SHIMADZU

Application News

High Performance Liquid Chromatography SSI LC 001

A Practical Approach to Conserving Acetonitrile

Introduction

Faced with dwindling acetonitrile supplies that seem likely to remain throughout 2009, labs are looking for ways to reduce their consumption of this common HPLC solvent.

Shimadzu offers reasonable and practical analytical tools and solutions to the global acetonitrile shortage. The use of small particle columns with smaller column dimensions has become an increasingly popular way to reduce analysis times, and also provides the additional benefit of decreasing mobile phase consumption. A Shimadzu ultra-fast LC column, the Shim-pack XR-ODS, has an optimized particle size (2.2µm) and provides much lower pressure compared to commercial sub-2µm particle columns. Therefore, the XR-ODS is convenient to use with a traditional HPLC. The column helps reduce acetonitrile consumption without the need for specialized high pressure hardware.

The Shimadzu UFLC system was designed to achieve ultra-fast LC and maximize the potential of the Shim-pack XR-ODS column by utilizing conventional Prominence HPLC hardware. The Prominence UFLC system can provide high-speed analysis to minimize acetonitrile consumption, while retaining the flexibility to perform conventional analysis.

The Shimadzu Prominence UFLC*XR* system allows operation under pressures up to 66 MPa. This system allows the use of extended length small particle columns to provide the highest resolution possible for your analysis. Another benefit of the higher pressure tolerance is allowing the substitution of methanol for acetonitrile with the use of small particle columns. Developing new methods with methanol in place of acetonitrile will assist with the current lab shortage of acetonitrile.

The Shimadzu Prominence UFLC and UFLC*XR* autosamplers also provide the benefit of reduced solvent consumption. The autosamplers have been engineered for low carryover, so additional valve rinsing after injection can typically be eliminated or greatly reduced. Other autosampler designs require extensive rinsing with two solvents for adequate cleaning and use a large amount of solvent during this process. The injection cycle time of the Prominence autosampler is also a mere 10 seconds, reducing your mobile phase consumption even further.

High-speed and High-separation Column: Shim-pack XR-ODS

Migration to the Shim-pack XR-ODS column is the most reasonable solution to the acetonitrile shortage issue. The Shim-pack XR-ODS enables users to shorten analysis time and reduce the consumption of

acetonitrile drastically without extremely high pressure through the use of an optimized particle size (2.2µm). As shown in Figure 1, the column pressure is maintained below 30MPa in most fast LC conditions. This fact means that the column is compatible with conventional HPLC systems and provides the ability to reduce acetonitirle consumption without the need for specialized high-pressure hardware. In addition, the 2.2µm particle technology allows high-speed analysis without losing the separation efficiency. For the separation performance of the Shim-pack XR-ODS, please refer to Figures 2 and 3. The optimized backpressure and separation efficiency bring benefits to you in reduced acetonitrile consumption.

