

A PRACTICAL GUIDE TO HILIC

A tutorial and application book

Introduction

This guide aims at introducing hydrophilic interaction liquid chromatography (HILIC), which is a technique suitable for separation of very polar and hydrophilic compounds. It deals with the basic theory of HILIC and the practical aspects of this separation mode. This booklet will also introduce the reader to the SeQuant zwitterionic ZIC®-HILIC and ZIC®-pHILIC stationary phases, see Figure 1, and contains a range of application examples for different types of hydrophilic compounds. You can read more about this and lots of other things in this guide.

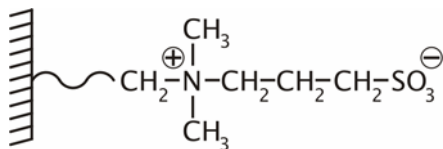


Figure 1: The functional group of the ZIC®-HILIC and ZIC®-pHILIC stationary phases.

If your HILIC questions cannot be solved with this compilation SeQuant is at your service. We first recommend you to visit the SeQuant homepage (www.sequant.com), where you always find material update, applications and technical data on our products. If you need additional information the SeQuant staff will be happy to further assist you.

Why HILIC?

Despite the fact that reversed phase liquid chromatography (RPLC) is the overall most applied separation technique, and that it can be used for a variety of applications in junction with the most common detection principles, certain solutes, especially polar and hydrophilic compounds, are not retainable in a simple fashion. Over a long period normal phase liquid chromatography (NPLC) has been the technique

of choice for this purpose, using non-aqueous mobile phases not very friendly to the environment. Yet, under such experimental conditions it is difficult to dissolve polar and hydrophilic compounds. In its place, HILIC is the alternative as the elution order is likewise inverted to RPLC, as illustrated in Figure 2. In other words, solutes that have little or no retention on RPLC columns generally experience strong retention on HILIC columns.

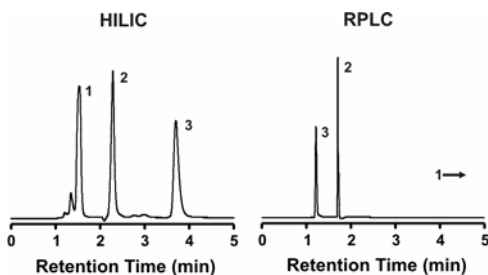


Figure 2: Separation of peptides under HILIC and RPLC conditions. Eluents; (HILIC) 60:40 acetonitrile / 10 mM ammonium acetate, pH 7, (RPLC) 5:95 acetonitrile / 10 mM ammonium acetate, pH 7. Legend; (1) Phe-Gly-Gly-Phe, (2) Leu-Gly-Gly, (3) Gly-Gly-Gly.

The HILIC technique thus bears similarities with traditional NPLC, but with the important difference that HILIC employs semi-aqueous mobile phases. Consequently, with respect to analyte solubility in the eluent and matrix compatibility, HILIC is superior, as the mobile phase compositions used are comparable to RPLC separations. Typical eluents for HILIC consist of 40-97% acetonitrile in water or a volatile buffer. HILIC is thus a very mass spectrometry (MS) friendly technique, and by changing from RPLC to HILIC a 10-1000 fold increase in sensitivity is often observed for hydrophilic analytes. Ion-pair reagents are also completely avoided, which is advantageous for preparative chromatography.