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Improve HPLC Methods by Increasing Sample Throughput Using the ACQUITY Arc System

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Abstract

This Application note demonstrates the capabilities of the ACQUITY Arc System to reduce the run time of an established HPLC analysis.

In this work, an existing HPLC method with a twelve minute analysis time was migrated to a UHPLC method with a reduced analysis time of four minutes. The ACQUITY Arc System provides the capability of maximizing analytical productivity by providing the capability of enhancing HPLC methods into quick and efficient UHPLC methods.

Benefits

- Reduces the run time of an HPLC method from twelve minutes to a UHPLC method with a run time of four minutes
- By using the solid-core CORTECS 2.7 µm particle size column, the method operates at higher linear velocity and flow rate without efficiency or resolution compromise
- Reduce solvent consumption by reducing the volume of the column

Introduction

Laboratories that use routine and established HPLC methods can require or desire enhancements for many different reasons. Established HPLC methods generally either do not need to be altered or only need minor improvements since they have been validated for use in a regulated environment. The most common reasons for altering an existing method include reducing run time, reducing solvent consumption, reducing waste generation, and the cost per sample. Such methods can still benefit from incorporating modern instruments and separation chemistries to improve laboratory efficiency without compromising analytical quality. Some of the aspects of the method that should not be altered include sensitivity, linearity, accuracy, and precision.

The goal of this work is to demonstrate the capabilities of the ACQUITY Arc System to reduce the run time of an established HPLC analysis. In this work, an existing HPLC method with a twelve minute analysis time was migrated to a UHPLC method with a reduced analysis time of four minutes. The capabilities of the ACQUITY Arc System can be used to improve the resolution when pairing the system’ s lower dispersion volume with an appropriately selected volume of the separation column.[1,2] In addition, the upper pressure limit of 9500 PSI enables users to accommodate columns with particles as low as 2 µm. In this example, separation integrity is preserved at the higher flow rate by using a solid-core CORTECS Column chemistry.[3] The combination of column chemistry, solid-core technology, and instrumentation are effective tools to improve the analytical efficiency of an HPLC method.

Figure 1. The ACQUITY Arc System with PDA. The system allows a user to replicate current HPLC methods as well

improve methods by scaling to smaller 2.x µm particle sizes.

Experimental

The sample mixture was separated using conventional HPLC conditions on a fully porous XBridge BEH C_{18} 5 µm, 4.6 mm x 50 mm column. To take advantage of the higher efficiency of solid-core particles,[4] the column was changed to a CORTECS C_{18} 2.7 µm, 3 mm x 50 mm column for UHPLC analysis. The dimensions of the CORTECS Column were chosen since it maintains the same resolution at a higher linear velocity, as well as reducing the solvent consumption. When changing the column, the Columns Calculator (Figure 2) can be used to determine the improved analysis gradient table and injection volumes. In this example, increasing sample throughput is the main goal, thus the flow rate of 1.5 mL/min was selected.