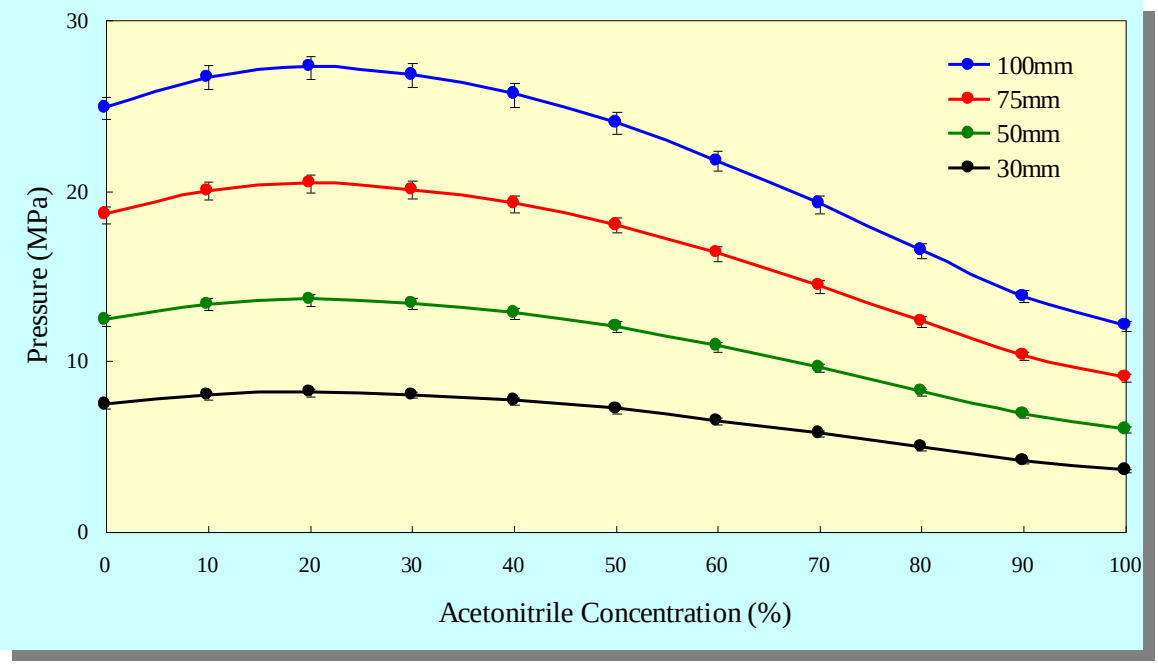

Figure 1*:* Relation of column pressure and acetonitirle concentration in mobile phase
Mobile phase: water/acetonitrile, flow rate: 0.9mL/min, temperature: 40°C.

Reduction of Acetonitrile Consumption by Ultra-fast LC

Figure 2 shows the separations of alkylphenones performed using two Prominence systems: conventional Prominence HPLC with a Shim-pack VP-ODS column (5µm particles) and Prominence UFLC with a Shim-pack XR-ODS column (2.2µm particles). By using 2.2µm particles and Prominence UFLC, the analysis time was five times faster than that of the 5µm particles. In this case, a 3mm I.D. column was used for reducing acetonitrile consumption. The analysis by conventional HPLC with a 5µm column required 10.5 mL of acetonitrile for the analysis, while the Prominence UFLC system with a Shim-pack XR-ODS column required only 2.3 mL of acetonitrile. Due to the excellent separation efficiency and use of the 3.0mm I.D. column, the acetonitrile consumption could be reduced down to nearly 1/5 without sacrificing separation efficiency.

