analytes between these two 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.

More polar compounds will have stronger interaction with the stationary aqueous layer than less polar compounds – resulting in a stronger retention.

Nonpolar compounds exhibit faster elution profiles due to minor hydrophobic interactions. Thus, as shown for the separation of uracil and naphthalene the elution order is quite often inverse on HILIC columns compared to RP columns.

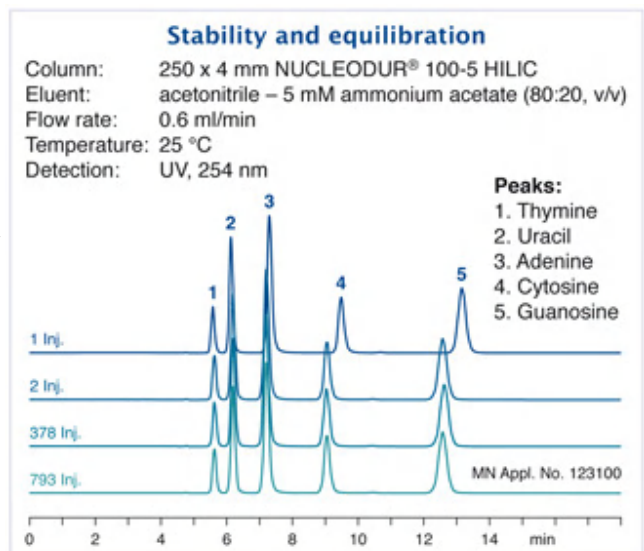
In comparison with medium polar aminopropyl phases or modification with less balanced charge equalisation NUCLEODUR® HILIC shows a superb separation and peak shape for critical compounds like adenosine and its phosphate derivatives.



Stability features

Due to an advanced and unique surface modification procedure (pat. pend.) NUCLEODUR® HILIC columns provide short equilibration times – after just 5 min equilibration already the 2nd injection shows stable and reproducible results.

Beyond this, NUCLEODUR® HILIC columns are characterized by an outstanding column life time - even after nearly 800 runs the columns show no loss of pristine performance - peak shape and retention are still immaculate.



Fields of application of HILIC phases

Due to its high loadability NUCLEODUR® HILIC is absolutely suitable for preparative and semi-preparative applications.

Based on their special interaction and retention features HILIC-columns are ideal for analysis of medium-polar and polar compounds. Especially since this group of substances is gaining an increasing interest.

Thus not only determinations of polar compounds in food, such as currently melamine in milk or acrylamide in bakery products are necessary, but also separations of vitamins and organic acids in foodstuff and pharmaceutical products. All these separations are efficiently performed on HILIC columns.

Stable and effective application notes were developed on NUCLEODUR® HILIC also for pharmaceutical and physiological substances, like the chemotherapeutic agent 5-fluorouracil, the energy carrier of muscle metabolism creatine, of catecholamines and amino acids.

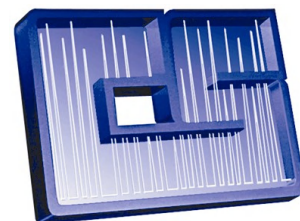
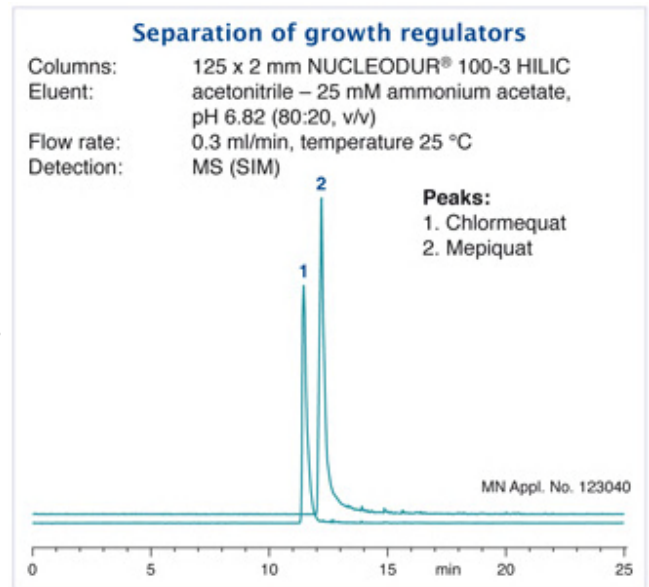
In bioanalysis nucleotides, as well as pyrimidine and purine bases can be determined efficiently.

Because well-biodegradable, but more and more polar pesticides have to be analyzed, the determination of chlormequat, mepiquat, paraquat and diquat gains increasing importance.

Determinations of hydrocarbons, peptides and glycolised or phosphorylated compounds are other successful applications of HILIC phases.

Advantages of NUCLEODUR® HILIC

- NUCLEODUR® HILIC is outstandingly suited for the chromatography of medium-polar, polar and ionic compounds.
- It stands out due to short equilibration time and good lifetime.
- Besides the established detection systems UV and fluorescence an application with highly sensitive LC-MS, LC-MS/MS and light scattering detectors is possible.
- It is **the alternative** to RP-18 and further RP phases, to NP and ion exchanger phases **for a reliable analysis**.



To find out more about Macherey-Nagel's NUCLEODUR® HILIC columns, contact the Chromatographic Specialties Inc. Technical Support Team at 1-800-267-8103 or email tech@chromspec.com