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HILIC Mobile Phase: Salt Content

The addition of salt to HILIC mobile phases is strongly recommended to improve reproducibility and peak shape. Do you know how to solubilize the salt in mobile phases at high acetonitrile content?

Salts are usually added to the mobile phase for controlling electrostatic interactions between charged analytes and the stationary phase. This results in the improvement of peak shape and in the reduction of peak tailing.

Salts for HILIC: Salts commonly used in HILIC are ammonium acetate and formate, but also bicarbonate, due to their good solubility in mobile phases with high levels of organic solvent. Moreover, they are compatible with mass spectrometric detection. The use of less soluble salts, such as phosphate, or ion pair reagents, such as TFA, should be avoided.

The majority of HILIC mobile phases are prepared with the addition of ammonium formate or acetate. The two salts are not equivalent because ammonium formate imparts a more acidic pH to the mobile phase, with ensuing possible variation of the separation. Ammonium bicarbonate solutions are less preferred because they are quite unstable and prone to changes of pH, which tends to become more basic due to the release of carbon dioxide.

Effect of the salt content: The principal effect of the increase



of the salt concentration is the decrease of the electrostatic interactions in the case of charged analytes on charged or zwitterionic columns (see HILIC Solution #3). In the presence of electrostatic attractions between solute and stationary phase, this leads to decreased retention, whereas in the case of electrostatic repulsions, it leads to increased retention. The second effect of the increase of the salt concentration is on the thickness of the water layer. The high content of organic solvent in the eluent causes salt to partition preferentially into the water layer. Increasing the salt concentration leads to an increase of the water layer, with ensuing stronger retention for all the hydrophilic analytes. Therefore, in HILIC mode the salt contributes to the retention also of the neutral compounds, for which electrostatic interactions are not present.

Preparation of mobile phase: For better reproducibility and peak shape, it is recommended to maintain a minimum buffer concentration of at least 5 mM in the final mobile phase and to keep it constant during the run. The solubilization of salt in mobile phases at high organic content can be problematic and precipitation phenomena can occur.

Figure 1

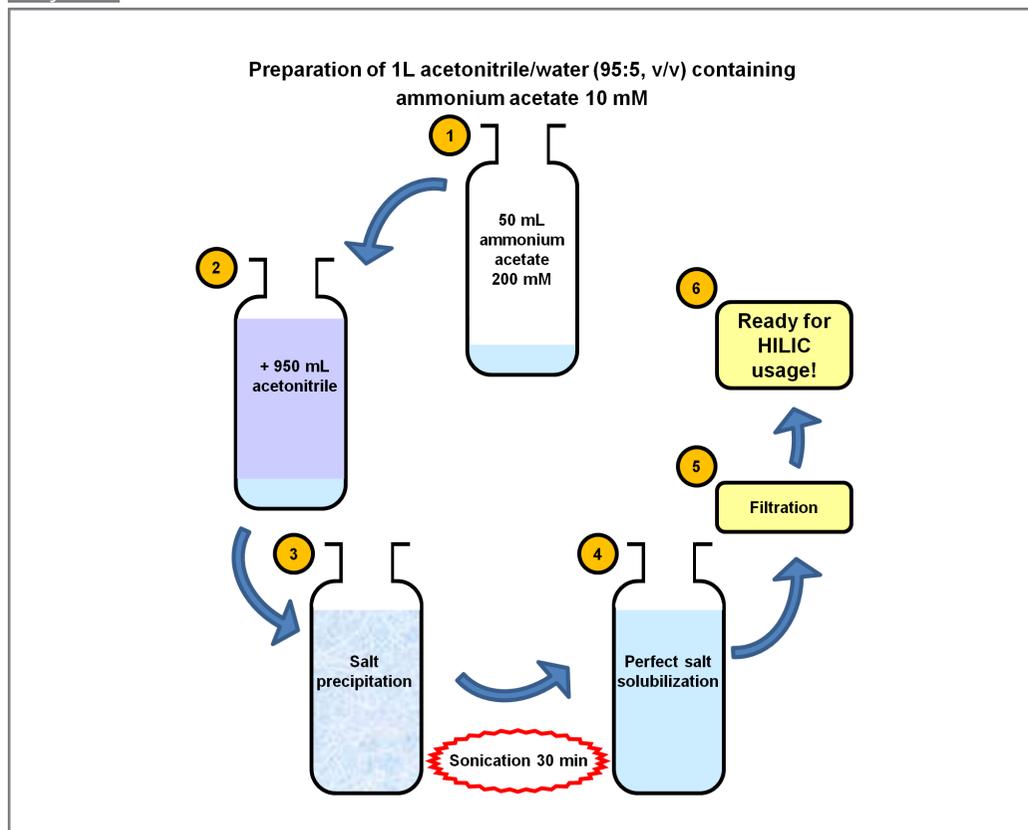


Figure 1: Scheme of recommended procedure for the preparation of HILIC mobile phases. The example represents the preparation of 1 L of acetonitrile/water (95:5, v/v) containing 10 mM ammonium acetate.

First of all, at 95% acetonitrile in the mobile phase, the salt concentration has to be kept necessarily below 15 mM, due to upper limits of solubility. In addition, the preparation of the mobile phase should be particularly careful to avoid any precipitation. A convenient procedure for the preparation of HILIC mobile phases is represented in Figure 1. Let us consider that we want to prepare 1 L of acetonitrile/water (95:5, v/v) containing 10 mM ammonium acetate. It is extremely important that the ammonium acetate is previously solubilized in pure water. In this case, we have to prepare 50 mL of 200 mM ammonium acetate solution in pure water. Simple stirring of the solution will facilitate the solubilization of the salt in few minutes. Thereafter, we can slowly add 950 mL of acetonitrile to the 50 mL of 200 mM ammonium acetate solution. The final solution will appear opaque and little drops on the bottom may be present. That indicates that the salt is not perfectly dissolved. The use

of transparent glass will help us to observe this process. Vigorous stirring, even overnight, will not improve the salt solubilization. In order to obtain a complete dissolution of the salt, the solution is recommended to be sonicated until it appears transparent with the absence of any particles. In general, 30 min of sonication are sufficient. After the total solubilization is achieved, the solution can be filtered as usual and, then, it will be ready for usage.

This procedure can be adapted for the preparation of any other HILIC mobile phase. Water content, salt type and its concentration in the mobile phase can be freely selected on the basis of your applications. In any case, always keep in mind that the sonication is the key step for the preparation of HILIC mobile phases.

In the forthcoming instalment, we will continue our discussion of the chromatographic parameters in HILIC mobile phases focusing on mobile phase pH. [QA1: Edit OK?]

Next Tip: HILIC mobile phase: pH

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.