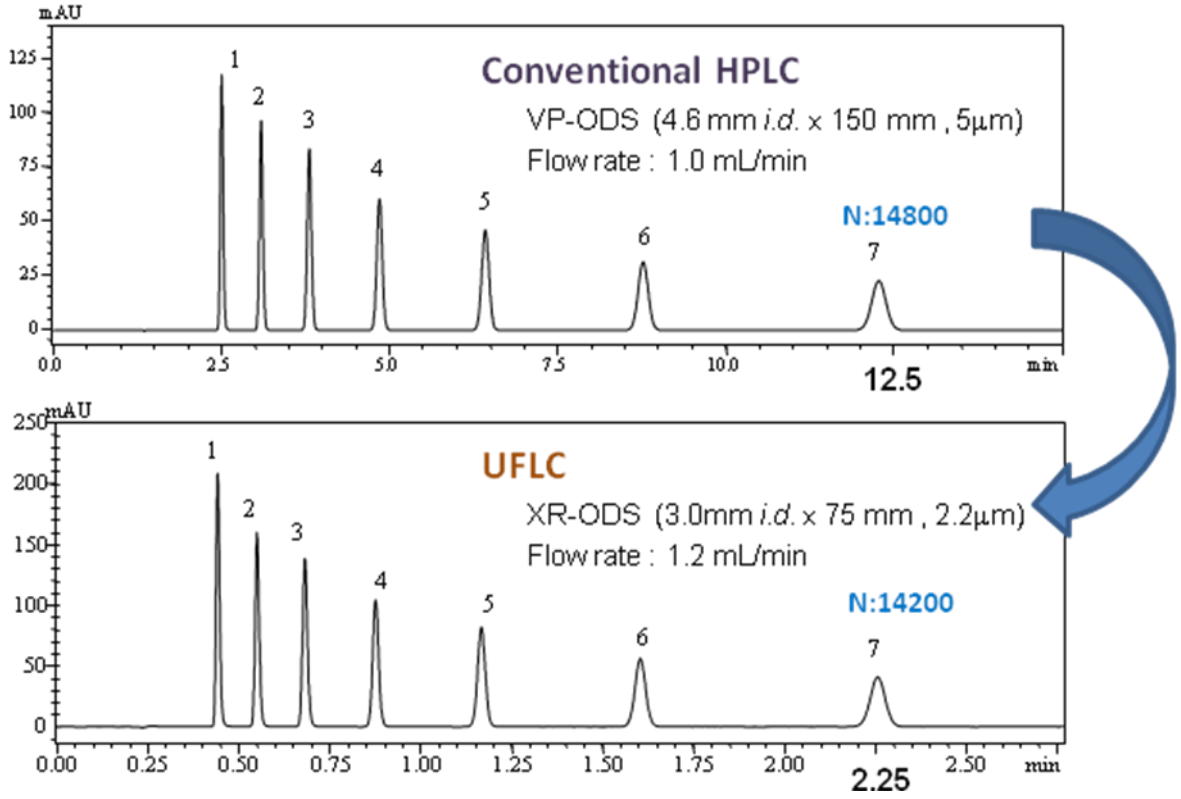

Figure 2: Alkylphenones separation by conventional Prominence and Prominence UFLC
Mobile phase: water/acetonitrile (3/7 v/v), temperature: 40°C, detection: absorbance at 245 nm,
Peaks: 1: actophenone, 2: propiophenone, 3: butyrophenone, 4: valenophenone, 5: hexanophenone, 6: heptanophenone, 7: octanophenone

Figure 3 shows an example of the analysis of a standard mixture of 12 cephem antibiotics performed by conventional Prominence HPLC with a Shim-pack VP-ODS column (5µm particles) and Prominence UFLC with a Shim-pack XR-ODS column (2.2µm particles). Table 1 shows the analytical conditions. In the case of the conventional HPLC, the total cycle time of 1 analysis, including column wash, was 45 minutes and the acetonitrile consumption was 12.8mL, while the total cycle time and acetonitrile consumption for the Prominence UFLC were 6.5 minutes and 1.7mL, respectively. In this case, acetonitrile consumption could be reduced down to 1/7 by using Prominence UFLC with a 2.2µm particle column. If you scale the column size down to 2.0mm I.D, acetonitrile consumption will be further reduced.

