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HILIC Solutions

Column Equilibration in HILIC

Giorgia Greco

Thomas Letzel

It is matter of fact that the samples most chromatographers have to face are rather complex for sample number and compound variety.

In these situations, gradient elution is largely preferred for the separation of analytes with different retention factors. In this way it is possible to narrow the peak width of the later eluted compounds and shorten the run time.

However, gradient separations have a principal drawback: after each run they require column equilibration with the starting concentration of the mobile phase before the next sample can be injected.

In comparison to RPLC stationary phases, HILIC phases need gradients with lower slope and longer equilibration times. This is because in HILIC the separation is driven by the thickness of the water layer that originates from the water in the mobile phase. Changing the mobile phase composition will change the water layer. However, this is a slow process and requires several column volumes before the water in the water layer reaches equilibrium with the mobile phase.

A HILIC mobile phase must always contain at least 2–3% of water in the final mobile phase in order to allow the formation of the water layer on the surface of stationary phase. Then a gradient elution should be performed increasing the water content up to a maximum of 40–50% in 20 minutes. Each HILIC column requires a different amount of column volume in order to reach a complete equilibration. Let's consider a general column of dimensions 2.1 mm × 150 mm. The column volume is ~300 µL (for calculation of the column volume, see *HPLC Solutions #101: Estimating Column Volume*). HILIC columns need 20 column volumes, or more, for proper equilibration. This means that at a mobile phase flow-rate of 200–400 µL/min, the equilibration time will be between 30 and 15 minutes. Insufficient column equilibration will cause shifting of the retention times, resulting in poor reproducibility (Figure 1).

In order to shorten the equilibration time it is possible to perform the equilibration step at a flow-rate higher, even two-fold, than the one used for the analysis. In HILIC this is possible owing to the low viscosity of the mobile phase at high organic content, which gives a low back pressure.
Another possibility of increasing the analysis throughput shortening the equilibration step is to run the samples continually allowing a fast but constant equilibration time among the runs. In this case, the column reaches a dynamic equilibrium and even if there is not a complete equilibration, it is possible to obtain good reproducibility.

In the forthcoming instalment, we will discuss the effect of sample diluent on HILIC retention and peak shape.

Figure 1

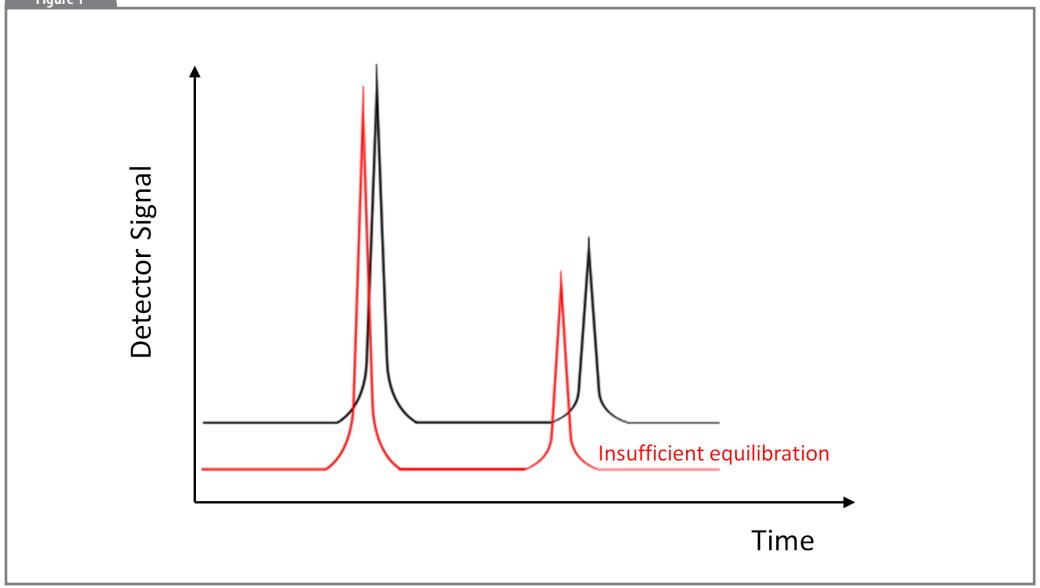

Figure 1: Retention time shift between runs due to insufficient column equilibration.

Next Tip: Sample diluent

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.