

The importance of sample diluent in HILIC

ABSTRACT

When working with Hydrophilic Interaction Liquid Chromatography (HILIC), the choice of sample diluent can have a profound impact on peak shape. An incorrect choice can lead to poor peak shape, peak splitting and unstable retention times. This ACE Knowledge Note discusses how a suitable choice can be determined during HILIC method development.

Introduction

Ideally, the sample diluent in HILIC should have a composition as close as possible to that of the mobile phase used for the separation i.e. the diluent should be composed of a high percentage of the weaker solvent, such as acetonitrile. For gradient HILIC chromatography, the sample diluent should be similar to the gradient starting conditions. In some cases, this may present issues with sample solubility as the polar analytes encountered in HILIC may be relatively insoluble in high concentrations of organic solvents.

Sometimes, peak shape problems encountered in HILIC can be resolved by addressing the choice of sample diluent. Samples dissolved in high aqueous diluents are problematic in HILIC as water is the strong solvent, possessing high elution strength. The presence of a large amount of water in the sample diluent therefore disrupts partitioning of the analyte into the water-rich layer which surrounds the HILIC stationary phase surface.

This can lead to poor peak shape and shifts in retention, particularly for weaker retained analytes. Figure 1 shows a comparison of the peak shape obtained for 2'-deoxyuridine when injected in mobile phase and 100% water. When the analyte is dissolved in water, a broad, almost split peak is observed. By changing the sample diluent to mobile phase, a dramatic improvement in peak shape and signal intensity is obtained.

The choice of sample diluent in HILIC tends to be application dependant and therefore, resource should be allocated to study the effect of sample diluent during method development. Often the effect of diluent strength can be analyte dependant and can also be influenced by stationary phase and eluent conditions. It is therefore recommended that a stepwise investigation should be carried out to investigate the effect of increasing the percentage of organic solvent on peak shape and method performance. Increments of 10% acetonitrile between 50 and 90% acetonitrile:buffer can help to understand how to achieve the optimum peak shape for target analytes.

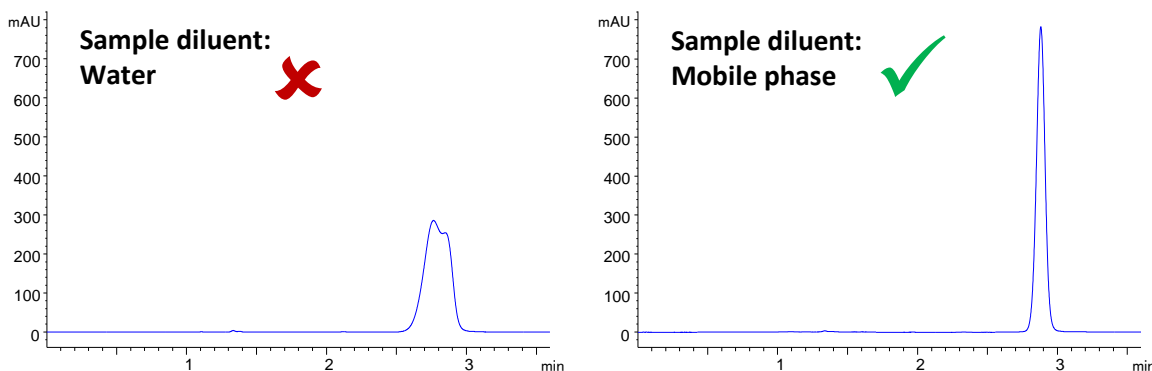


Figure 1: Effect of sample diluent on the peak shape of 2'-deoxyuridine. Column: ACE 5 HILIC-N, 150 x 4.6 mm. Flow rate: 1.5 mL/min. Mobile phase: 10 mM ammonium formate pH4.7 in MeCN:water 9:1. Injection volume: 5 μ L. Temperature: 25 $^{\circ}$ C. Detection: UV, 254 nm.

