Expert tip: Sample solvent

The sample solvent can have a significant impact on peak shape and therefore on the separation efficiency. Dissolving and injecting a sample in a solvent that is of higher elution strength than the mobile phase reduces the interaction of the sample molecules with the stationary phase. Only sufficient mixing of the stronger sample solvent with the mobile phase will lead to the desired equilibration of concentration of sample molecules distributed in the mobile and stationary phases. Insufficient mixing is reflected in the chromatogram by the following phenomena:

- fronting / loss of resolution
- peak splitting
- retention time shift

To avoid these negative effects a sample solvent should be selected that corresponds to the mobile phase in elution strength or is even weaker. When using gradient elution, the mobile phase composition at the time of injection should be used as the basis for selection.
If the use of a stronger sample solvent is inevitable e.g. due to sample solubility reasons, a low injection volume should be chosen. As mixing of a small volume of strong solvent with the mobile phase is faster, its influence is reduced.

In the following example the impact of sample solvent is shown using the chiral separation of the fungicide cyproconazole. Cyproconazole is a triazole and has four stereoisomers. For the illustrated application the fungicide was dissolved in acetonitrile, dichloromethane, chloroform and THF. Depending on the sample solvent, the resulting peak shapes and hence the separation efficiency differed significantly (see figure 1).

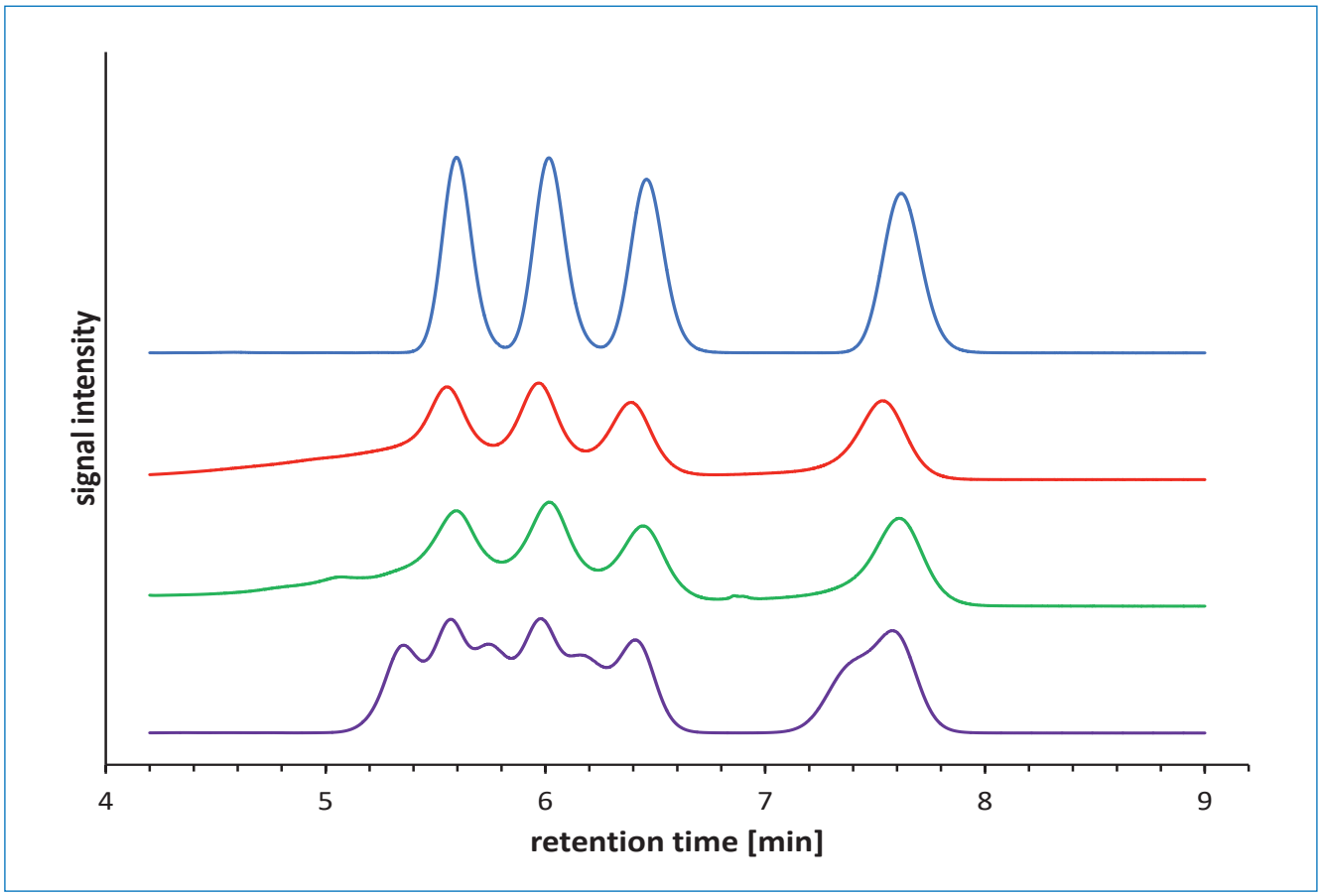

Figure 1: Influence of sample solvent on peak shapes; blue = acetonitrile, red = dichloromethane, green = chloroform, purple = THF.

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When dichloromethane or chloroform was used, severe peak fronting led to a reduced resolution. The use of THF as sample solvent even induced peak splitting. In these cases, no base line separation of the four stereoisomers could be achieved. Using acetonitrile, the weakest of the four solvents, as sample solvent however resulted in symmetrical peaks and an efficient separation.

Table 1: Chromatographic conditions.

Column	**CHIRAL ART Cellulose-SC** 5 µm, 250 x 4.6 mm ID KSC99S05-2546WT
Mobile phase	water / acetonitrile (48/52)
Flow rate	1.2 mL/min
Temperature	25 °C
Detection	UV at 220 nm
Injection	10 µL (1 mg/mL)

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