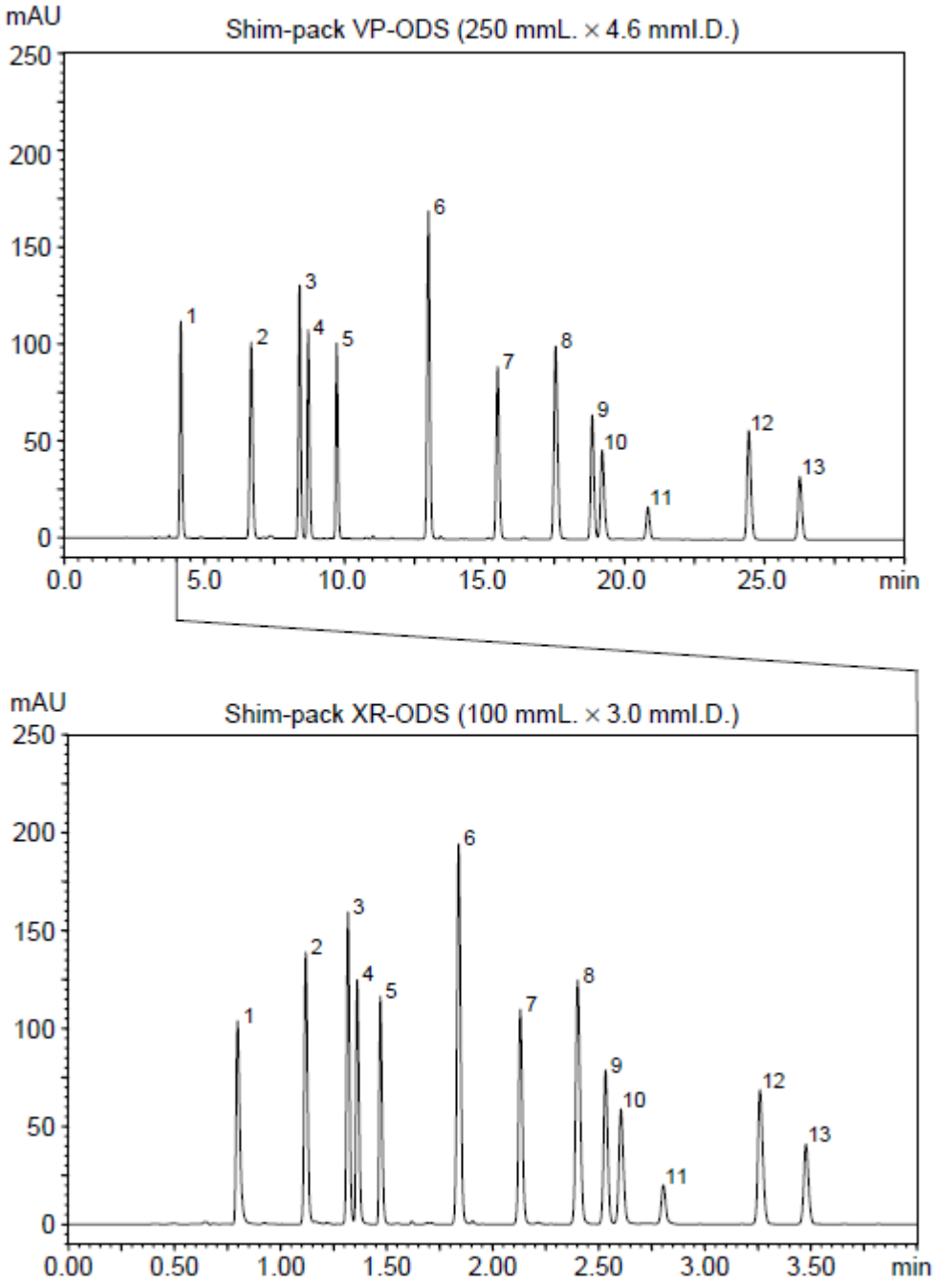

Figure 3: HPLC chromatogram of a standard mixture of 12 cephem antibiotics by conventional Prominence with Shim-pack VP-ODS (upper) and Prominence UFLC with Shim-pack XR-ODS (lower)
Peaks: 1: cefadroxil, 2: cephaprin, 3: cefaclor, 4: cefalexin, 5: cephradine, 6: cefotaxime. 7: cefazolin, 8: cefuroxime, 9: cefoperazone, 10: cefloxitin, 11: cefamandole A, 12: cephalothin, 13: cefamandole B (50mg/each).

Table 1 Analytical Conditions

Column	: Shim-pack XR-ODS (100 mm L. × 3.0 mm I.D., 2.2 µm)
	Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile Phase	: A ; 0.1 % Formic Acid-Water
	B ; Acetonitrile
Time Program	: [XR-ODS]
	B.Conc. 15 % (0 min)→55 % (3.5 min)→15 % (3.51 - 6.5 min)
	[VP-ODS]
	B.Conc. 15 % (0 min)→55 % (30 min)→15 % (30.01 - 45 min)
Flow Rate	: 1.0 mL/min (XR-ODS)
	1.0 mL/min (VP-ODS)
Column Temp.	: 40 °C
Injection Vol.	: 4 µL (XR-ODS)
	10 µL (VP-ODS)
Detection	: SPD-M20A at 260 nm
Flow Cell	: Semi-micro Cell (XR-ODS)
	Conventional Cell (VP-ODS)

Method Transfer to Ultra-fast LC

When transferring methods from conventional HPLC to Prominence UFLC/UFLC*XR*, we need to consider modifications of some method parameters to duplicate the chromatographic pattern, without losing peak resolution. Our method transfer program (Figure 4) can automatically calculate optimal parameter settings for flow rate, column size, gradient time programming, etc. by entering the current method conditions. The program will assist with method development for solvent savings.