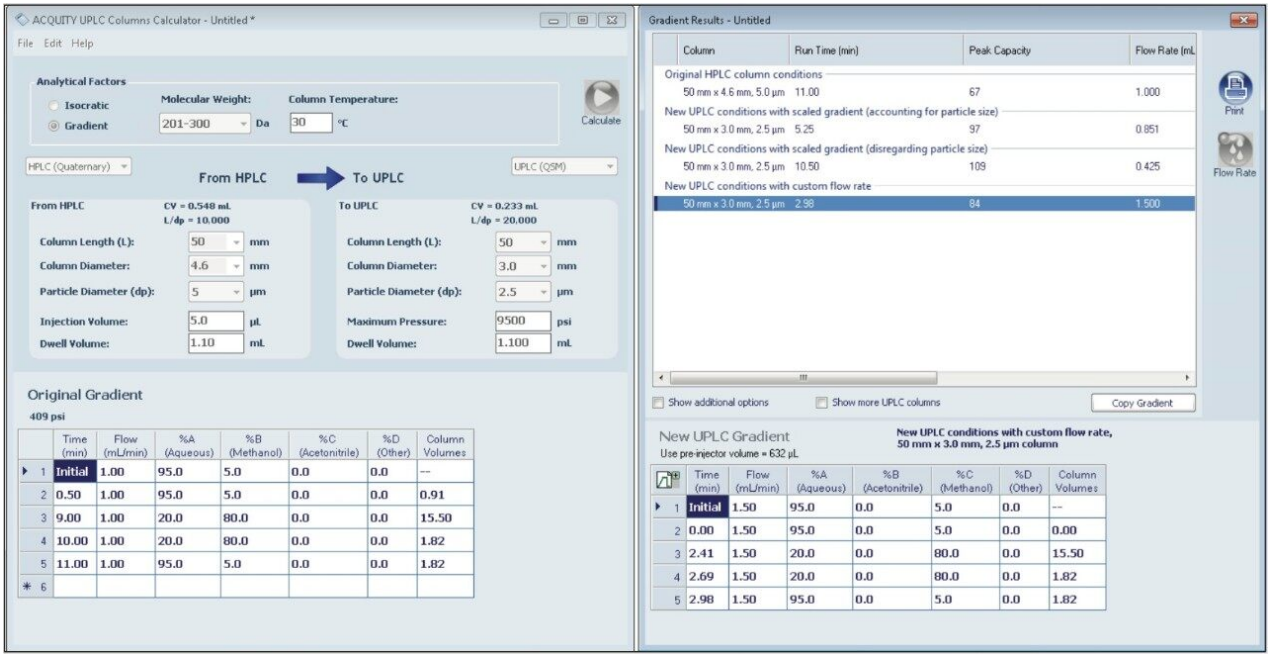

Figure 2. Columns Calculator. In order to calculate the gradient for the modified method the following information is entered for the current method and the new method (left): Column Length, Column Diameter, Particle Diameter, Injection Volume, and the system Dwell Volume and Maximum Pressure for the new method. Once the original gradient is entered the new parameters for the new column are calculated. Since the superficially porous materialcan provides optimal results at a higher linear velocity than the porous material, a value of 1.5 mL/min was chosen rather than the scaled 0.85 mL/min flow rate in order to accelerate the analysis.

Sample description and preparation

The analysis of a five compound sample (Waters UPC^2 Gradient Standard P/N:186006551) is performed on two columns on the ACQUITY Arc System. In a vial, combine 100 µL of the UPC^2 Gradient Standard with 900 µL of the 50/50 Water/Methanol solution.

HPLC/UHPLC conditions

LC system: ACQUITY Arc System with 30-cm Column Heater

2998 Photodiode Array Detector with 10 mm analytical flow cell:

Wavelength: 254 nm

Sampling rate: 20 Hz

Filter time constant: Normal

Data management: Empower 3 FR2 SR1

	HP LC method	UHPLC method
Column	XBridge BEH C_{18} 5 µm, 4.6 mm x 50 mm	CORTECS C_{18} 2.7 µm, 3 mm x 50 mm
Column temperature	30 °C	30°C
Sample temperature	10 °C	10 °C
Injection volume	5.0 µL	2.1 µL
Flow rate	1.0 mL/min	1.5 mL/min
Flow path	Path 1	Path 1
Mobile phase	Solvent A: 0.1% Formic acid in MilliQ water Solvent B: 0.1% formic acid in Methanol	Solvent A: 0.1% Formic acid in MilliQ water Solvent B: 0.1% formic acid in Methanol
Gradient	5 to 80% B over 9 min	5 to 80% B over 2.5 min
Solvent consumed per injection	12 mL	6 mL

Results and Discussion

The ACQUITY Arc System can be utilized for both HPLC and UHPLC analysis. A typical HPLC method was used on the ACQUITY Arc System as shown in Figure 3. The method was then modified to decrease run time with the column chemistry and UHPLC instrumentation. The chemistry component was modified by replacing the XBridge C_{18} Column with a solid-core particle column.[5] The smaller particle size provides the same resolution at a higher linear velocity while simultaneously using less solvent. Scaling down the column diameter from a 4.6mm to a 3.0mm diameter results in lower solvent consumption as well. The solid-core particle provides higher efficiency under these operating conditions; therefore, the resolution is similar to the resolution that is observed with a smaller particle fully-porous column. Solid-core columns maintain high efficiency at a higher linear velocity so the net effect is to observe better resolution in less time.

