HILIC – New Separation Principle in Chromatography?

In the field of liquid chromatography/HPLC, so-called HILIC separation phases have been used increasingly in recent years and for a broad area of applications. This concerns a special variant of normal-phase chromatography (NPC), whose popularity was long considered superseded by so-called reversed-phase chromatography (RPC).

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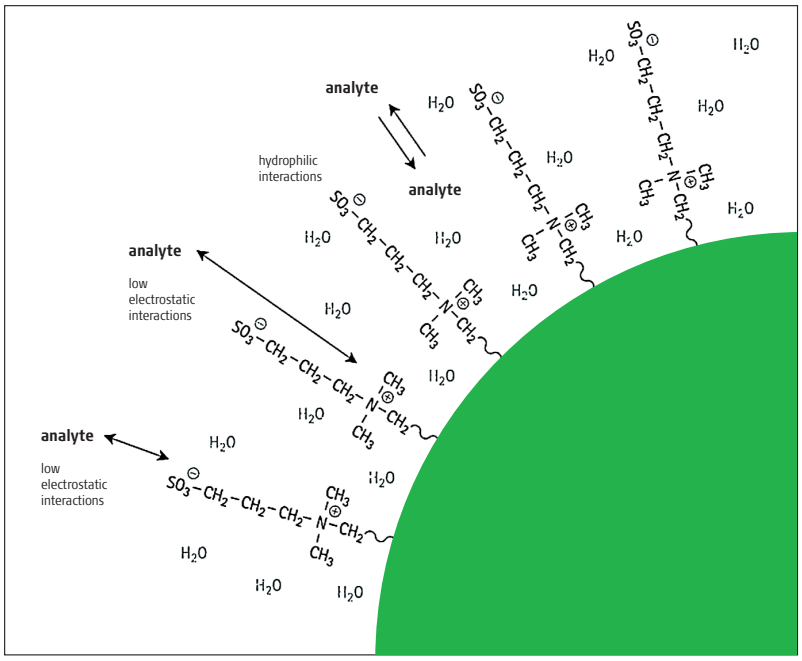
The acronym HILIC stands for "hydrophilic interaction liquid chromatography" and was first defined in 1990 by A.J. Alpert, who specified an alternative chromatographic procedure for separating highly polar compounds. Analogue to NP chromatography, HILIC uses polar stationary phases, but – in contrast to NP chromatography – applies typical polar RP eluents (such as methanol, acetonitrile, or water). Elution strength is inverse to the RP, so that water functions as the most concentrated eluent.

Today HILIC is understood to be more of a variation of NP chromatography and is hence frequently termed "aqueous normal-phase chromatography" or "reversed-phase chromatography". It is considered a link between classic NP chromatography and diversely used RP chromatography and ion chromatography (IC).



Long before the term HILIC had established itself in the world of chromatography, Samuelson and Sjöström developed a first HILIC application. In 1952, the researchers succeeded in separating monosaccharides using an Amberlite IRA ion-exchange column with an eluting gradient composed of ethanol and water.

In recent years HILIC has become increasingly significant as a supplement to RP. Particularly since determining highly polar analytes using RP is difficult, or even impossible, due to lack of retention.

Separation principle

A characteristic of hydrophilic interaction chromatography is the use of a polar stationary phase and an eluent composed of an aqueous buffer system and an organic water-soluble modifier (preferably acetonitrile). The prerequisite for this is the use of salts with good solubility in the organic solution. In HILIC an eluting gradient mostly starts with a high acetonitrile (ACN) share (< 70%) and ends with a high water/buffer share in the mobile phase. Separation of the analytes is based on their respective hydrophilicity or polarity. The corresponding separation principle is not yet comprehensively clarified, however it is believed that a water/buffer layer forms on the polar surface and that partition chromatography occurs between the analyte and the stationary phase. The analytes elute in ascending order of polarity since the retention times of polar and hydrophilic compounds expand due to greater interaction with the stationary phase.

In addition, since other effects and interactions also play a significant role in applying this separation method, different stationary HILIC phases also lead to different results in practice. Basically speaking, HILIC phases can be divided into three groups. The neutral phrases, e.g. diol phases, the loaded phases, e.g. silica or amino phases, and the zwitterionic phases, characterised by good selectivity.

As regards detection, HILIC applications are easily combined with standard detection methods such as UV, fluorescence, and refractive index. In addition, LC-MS detection, LC-MS/MS detection, and evaporative light scattering detection (ELSD) is seeing increasing use, for HILIC – due to its LC-MS-convergent aqueous buffer systems – offers good compatibility with MS and ELSD. When using ESI-MS (electrospray ionisation combined with mass spectrometry) the sensitivity of the separation method increases significantly due to the high concentration of organic modifiers compared to RP.

Application examples

For example, in food analysis HILIC is used in detecting acrylamide, methacrylamide, and methacrylic acid and in separating water-soluble vitamins such as dehydroascorbic acid/ascorbic acid, panthenol, and thiamine. What is more, this chromatographic procedure is used in residue analysis for routine determination of veterinary medicine residues, grower feed residues, and growth regulator residues. In addition, its use is also documented for analysing organic acids, amino acids, and carbohydrates. The LCI recently established an HILIC method for the chromatographic separation of imidazoles. SV