

HILIC Solutions

HILIC Mobile Phase: pH

The pH of the mobile phase should be selected in order to enhance the hydrophilicity of the analytes.



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All the analytical scientists that work with reversed-phased liquid chromatography (RPLC) know really well that the pH of the mobile phase determines the ionization state of the analytes. That remains valid also in HILIC, but differently from RPLC, the pH of the HILIC mobile phase should be selected with the intention of bringing the analytes in their ionic form. Indeed, the charged form of a compound is in general more hydrophilic in comparison with its neutral form, and, therefore, it will be more retained in HILIC conditions.

In order to choose the most appropriate pH of mobile phase you may want to have a look at the pK_a values of your analytes. The pH of the mobile phase is recommended to be above the pK_a values of acid compounds and below the pK_a values of basic ones. Commonly, the pH of HILIC mobile phases is neutral (pH \sim 7) for the separation of acid solutes and acidic (pH $<$ 7) for basic solutes.

Figure 1 shows the relative concentration of benzoic acid ($pK_a = 4.1$) and its anionic form in function of the pH. At neutral pH the solute is completely ionic and consequently more hydrophilic.

An alternative approach for the selection of the mobile phase pH is the examination of the log D values of your solutes. We remind you that log D is a parameter that well describes the hydrophilicity of a compound (please, visit HILIC Solutions #1

for more details). With a good approximation, it can be stated that the lower the log D value is, the more hydrophilic is the molecule.

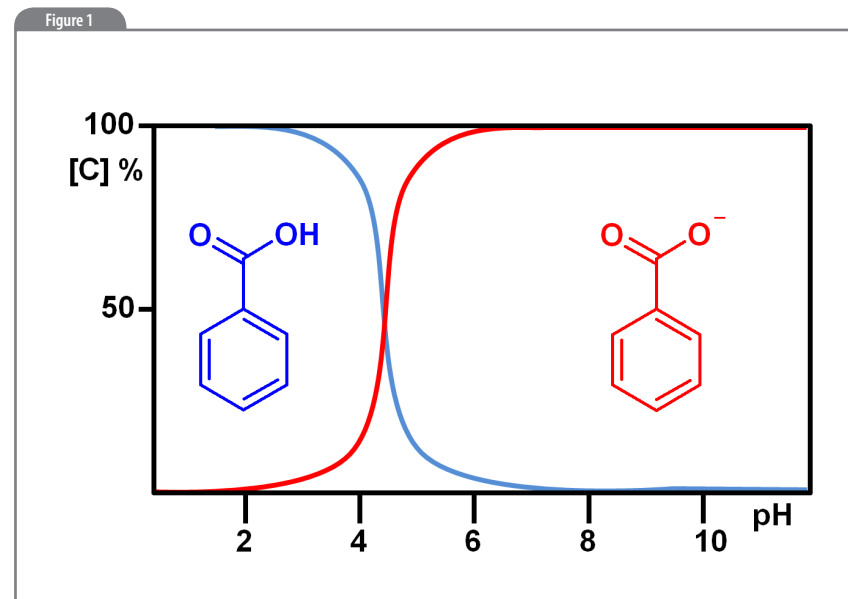


Figure 1: Relative concentration of benzoic acid ($pK_a = 4.1$) and its anionic form in function of the pH. Data is adapted from www.chemicalize.org.

Figure 2

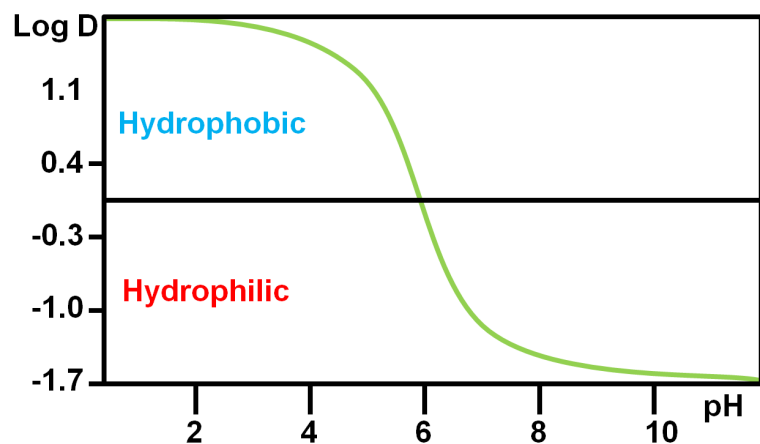


Figure 2: Variation of log D value of benzoic acid in function of the pH. Data is adapted from www.chemicalize.org

The log D value is a parameter that is pH dependent. Therefore, the choice of a mobile phase at a pH value – for which the log D is lower – will ensure that you have higher retention. This approach is particularly useful for molecules with several ionizable groups, for which the analysis of pK_a can not always be straightforward.

You can find the log D values for numerous molecules on free databases such as www.chemicalize.org and www.chemspider.com.

Figure 2 shows the variation of log D value of benzoic acid in function of the pH. At neutral pH the log D values are negative, indicating that the compound is hydrophilic.

The pH of the mobile phase should always be between 3 and 8 for silica-based stationary phases, whereas polymeric phases can often be used in an extended pH interval. In the column manufacturer's instruction you can find the exact pH stability range for your column.

The pH of the mobile phase has in general no effect on the charged state of the stationary phases commonly employed in HILIC because they are pH independent. However, silica phases, that are the most used HILIC phases, are also the principal exception. At pH values above 4–5, the surface silanol groups are deprotonated, so that the phase is negatively charged. This leads to attractive electrostatic interactions with positively charged analytes, as amino compounds, that will be strongly retained. In contrast, on silica phases

at neutral pH, acidic analytes may be poorly retained or not at all, due to the repulsive electrostatic interactions.

In addition to that, all the silica-based phases may contain underivatized silanol groups, whose pH-dependent behaviour influences the retention and selectivity in HILIC.

The most common pH modifiers are acetic acid and formic acid for the preparation of acidic eluents and ammonium hydroxide and ammonium bicarbonate for basic eluents. They have the advantages of being soluble at a high percentage of organic solvent and compatible with mass spectrometric detection.

There are two main possibilities for the preparation of HILIC mobile phase at a particular pH value. The first one is to adjust the pH of the water solvent — in many cases it is the stock salt solution, before mixing with acetonitrile. The second one is to add the same amount of pH modifier also to the acetonitrile fraction. The final pH of the mobile phase in both cases presumably deviates from the pH of the water solution, due to the high organic content. However, the specific pH deviation is generally unknown and is often neglected.

In the forthcoming instalment, we will discuss the effect of column temperature on HILIC retention and selectivity.

Next Tip: Column temperature in HILIC

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.