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The Basics of HILIC

How can we describe the mechanism at the basis of HILIC retention? We will answer this question and give you an insight into the main interactions that govern chromatographic separation in HILIC mode.

HILIC retention mechanism is more complex in comparison to other chromatographic separation modes and many scientists are still striving to understand it fully. It is often described as a mixed-mode mechanism, where several kinds of interaction contribute simultaneously to the overall retention.

In order to understand the HILIC mechanism, it is useful to have a look at the main retention model used to describe the widespread reversed-phase liquid chromatography (RPLC), familiar to most chromatographers.

RPLC is based on the use of hydrophobic stationary phases such as the C18 and aqueous mobile phases. As shown in Figure 1, the alkyl chains of the RPLC stationary phase create a hydrophobic environment near the surface of the phase, in contraposition to the hydrophilic eluent. Such a system can be regarded as a liquid-liquid separation system where the retention is governed by partition processes. Therefore, RPLC retention can be described by partitioning of the solutes between the hydrophobic environment near the phase surface and the hydrophilic aqueous mobile phase. The solubilization in one phase instead of the other is determined by the

Figure 1

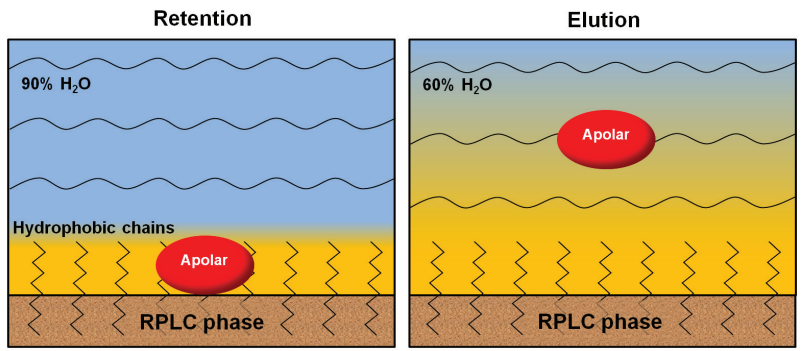

Figure 1: Scheme of RPLC partitioning of a general apolar analyte into the hydrophobic environment near the phase surface at high water content in the mobile phase, and its elution with the increase of the organic content in the mobile phase.

Figure 2

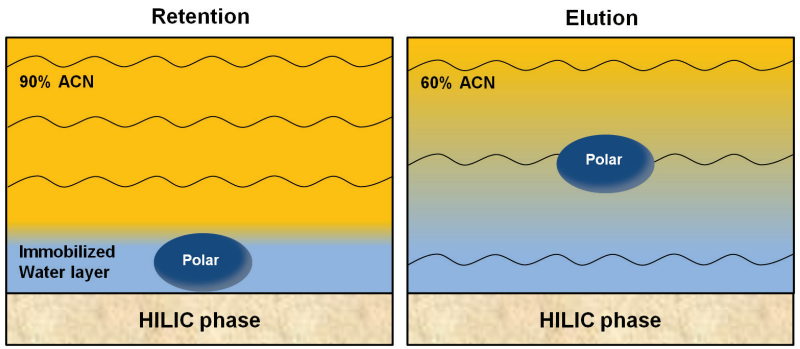

Figure 2: Scheme of HILIC partitioning of a general polar analyte into the water layer adsorbed on the hydrophilic phase surface at high acetonitrile content in the mobile phase, and its elution with the increase of the water content in the mobile phase.

analyte polarity (see HILIC Solutions #1). Apolar compounds solubilize in the hydrophobic environment, and, as a consequence, they are stronger retained by the stationary phase. When the percentage of organic solvent in the mobile phase increases, the difference in polarity between the stationary phase and the mobile phase decreases and the apolar analyte can transfer into the mobile phase, with ensuing elution.

The same partitioning model can be satisfactorily applied also to describe the retention in HILIC. It has to be noticed that everything works exactly the opposite, and this is the reason because HILIC is known as "reversed-RPLC". A schematic description of the retention of a polar analyte by a HILIC stationary phase is presented in Figure 2. HILIC separations are based on the use of polar stationary phases eluted with hydrophobic mobile phases, generally acetonitrile, containing a small percentage of water. Polar stationary phases retain water strongly on their surface. In these conditions, an eluent gradient is created, which ranges from a water rich layer near the hydrophilic surface of the phase to an acetonitrile rich bulk. The presence of an immobilized water layer adsorbed onto the surface of the hydrophilic stationary phase is the most important concept at the basis of HILIC retention. The water rich layer and the acetonitrile rich bulk constitute the liquid-liquid separation system here.

Therefore, HILIC retention mechanism is governed by the partitioning of the analyte between these two phases on the basis of its relative solubility. Contrary to RPLC, polar hydrophilic analytes are strongly retained, because they are preferentially solubilized in the water layer.

The increase of the water content in the mobile phase has the effect of decreasing the difference in polarity between the bulk and the adsorbed layer. This results in the solubilization of the analyte in the mobile bulk phase and in its elution.

In addition to partitioning, other kinds of interaction, such as hydrogen bonds and electrostatic interactions, support the HILIC retention. Solutes with hydrogen-donor or hydrogen-acceptor functionalities can interact directly with the stationary phase via hydrogen bonds. Moreover, charged analytes interact electrostatically with HILIC phases. These interactions can be attractive or repulsive depending on the charged state of both the analyte and the stationary phase. Electrostatic attractions between positive charged compounds and negative charged stationary phases, or vice versa, produce the increase of retention times, whereas electrostatic repulsions between analytes and phases with the same charge have the opposite effect.
With this in mind, in the next instalment we will present the main HILIC stationary phases and their effects on each specific interaction.

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.