Compound	HPLC method USP resolution	UHPLC method USP resolution
Thymine	–	–
Caffeine	36.6	32.7
Coumarin	15.0	13.1
Prednisone	14.1	14.4
Flavone	16.6	16.2

Table 1. Resolution values for each peak for the HPLC and UHPLC methods.

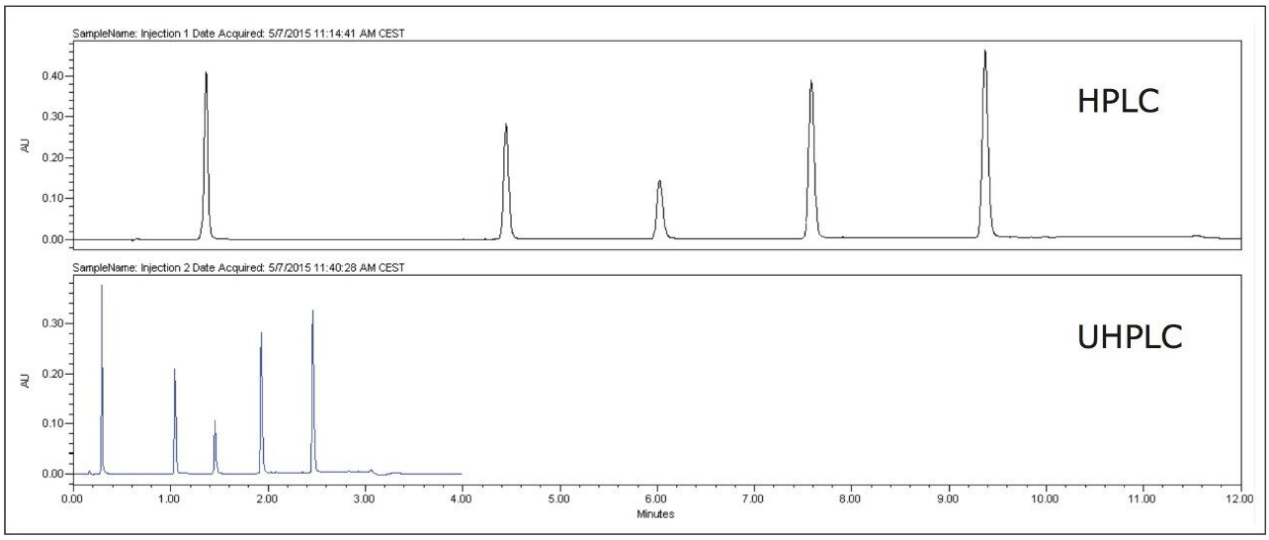

Figure 3. Comparison of the HPLC method and UHPLC method results on the ACQUITY Arc System. Elution order: thymine, caffeine, coumarin, prednisone, and flavone.

In combination with the column chemistry described, the ACQUITY Arc System features facilitate improvement of the HPLC method. The ACQUITY Arc System has lower dispersion passive solvent pre-heating as well as a lower dispersion analytical flow cell (2998 PDA) in comparison to a conventional HPLC system. An LC system with lower dispersion increases the sensitivity for the analytes with corresponding improved resolution when all factors are held constant. The ACQUITY Arc has a flow rate maximum of 5 mL/min along with a pressure limit of 9500 psi allowing for a broader range of methods to be supported. The original HPLC method had a maximum pressure of ~1900psi and the improved method had a maximum pressure of ~7500 psi which is well within the system limitations of 9500 psi.

By completing the method improvement from the original HPLC method we are able to obtain the same quality results in a much shorter time period. The HPLC method was shortened from twelve minutes down to four minutes for each injection. Even though the flow rate was increased, the amount of solvent consumed was cut in half for the UHPLC method using a total of 6 mL per injection versus a total of 12 mL per injection for the HPLC method. The resolution between each of the peaks is similar between the two methods. The injection volume was scaled to the column volume. Similar peak heights and areas are observed with the modernized method (Figure 3 and Figure 4).

