Chromatography Solutions

Knowledge note #0009

The Effect of Sample Diluent on Peak Shape

INTRODUCTION

Distorted peak shapes are one of the most commonly encountered problems in liquid chromatography. The choice of sample diluent is a critical aspect of any LC separation and can have a significant impact on analyte peak shape. This Knowledge Note explains the key considerations for diluent choice and discusses some of the negative effects that may be observed if an unsuitable diluent is selected.

SAMPLE DILUENT SELECTION

The sample diluent is an important aspect of any LC method and requires careful selection to avoid potentially detrimental effects on the chromatographic separation obtained. Often, sample diluent choice is at least partly driven by sample preparation techniques, whereas in other situations the analyst may have a less restricted choice.

In selecting a diluent, the most important attribute is that it should fully dissolve the analytes of interest. In some cases this may be a single analyte, such as a single active pharmaceutical ingredient (API), but in others the diluent may also be required to fully dissolve any related impurities or degradation products. Solubility for such samples may additionally be required over a wide range of concentrations e.g. during validation studies.

In other applications, the diluent may be required to solubilise a complex range of analytes with widely varying physical properties. Importantly, the diluent must not cause any degradation or alteration of the sample components. In selecting an appropriate diluent, all of these points require careful consideration of analyte and sample chemistry, where possible.

After considering these points and identifying a suitable diluent for the sample, it also pays to consider any potential impacts that diluent choice may have on the chromatographic system. When a sample is injected onto an LC system, it is introduced as a discrete plug within the flow path. At the head of the column, the sample analytes are adsorbed onto the stationary phase. Ideally, the sample diluent is identical in elution strength

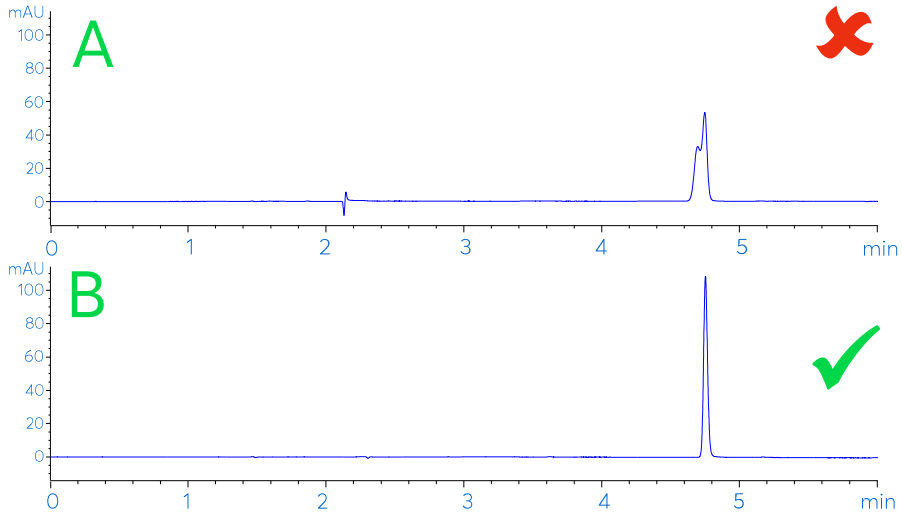

Figure 1: Injection of procainamide on a reversed-phase gradient using an Avantor® ACE® C18 column. (A) acetonitrile/water 1:1 v/v used as the sample diluent and (B) water used as the sample diluent.

to the mobile phase and the analytes are adsorbed as a discrete sample band. If this is not the case and the diluent has a significantly higher elution strength, it may interfere with the initial adsorption and result in distortion of the sample band.

Chromatographically, this manifests as broadening, tailing or fronting of the peak and, in some cases, split peaks. The solution to this issue is to adjust the diluent to reduce its elution strength so that it is more similar to, or weaker than, the mobile phase. Figure 1 shows an example for the injection of procainamide on a reversed-phase gradient. Injection using a 1:1 v/v mix of acetonitrile and water resulted in poor peak shape with an almost split peak. Changing the diluent to pure water fully resolved the problem, resulting in excellent peak shape.

In isocratic separations, this diluent mismatch may result in poor peak shape for all sample components, whereas in gradient chromatography, early eluting analytes are more likely to be affected.

In some applications, in particular those involving lengthy sample pre-treatment, correcting this issue may involve evaporation of the solvent, followed by reconstitution in a more appropriate diluent.

This is typically undesirable as it adds a time-consuming step to the sample preparation. In other applications, this approach can be useful for increasing analyte concentration, for example in trace analysis work.
In some applications, it may be possible to dilute the sample a little with a portion of weaker solvent. In others, reducing the injection volume may help to produce acceptable chromatographic results, however, dealing with the sample diluent as the root cause is a more appropriate long term solution.

Sample diluent is also very important for separations in HILIC mode and is one of the most common sources of chromatography problems. For further information on how to select a suitable diluent for HILIC separations, please refer to Avantor® ACE® Knowledge Note AKN0024.

CONCLUSION

In order to select a suitable sample diluent, the chromatographer must consider a variety of important aspects, including analyte solubility and stability. It is also important to consider the impact of diluent choice on chromatography, as an unsuitable sample diluent can easily lead to distorted peak shape and loss of chromatographic performance.