Is your sample soluble in your mobile phase?

Solubility of a sample is crucial for achieving an optimal chromatographic result. This must be considered not only for the solvent of injection, but for the mobile phase as well. When the sample solvent and eluent compositions are the same, solubility of the sample in the mobile phase is known. However, if sample solvent and mobile phase at the time of injection are different, sample precipitation can occur. Possible effects are:

- **increased backpressure,**
- **peak deformation,**
- **peak splitting,**
- **reduced resolution,**
- **retention time shift.**

This can be prevented by investigating sample solubility for different sample amounts in both media!

By way of example, in the following experiment, a defined amount of a poorly water-soluble chemotherapeutic agent was dissolved in acetonitrile and analysed using a standard procedure. The injection volume was 25 µL. The mobile phase at the time of injection comprised 40% acetonitrile and 60% water.

Even after a few injections increased backpressure and peak splitting were observed. As this indicated precipitation of the sample, investigation of solubility was conducted. Different volumes of sample solution were added to 0.5 mL of mobile phase.* Addition of 25 µL of sample solution resulted in a turbid mixture while for a sample volume of 15 µL or lower the mixture stayed clear.

Solution: reduction of injection volume

Accordingly, the injection volume of the sample used for the chromatographic analysis was reduced. Figure 1 compares chromatograms achieved from 20 µL and 10 µL injection volumes. While for 20 µL peak splitting and retention time shift was observed, retention and peak shape were unchanged for a sample volume of 10 µL.

In conclusion, by reduction of the injection volume not only sample precipitation can be avoided, but also a more efficient mixing of sample solvent and mobile phase takes place. The inefficient mixing with the 20 µL injection led to a reduced retention because the stronger eluent acetonitrile partially prevented interaction with the stationary phase. However, due to the efficient mixing, this was optimised with the 10 µL injection volume.

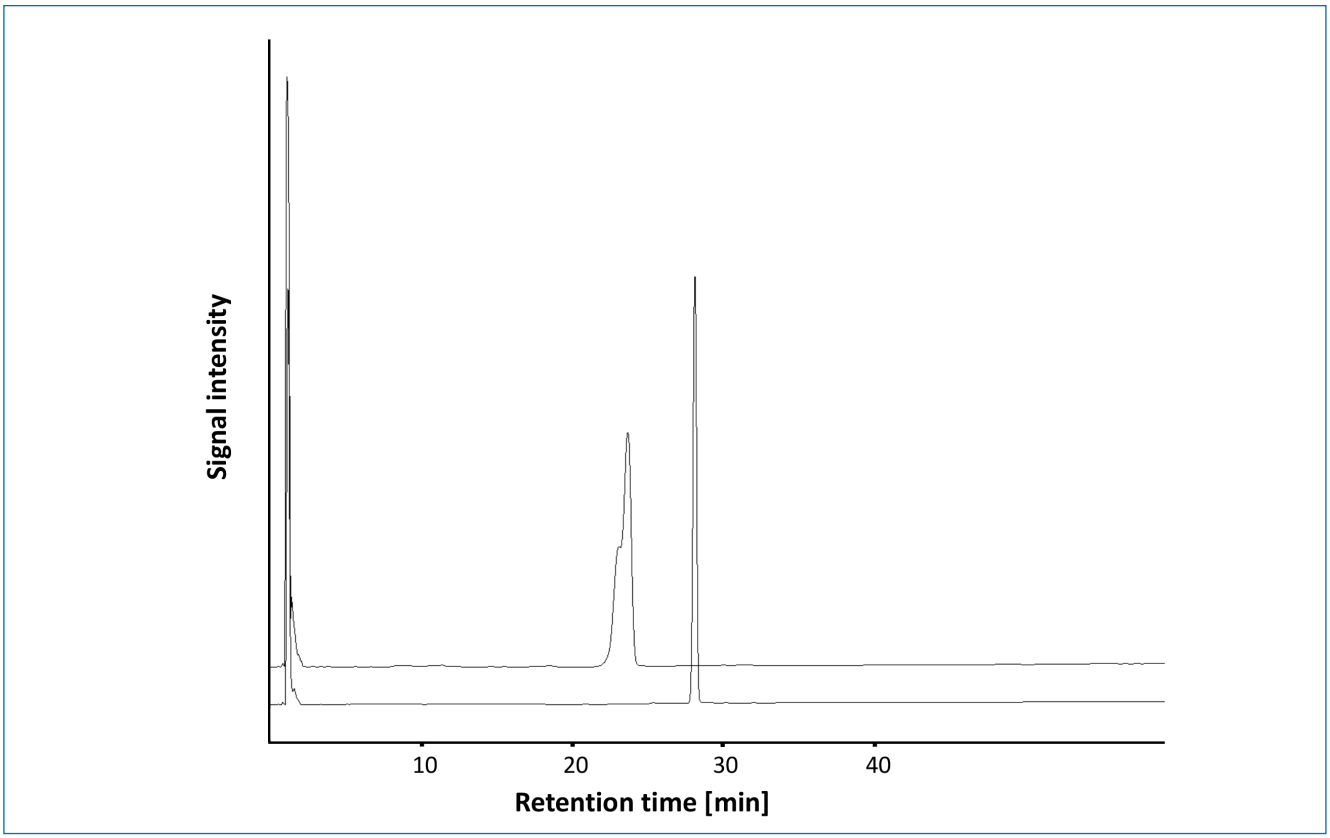

Figure 1: Chromatograms at 20 µL injection volume (top) and 10 µL injection volume (bottom).

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Table 1: Chromatographic conditions.

Column	**YMC-Pack ODS-A** 3 µm, 150 x 4.6 mm ID AA12S03-1546WT	
Temperature	35 °C	
Flow rate	1.2 mL/min	
Detection	UV at 220 nm	
Mobile phase	A: Water B: Acetonitrile	
Gradient	**t** [min]	**B** [%]
	0	40
	20	40
	60	94
	62	40
	70	40

* *Instruction: 0.5 mL of mobile phase are transferred to a clear and narrow sample vial. The sample volume is added using a microliter pipette. Do not shake. For visual inspection, the vial containing the mixture is held against a light source.*
Attention: in the column the sample is diluted with a volume less than 0.5 mL. Consequently, the likelihood of precipitation in the column is even higher.