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PO-CON1404E

Techniques for Reducing the Effects of Sample Solvent on UHPLC Analyses

Pittcon 2014 2050-11P

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Techniques for Reducing the Effects of Sample Solvent on UHPLC Analyses

Introduction

For HPLC, sample solvents that adequately dissolve target compounds are required. Therefore, sample solvents which contain a high concentration of organic solvent are often used for reversed phase chromatography. The problem is that these solvents sometimes cause peak broadening. In this poster, we discuss techniques for reducing the effects of sample solvents. Recent widespread UHPLC analyses are taken as examples.

Reducing injection volume is a simple technique to reduce peak broadening. However, the right autosampler should be selected because some autosamplers do not have good reproducibility when injecting small amounts of samples. This reproducibility is critical, especially for UHPLC, which uses smaller-volume injections than HPLC. To examine the effects of sample solvents, we injected 0.2-2 µL volumes of samples on a UHPLC analysis with an acetonitrile sample solvent and a mobile phase mixture of buffer and acetonitrile. Peak broadening was observed even when injecting only 1 µL of sample. In addition, an increase in sensitivity was not observed when the injection volume was increased from 1 µL to 2 µL due to peak broadening. We also evaluated the reproducibility of peak area on some autosamplers. When injecting small volumes of 1 µL or less, one autosampler could not achieve good reproducibility. On the other hand, the SIL-30AC autosampler, which is the autosampler of the Nexera series from Shimadzu, showed reproducibility of less than 1 % even when injecting only 0.1 µL of sample.

Diluting samples with a weak solvent is also a good chromatographic technique. The advantage is that the overall sensitivity can be improved because injecting a large amount of sample does not cause peak broadening if the sample solvent is weak enough. There is a problem in that diluting processes are tedious and time-consuming, but the SIL-30AC is equipped with pretreatment and over-lapping injection functions. The pretreatment function enables automated dilution of the sample and the over-lapping function makes it possible to start the pretreatment operations for the next analysis while the current analysis is in progress. In the UHPLC analysis above, we diluted the sample ten times with water and were able to inject 50 µL of the diluted sample without peak broadening, which resulted in a five-time increase in sensitivity.

Materials

Reagents and standards

Reagents: Acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, and acetylsalicylic acid were purchased from Sigma-Aldrich. Water was made in house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Acetonitrile and methanol was purchased from Honeywell. A cold medicine was purchased from a local pharmacy.

Samples: 100 mg of the cold medicine was extracted with 10 mL of acetonitrile and then the supernatant was filtered using a 0.22 µm filter and transferred to a 1.5 mL vial for analysis.

10 mg of acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, and octanophenone were dissolved in 10 mL of acetonitrile. It was diluted 10 times with water and transferred to a 1.5 mL vial for analysis.

10 mg of acetylsalicylic acid was dissolved in 100 mL of acetonitrile and then transferred to a 1.5 mL vial for analysis.

System

The samples were separated using a Shimadzu Nexera UHPLC system consisting of two LC-30AD pumps, DGU-20A5R degassing unit, SIL-30AC autosampler, CTO-30A column oven, SPD-M30A photodiode array detector, and a CBM-20A system controller.

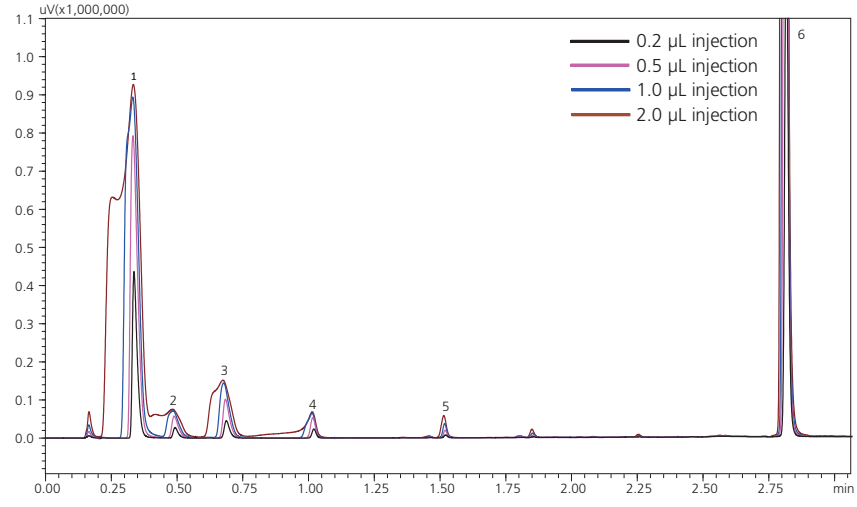
Results

Simultaneous analysis of several compounds with a broad range of polarity

When analyzing several compounds with a broad range of polarity simultaneously, the analytes are usually dissolved in 100 % organic solvent or a solvent which contains a high concentration of organic solvent. In this example, we analyzed a cold medicine. Cold medicines contain several active compounds with a broad range of polarity, so the cold medicine was extracted in 100 % acetonitrile. Table 1 shows the analytical conditions and Fig. 1 shows the chromatograms. Fig. 1 shows that peak broadening occurred when injecting 1.0 µL or more. Under these conditions, peak shape can be maintained with an injection volume of 0.5 µL or less.

