

# 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.

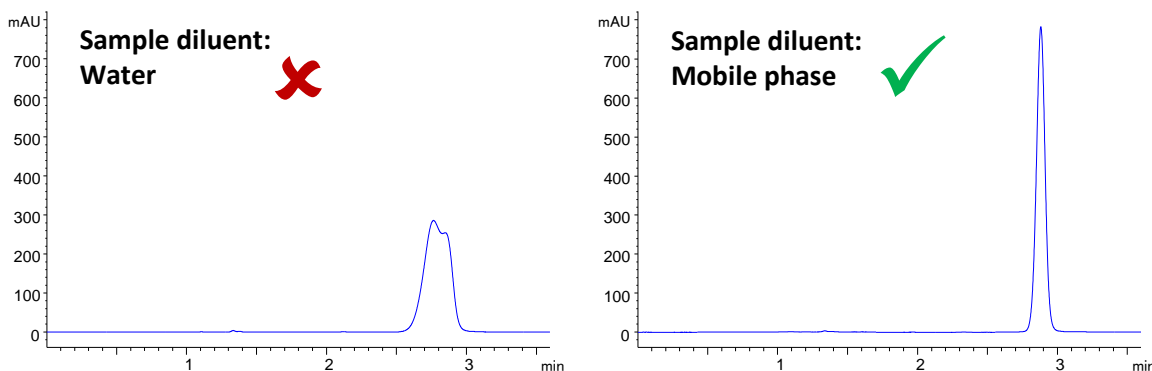
## Introduction

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.

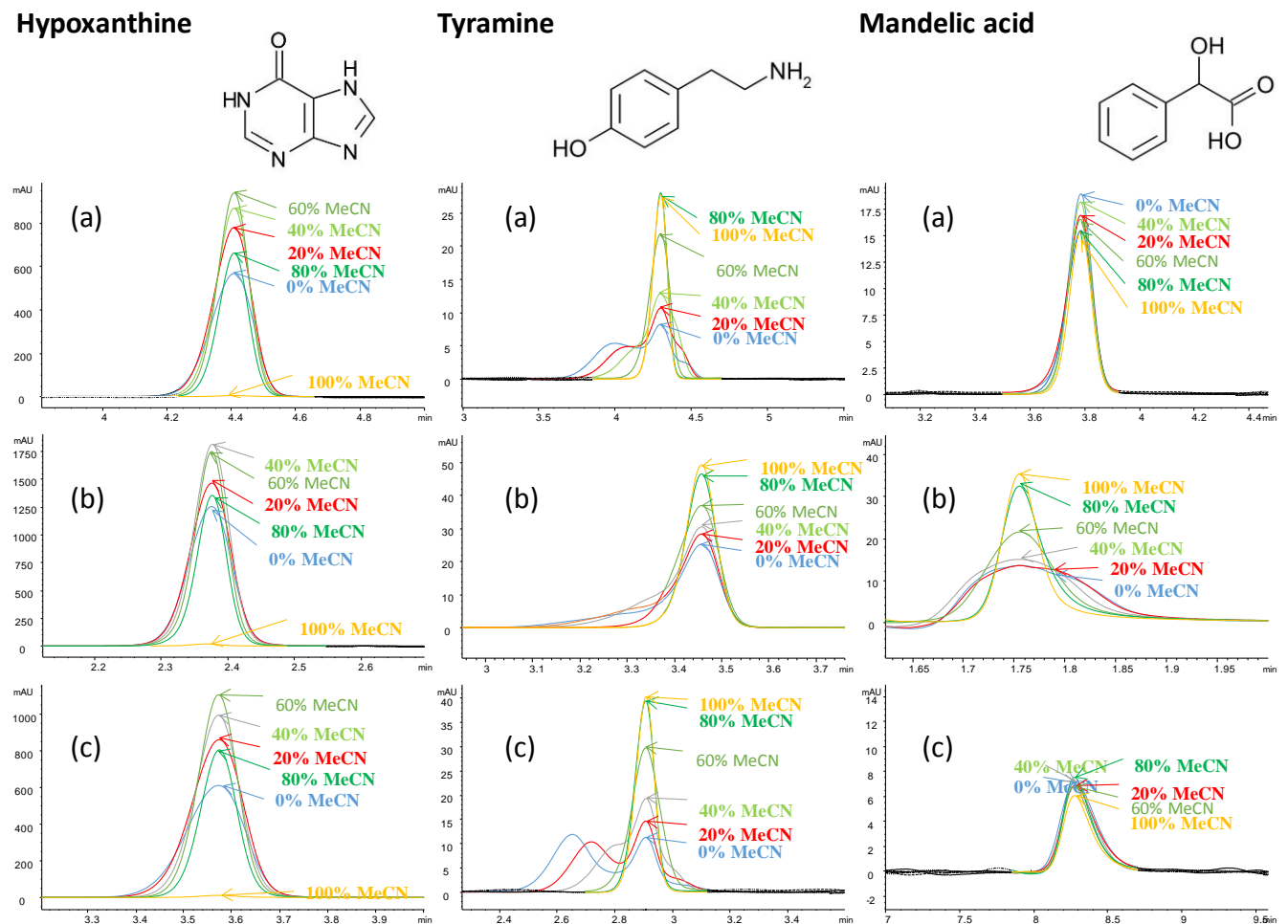


**Figure 1:** Effect of sample diluent on the peak shape of 2'-deoxyuridine. Column: ACE 5 HILIC-N, 150 x 4.6 mm. Flow rate: 1.5 mL/min. Mobile phase: 10 mM ammonium formate pH4.7 in MeCN:water 9:1. Injection volume: 5  $\mu$ L. Temperature: 25  $^{\circ}$ C. Detection: UV, 254 nm.

Figure 2 shows the results of a study on the effect of sample diluent for basic, acidic and neutral analytes on the three ACE HILIC phases (ACE HILIC-A, HILIC-B and HILIC-N) at a mobile phase pH of 3.0. The sample diluent was a mixture of acetonitrile and water and the percentage of water was varied systematically between zero and 100%.

The peak shape of hypoxanthine (polar neutral) generally improved with increasing concentration of acetonitrile, with an optimum around 60-80% organic.

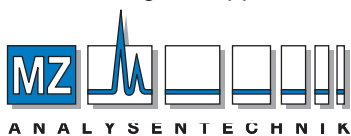
Tyramine (basic) gave a split peak on the HILIC-B and HILIC-N phases and poor peak shape on the HILIC-A at low percentage organic. Significant improvements in peak shape were obtained at acetonitrile concentrations greater than 60%. For mandelic acid, the peak shape was less affected by the diluent composition on the HILIC-B and HILIC-N phases, whilst on the HILIC-A, peak shape was poor at higher water concentrations. Overall, from this dataset a sample diluent composition of 60-80% is recommended for the application.



**Figure 2:** Hypoxanthine, tyramine and mandelic acid on (a) ACE 5 HILIC-N (b) ACE 5 HILIC-A and (c) ACE 5 HILIC-B, 150 x 4.6 mm, ammonium formate pH 3.0 in MeCN/H<sub>2</sub>O (90:10 v/v), flow rate: 1.5 mL/min, temperature: 25 °C, detection: 254 nm, injection volume: 5 µL.

## CONCLUSION

The choice of sample diluent is important for peak shape and method performance in HILIC. In general, the sample diluent should be composed of as high a percentage of the weaker mobile phase solvent as possible (typically acetonitrile in HILIC). The use of high aqueous sample diluents should be avoided where possible. A systematic assessment of sample diluent for a given application can help to eliminate any undesirable peak distortion effects.



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