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# HILIC Mobile Phase: Solvents

In HILIC, small variations of the elution conditions often result in differences in the retention greater than the ones observed in RPLC. Therefore, particular attention has to be paid to the selection and the preparation of the eluents.

It is important to know that, generally, the impact on the retention in HILIC is observed for modification of the chromatographic parameters in the following order.

organic solvent content > salt content ~ pH > column temperature

On this basis, the first and most important parameter to optimize is the organic solvent content. As already emphasized, the mobile phase in HILIC is constituted by a mixture of water– water miscible organic solvent, such as in RPLC. However, the elution strength for common HILIC solvents follows the order:

methanol > ethanol > isopropanol > tetrahydrofuran > acetonitrile

Acetonitrile is the most used solvent for HILIC separations and it represents the best choice during a first screening of the elution conditions. Other types of water-miscible organic solvents that are occasionally employed include alcohols and cyclic ethers. Alcohols provide the lowest retentions and are preferred just in case of strong interactions with the column.

Independently from all the other chromatographic conditions, a general rule for identifying a HILIC mechanism is the observation of an increase in the retention times with the increase of the organic



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Figure 1

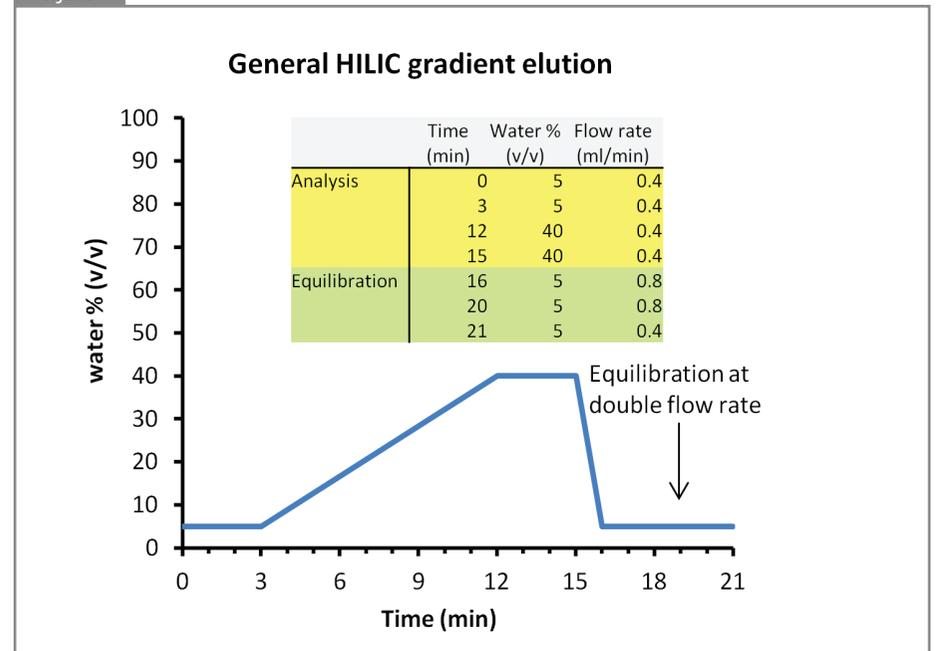


Figure 1: Gradient elution with a mixture of water/acetonitrile, useful as first step during method development. General conditions for an analytical column. The flow-rate should be selected according to column dimensions.

solvent content in the mobile phase.

In contrast to RPLC, water in HILIC represents the stronger eluting solvent. The water content should be at least 3% of the mobile phase to create the water layer on the surface of the HILIC stationary phase and to allow analyte retention. Then, the elution is promoted increasing the water content up to 40% of the mobile phase, according to the mechanism described in HILIC Solutions #2. A classical gradient elution with a mixture of water/acetonitrile is represented in Figure 1 and it can be used as the first step during method development in order to have a rough idea about the compounds retention.

HILIC is more sensitive than RPLC to small changes of eluent composition and the following precautions always have to be considered.

1. Injection solvent: keep the acetonitrile content as high as possible, according to analyte solubility. Methanol content up to 20% can help to increase analyte solubility. In any case, try to avoid water or do not exceed 5% of water.
2. Wash solution: always use acetonitrile with 5–10% of water, without the addition of acids or salts.
3. Premixed eluent: HILIC retention varies significantly for small variations of water content. The use of premixed solutions of acetonitrile/water helps to increase the reproducibility of the analyses.
4. Column equilibration: longer equilibration times are required in HILIC. Typically, 15–20 column volumes ensure the complete column equilibration. Consider that the equilibration time can be accelerated increasing the flow-rate. The lower viscosity of acetonitrile will lead to a relative low backpressure also at higher flow-rates. Alternatively, it is possible to obtain a partial column equilibration using shorter equilibration times. This situation, called dynamic equilibrium, allows reproducible measures, but the equilibration time among the runs must always be kept constant.

In the forthcoming instalment, we will discuss the second most important chromatographic parameter in HILIC mobile phases: the salt content. In particular, we will describe an easy procedure to solubilize the salt in mobile phases at high acetonitrile content.

**Next Tip:** HILIC mobile phase: salt content

Giorgia Greco is currently a Post Doc researcher at the Analytical Research Group at the Technische Universität München, Germany. She received a PhD in Chemistry at the University of Naples, Italy. During her research, she specialized in the analysis and separation of metabolites from human and food matrices, as well as of organic contaminants in waste water samples, by hyphenated RPLC/MS

and HILIC/MS techniques. She has also focused on the theoretical elucidation of the HILIC retention mechanism with the aim of providing scientific bases for the fast development of HILIC separations.

Thomas Letzel, Associate Professor, is head of the Analytical Research Group at the Technische Universität München, Germany. He received his PhD in Chemistry with Aerosol Analysis, worked as Post-Doc performing pharmaceutical analysis, built up his research group in bioanalysis with Habilitation in 2009 and extended his analytical experience from then in food and water analysis. In all areas he developed novel analytical platforms based on LC–MS for the characterization of organic molecules in complex matrices. Thereby techniques (such as HILIC or RP-UHPLC) are applied for new analytical solutions often in direct flow-coupling with (bio)functional assays. He is the author of more than 50 publications and two books.

Thomas Letzel wants to share his experience in liquid chromatography, especially in HILIC, with the community to accelerate the dissemination about HILIC theory and practical handling.