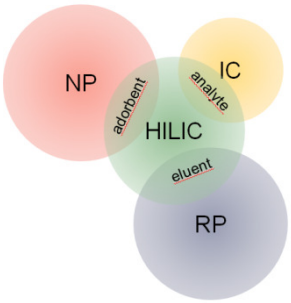
HPLC · SMB · Osmometry

KNAUER HILIC method development guidelines



How can I take advantage of KNAUER HILIC columns?
First steps in the HILIC direction

This guideline will help users who are not so familiar with HPLC in the HILIC mode to start a method development in an easy way.

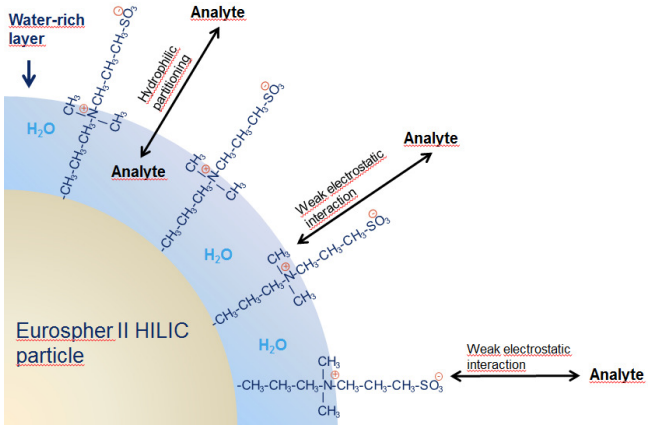
What is HILIC?

Hydrophilic **I**nteraction **Li**quid **C**hromatography or HILIC is normal phase (NP) chromatography of polar and ionic compounds under reversed phase (RP) conditions. The main separation mechanism is caused by an aqueous layer built on the stationary phase and partitioning of the analytes between the very polar stationary phase and the less polar mobile phase. This leads to the retardation of polar and hydrophilic compounds like Uracil for example, which is used as a non retarded dead time marker in reversed phase HPLC. Unpolar compounds like Toluol for example are not retarded and can be used as a dead time marker in HILIC.
This leads to HILIC having an elution order that is often inverse compared to reversed phase separations.

Relative solvent strength in HILIC Mode: Acetone < Acetonitrile < Isopropanol < Ethanol < Methanol < Water

The HILIC Retention mechanism

The retention mechanisms in HILIC are complex and lead to different retention patterns on different HILIC stationary phases. Main mechanisms are partitioning between a water-rich layer on the surface and the organic enriched mobile phase, specific adsorption of polar functional groups on the stationary phase, ionic retention on ionized groups or on ionized silanols of the base silica and reversed-phase retention on the hydrophobic portions of bonded ligands.