Table 1: Analytical Conditions

System	: Nexera UHPLC System
Column	: Shim-pack XR-ODSIII (75 mm L. x 2.0 mm I.D., 1.6 µm)
Mobile Phase	: A: 10 mmol/L Phosphate (potassium) buffer (pH 2.6) containing 100 mmol/L sodium perchlorate B: Acetonitrile
Time Program	: B Conc. 20 % (0 min) - 100 % (3 min) - 20 % (3.01-5 min)
Flow Rate	: 0.6 mL/min
Column Temperature	: 40 °C
Injection Volume	: See Fig. 1
Detection	: SPD-M30A at 210 nm
Cell	: Standard cell



■ Peaks
1. Caffeine, 2. Dihydrocodeine, 3. Methylephedrine, 4. Hesperidin, 5. Chlorpheniramine, 6. Ibuprofen

Figure 1: Chromatograms of cold medicine

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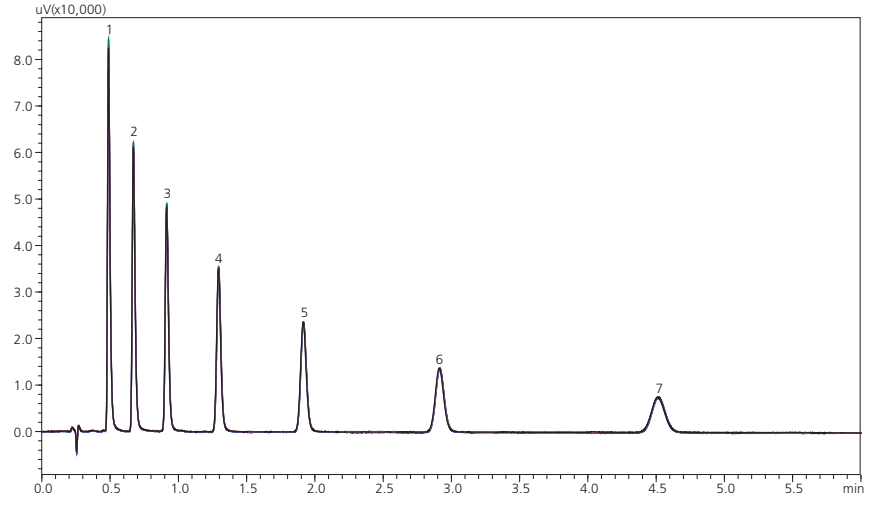
Reducing the injection volume is a good technique to maintain peak shape, but reproducibility at low injection volumes should be evaluated because there is a tendency that lower injection volumes lead to worse reproducibility. In order to check reproducibility at low injection volumes, an alkylphenone mixture was analyzed using the SIL-30AC and two competitor's autosamplers.

Table 2 shows the analytical conditions, Figure 2 shows the chromatograms and Table 3 shows the results. When injecting small volumes of 1 µL or less, one autosampler could not achieve good reproducibility. On the other hand, Shimadzu's SIL-30AC Nexera series autosampler showed reproducibility of less than 1 % even when injecting only 0.1 µL of sample.

Table 2: Analytical Conditions

Column	: ODS column (50 mm L. x 2.1 mm I.D., 1.7 µm)
Mobile Phase	: Water/acetonitrile=40/60 (v/v)
Flow Rate	: 0.4 mL/min
Column Temperature	: 40 °C
Injection Volume	: See Table. 3
Detection	: PDA at 245 nm
Cell	: Standard cell



■ Peaks
1. Acetophenone, 2. Propiophenone, 3. Butyrophenone, 4. Valerophenone, 5. Hexanophenone, 6. Heptanophenone, 7. Octanophenone

Figure 2: Chromatograms of mixture of alkylphenones

Table 3: Area Reproducibility at each injection volume

Injection volume	0.1 µL			0.5 µL			1.0 µL			2.0 µL			4.0 µL		
	SIL-30AC	Competitor's autosampler A	Competitor's autosampler B	SIL-30AC	Competitor's autosampler A	Competitor's autosampler B	SIL-30AC	Competitor's autosampler A	Competitor's autosampler B	SIL-30AC	Competitor's autosampler A	Competitor's autosampler B	SIL-30AC	Competitor's autosampler A	Competitor's autosampler B
Acetophenone	0.786	1.620	2.350	0.091	0.844	0.483	0.101	0.499	0.377	0.029	0.227	0.265	0.064	0.115	0.255
Propiophenone	0.727	1.479	1.619	0.110	1.012	0.533	0.139	0.768	0.149	0.026	0.236	0.209	0.032	0.112	0.257
Butyrophenone	0.605	1.636	2.769	0.163	1.161	0.515	0.096	1.133	0.241	0.050	0.265	0.255	0.028	0.115	0.237
Valerophenone	0.625	1.353	2.306	0.105	1.131	0.473	0.090	1.462	0.240	0.036	0.278	0.214	0.073	0.115	0.257
Hexanophenone	0.762	1.578	2.091	0.089	1.300	0.458	0.124	1.694	0.215	0.077	0.360	0.177	0.069	0.109	0.236
Heptanophenone	0.746	1.403	2.487	0.160	1.423	0.337	0.128	1.956	0.220	0.045	0.326	0.206	0.070	0.130	0.257
Octanophenone	0.614	1.375	3.210	0.198	1.394	0.495	0.109	2.018	0.310	0.046	0.330	0.155	0.074	0.179	0.272

