

HILIC Solutions

RPLC and HILIC Coupling



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Numerous research fields, such as metabolomic or environmental analysis, need to collect information about a huge number of compounds in solution that present different physical-chemical properties. For this reason, a single chromatographic step is generally not sufficient to achieve an acceptable separation of all the analytes.



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A valid analytical approach that can ensure a comprehensive analysis is the coupling of two chromatographic techniques with a high degree of orthogonality. In this regard, the coupling of RPLC to HILIC is very attractive. Ideally, compounds strongly retained in RPLC are almost not retained in HILIC. In addition to their orthogonality, common solvents are used: both RPLC and HILIC employ water and acetonitrile, which are readily compatible to ESI sources for MS detection. Despite the use of the same solvents in both techniques, the starting condition in HILIC requires high organic content in the mobile phase, while high water content in RPLC. This solvent strength mismatch must be carefully taken into consideration in the development of a coupling setting.

RPLC and HILIC coupling can be realized in two main modes: off-line or on-line.

Off-line coupling of RP and HILIC is really easy to realize and does not require special equipment. Fractions from the first separation are collected and reinjected into the second system. In the off-line coupling solvent mismatch is not a problem, since the fraction can be evaporated prior to the second separation. As a downside, off-line collection requires significant sample handling, making it time-consuming and less reproducible.

Generally, the implementation of the coupling in on-line mode is more desirable. In this case, a short column is utilized on-line for trapping and transferring all the compounds eluted near the void volume of the first one to the second column, according to the “heart-cutting” method. Systems with switching valves are essential (i) to direct analytes from the first column either to the detector or to the second column and (ii) to adjust the strength of the mobile phase (1,2).

A simple and effective approach without the use of valves is a linear on-line coupling of HILIC to RPLC. In this case the sample is injected on a RP column. The hydrophobic compounds are then retained, while the polar ones are directed via a T-piece towards the HILIC column. The key aspect of this setting is the use of a RP column with a smaller

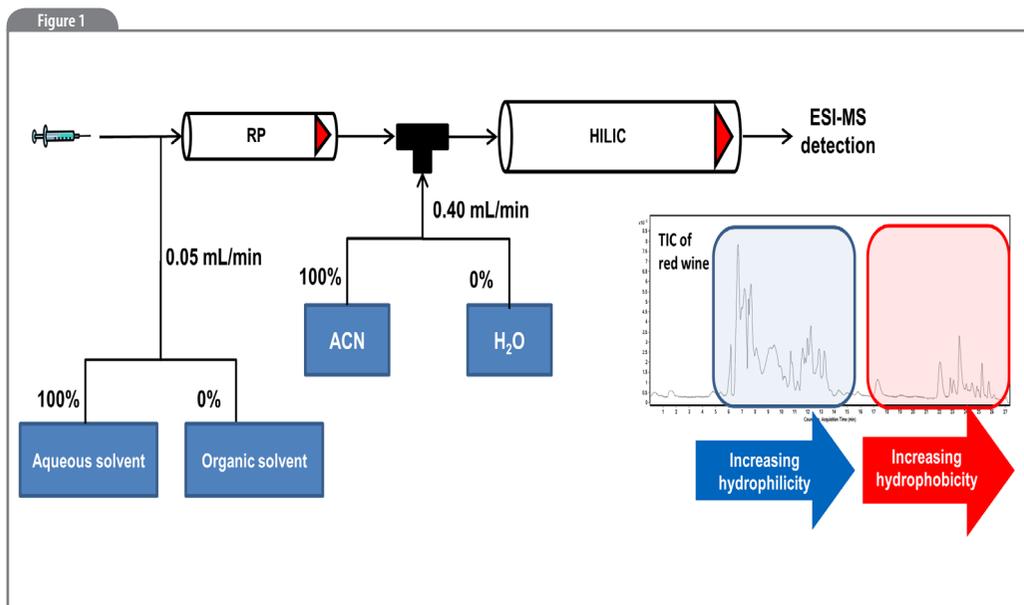


Figure 1: Schematic of linear on-line coupling of RPLC and HILIC with MS detection for the analysis of red wine.

dimension than the HILIC one. This makes the use of a higher flow-rate for the HILIC column possible. Indeed, the mobile phase eluting from the RP column is connected via the third port of the T-piece to a second pump, with which the organic content in the mobile phase is increased (i.e. $\geq 80\%$), for the trapping of the polar fraction onto the HILIC column. The polar analytes are eluted from the HILIC column increasing the water content in the mobile phase of the second pump. In the same time, the high water content in the first dimension ensures the retention of the RP-retained analytes. Subsequent to the HILIC analysis, the RP analysis is performed. All the hydrophobic compounds, before the detection, pass through the HILIC column with almost no retention. RPLC-HPLC linear on-line coupling is also suitable for several aqueous samples that requires peak capacity (by extended polarity) to be increased. Examples comprise drug development studies, wine analysis, algae toxins and wastewater analysis (3,4,5,6). Although linear on-line coupling is easier to realize, it has to be considered that it may be not ideal for trace analysis, since considerable sample dilution occurs. In contrast the mostly applied mass spectrometric detections becomes more and more sensitive.

This is the last instalment of the 'HILIC Solutions Series'. We hope that this series has helped more and more people to get familiar with the HILIC world (7).

We would like to thank you and wish you nice separations!

References

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Dr. Giorgia Greco is currently working as an Application Scientist with Thermo Fisher Scientific in Germany focusing on LC-MS applications for food and environmental analysis. She received a PhD in Chemistry and worked as Post Doc researcher at the Technische Universität München, Germany. During her research, she specialized in the fundamental of LC-MS and in the separation and analysis of metabolites from human and food matrices, as well as organic contaminants in waste water samples, by hyphenated HPLC/MS and HILIC/MS techniques.

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.