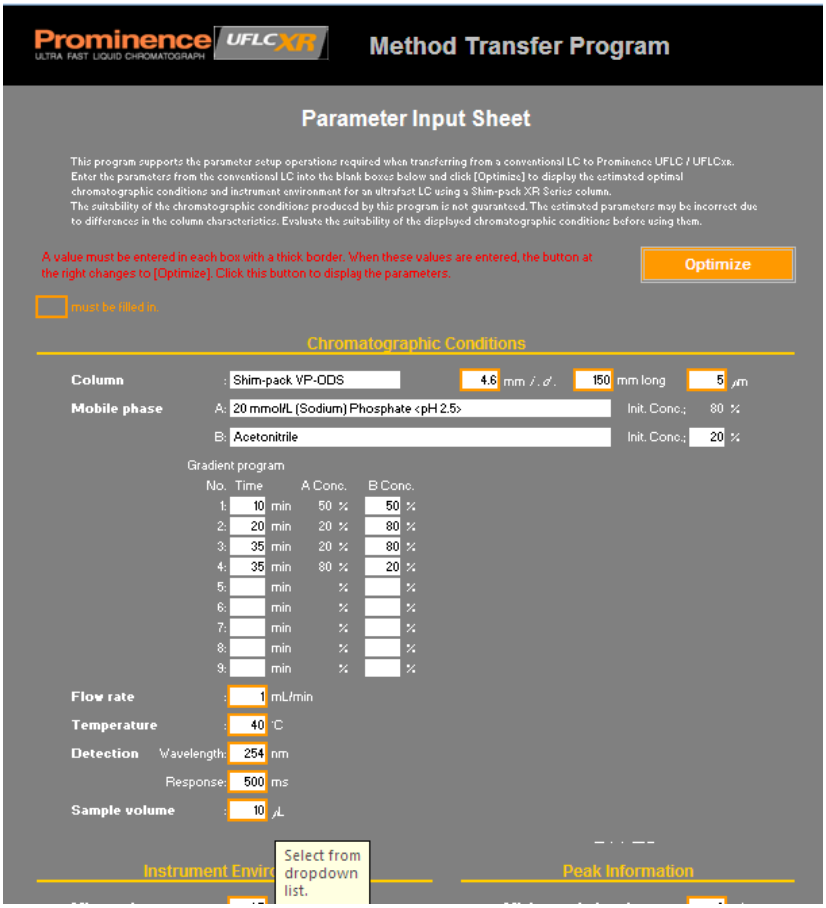

Figure 4: Prominence UFLC/UFLC*XR* method transfer program

Analysis by Methanol Mobile Phase

Prominence UFLC*XR* enables not only the ultra-fast analysis of Prominence UFLC but also the usage of high-pressure conditions (maximum: 66MPa, 9570psi), which enables the use of a longer column with sub-3µm particles to obtain higher separation efficiency. Prominence UFLC*XR* also allows ultra-fast analysis by a methanol mobile phase, which provides higher backpressure compared to acetonitrile. Figure 5 shows a chromatogram of food additives by the Prominence UFLC*XR* system. Methanol was used in place of acetonitrile as the strong solvent in the mobile phase. The UFLC*XR* system is applicable for a wider range of applications due to the increased pressure tolerance.

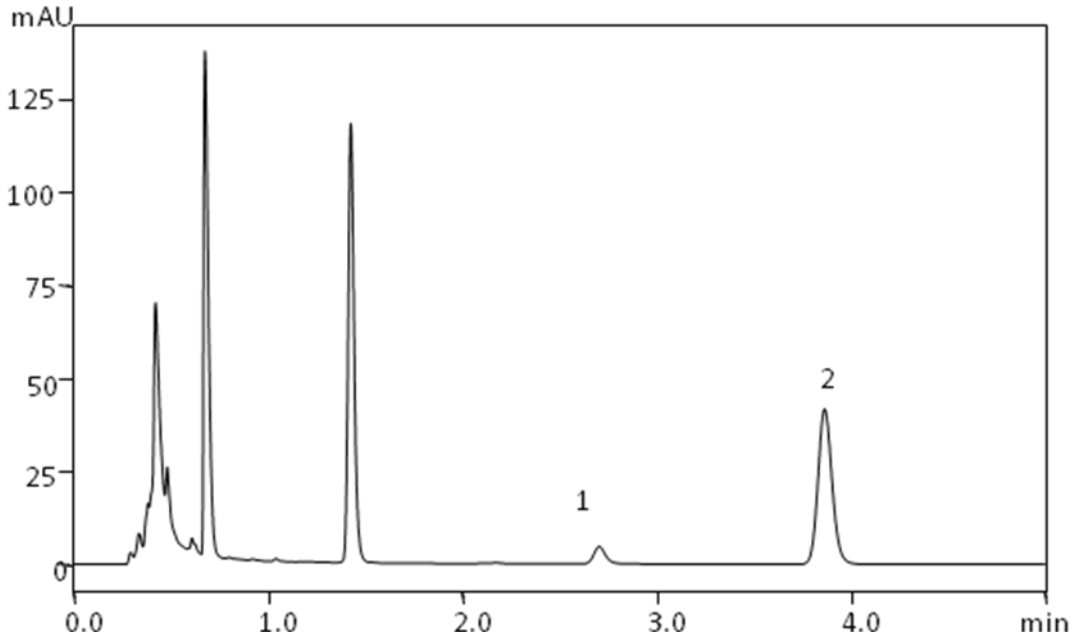

Figure 5: Analysis of food additives in soft drink by Prominence UFLC*XR*
Column: Shim-pack XR-ODS II (75mm×3mm I.D., 2.2 µm particles), mobile phase: 40mmol/L (sodium) acetate buffer pH4.0 / methanol =4/1 (v/v), flow rate: 1mL/min, column temperature: 40°C, detection: 250nm
Peaks: 1: aspartame, 2: benzoic acid

Conclusion

Prominence UFLC/UFLC*XR* was designed to achieve ultra-fast LC by utilizing the conventional Prominence hardware series; consequently, these systems can be used for a broad range of applications, including ultra-fast LC and conventional LC. A specialized instrument that can operate under ultra-high pressure is not required. In addition, the Shim-pack XR-ODS column enables you to shorten analytical time and reduce acetonitrile consumption easily.

Therefore, Prominence UFLC/UFLC*XR* offers a smooth transfer from conventional methods to fast LC methods, reducing mobile phase consumption in a realistic and reasonable way.