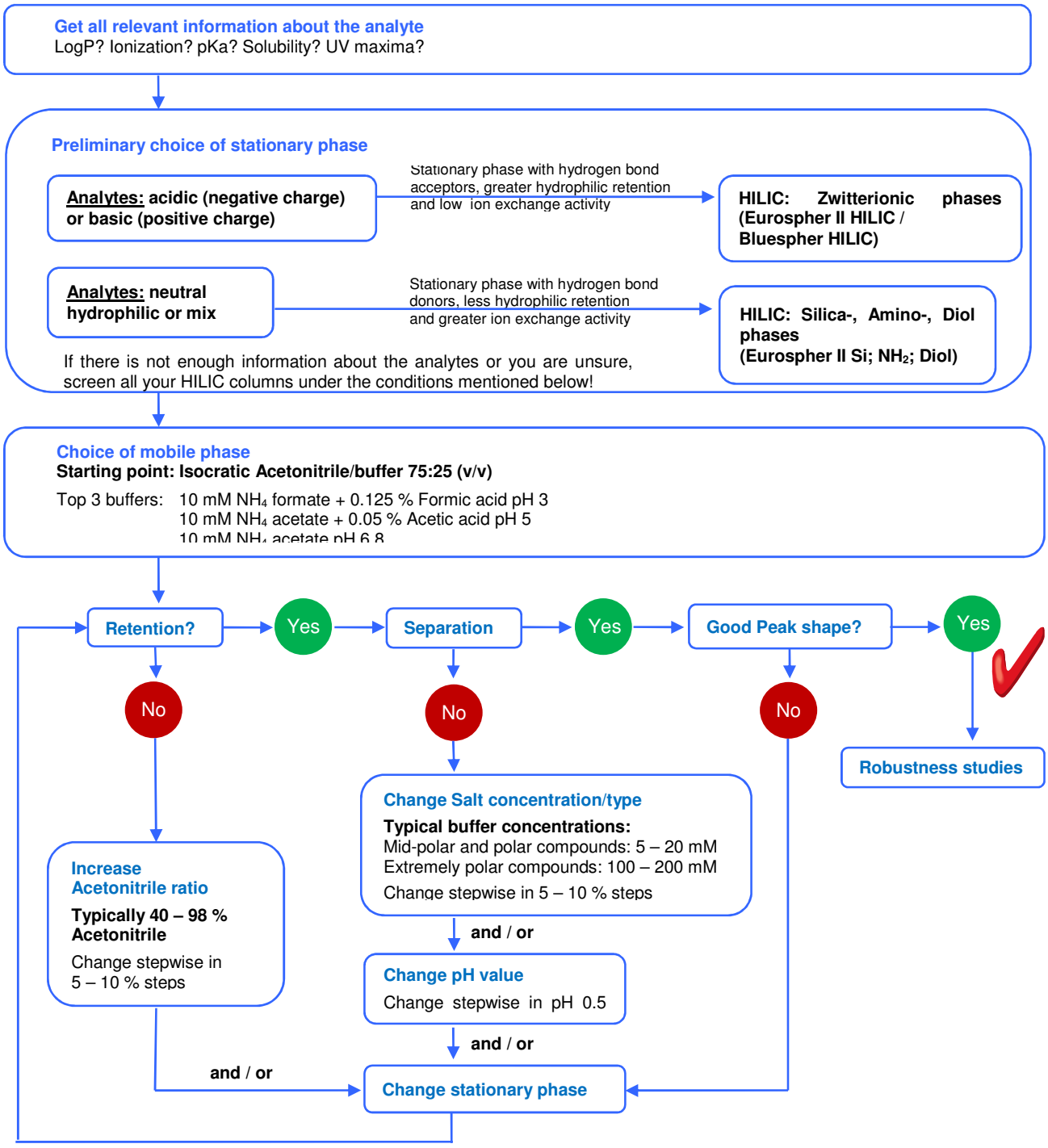
A significant discrimination between HILIC columns is whether they rely mainly on adsorption and hydrogen bonding, or hydrophilic partitioning and multipoint interactions. All plain silica columns exhibit adsorption selectivity, whereas zwitterionic columns generally exhibit a selectivity pattern that could be attributed to partitioning.
With the Eurospher II HILIC and the Bluespher HILIC phases, KNAUER offers an ammonium– sulfonic acid modified HILIC phase. This is a special zwitterionic modification with a neutrally charged but highly polar surface**.**
Also Silica-, Amino-, Diol- and Cyano phases are quite often used for HILIC separations.

The HILIC method development – Starting information

As in RP method development, first information about the analytes has to be collected to estimate the potential for a HILIC method and to find the right starting parameters for method development. When the Partition Coefficient LogP is available for the analyte, it gives a good idea about the usability of HILIC. If LogP is near to zero or even below, HILIC will work really well. The acid dissociation constant pKa is also an important factor. It gives information about the ability of the molecule to accept or release protons subject to the pH of the mobile phase. It is an important factor to choose the right stationary phase.
Typical HILIC eluents consist of 40 – 98 % acetonitrile in water or a volatile buffer. It is recommended to use isocratic elution in HILIC. Sometimes gradients can also be usable. Here again the gradient will be in the “opposite direction” as in RP mode. The polarity of the mobile phase will be increased by decreasing the content of organic solvent. ***It is very important that HILIC phases are in general less tolerable to fast gradients and need longer equilibration times than RP phases.*** This is caused by the aqueous layer on the stationary phase, which is dependent on the composition of the mobile phase. ***For this reason, HILIC gradients should also not be run from 0 % to 100 % aqueous phase. Never use less than 2 % aqueous phase to ensure sufficient hydration of the stationary phase.*** If it is needed to separate solutes with widely different retention factors during the same run, use a linear gradient from 90 % to 40 % Acetonitrile as a starting point.

In the following you can find a flow chart that will help you during method development in the HILIC mode.
Just follow this guideline and you will get a good starting point for your HILIC separation.

HILIC method development Flow chart



Contact information

Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
14163 Berlin, Germany

Tel: +49 (0)30 / 809727-0
Fax: +49 (0)30 / 8015010
E-Mail: info@knauer.net
Internet: www.knauer.net