Analysis of a compound which is unstable in water

A compound which is unstable in water should be dissolved in organic solvent as well. For example, Acetylsalicylic acid is gradually hydrolyzed in water, so it is desired to be dissolved in organic solvent.
However, if the sample solvent is strong, such as 100 % organic solvent, enough sensitivity might not be obtained because a large injection volume causes peak distortion and does not contribute to enhance peak height. In this case, diluting the sample with a weak solvent such as water right before injection and injecting a large volume is a good technique because it can minimize hydrolysis and enhance peak height. In order to examine how effective this technique is, acetylsalicylic acid in acetonitrile was injected. Table 4 shows the analytical conditions and Figure 3 shows three chromatograms. The black, pink and blue chromatogram are that of a 1 µL injection of undiluted sample, a 5 µL injection of undiluted sample and a 50 µL injection of ten-time diluted sample, respectively. When injecting the sample directly, increasing injection volume caused peak distortion and did not contribute to enhance peak height. On the other hand, when injecting a ten-time diluted sample, even a 50 µL injection volume did not cause peak distortion and achieved approx. five times higher peak height than that of a direct injection. The SIL-30AC has an automated dilution function, so there was no need to dilute each sample manually. In addition, the SIL-30AC has an over-lapping function which makes it possible to start the pretreatment operation for the next analysis while the current analysis is in progress, so there was no need to take additional time to wait for the pretreatment operation.

Table 4: Analytical Conditions

System	: Nexera UHPLC System
Column	: Kinetex C18 100A (50 mm L. x 2.1 mm I.D., 1.3 µm)
Mobile Phase	: 0.085% Phosphoric acid in water/methanol=65/35 (v/v)
Flow Rate	: 0.3 mL/min
Column Temperature	: 40 °C
Injection Volume	: See Fig. 3
Detection	: SPD-M30A at 230 nm
Cell	: Standard cell

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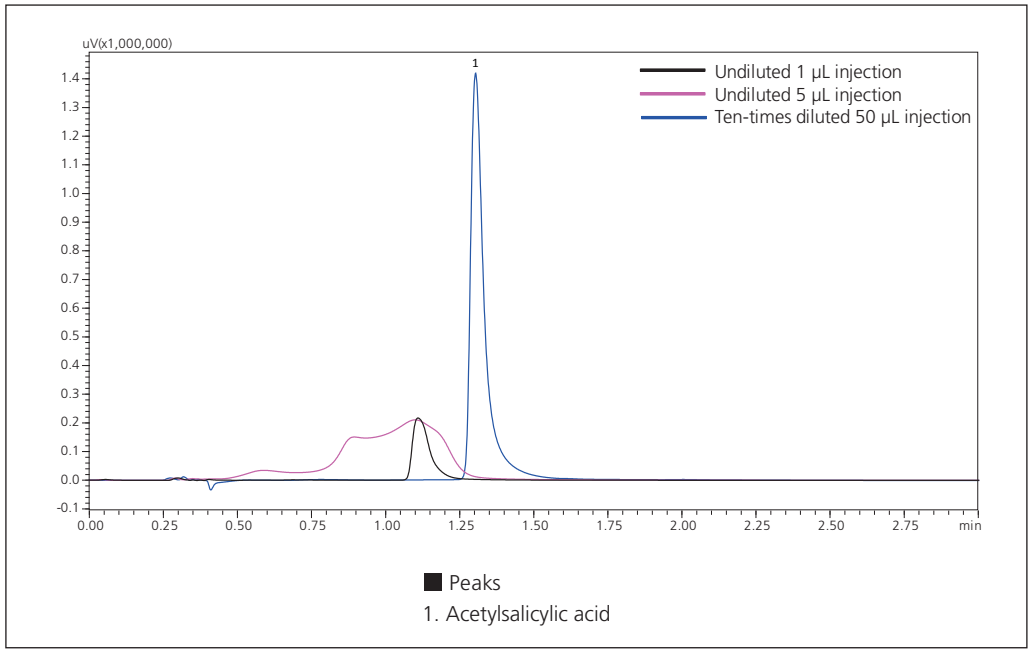

Figure 3: Chromatograms of acetylsalicylic acid

Conclusions

1. Reducing injection volume is a good technique for reducing the effect of sample solvent and the SIL-30AC autosampler enables good reproducibility, even at very low injection volumes.
2. Diluting sample with a weak solvent is also a good technique and the Nexera SIL-30AC autosampler enables automated sample dilution and features an over-lapping pretreatment function.

First Edition: March, 2014

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