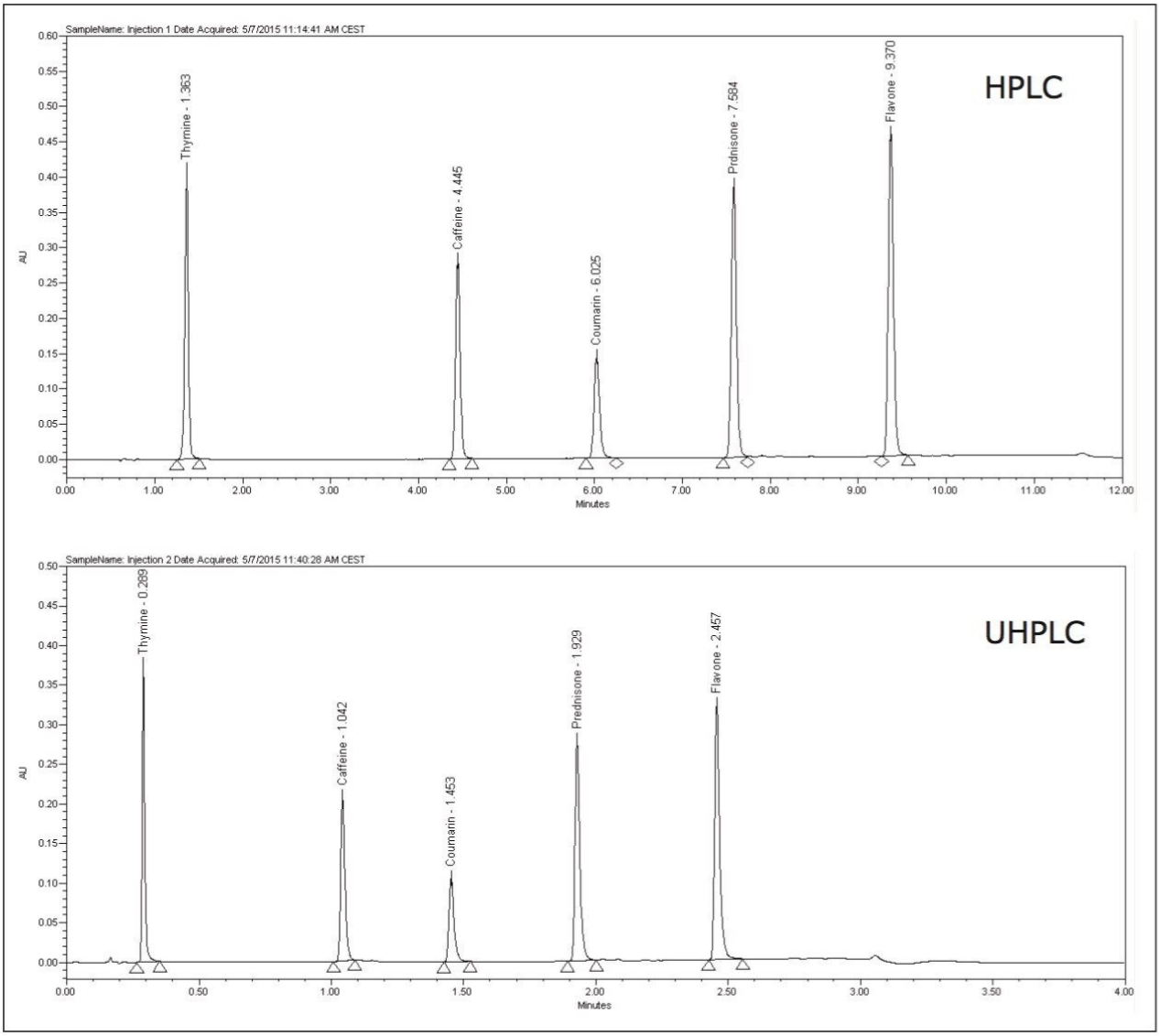

Figure 4. Comparison of the HPLC and UHPLC method results on the ACQUITY Arc System with independent x-axis values. The comparison demonstrates how the chromatographic profile remains similar.

Conclusion

The analysis of a five compound sample is performed under two sets of conditions to demonstrate how the combination of the ACQUITY Arc System and solid-core column technology can convert an HPLC method to a UHPLC separation. It is often desirable to reduce the run time of a method while maintaining analytical resolution. This brings additional analytical efficiency to the laboratory. Changing from a 4.6 mm to a 3 mm

diameter column reduces the solvent consumption by half. Scaling the method to CORTECS solid-core packing material increases the throughput by a factor of three from the original method. The ACQUITY Arc System provides the capability of maximizing analytical productivity by providing the capability of enhancing HPLC methods into quick and efficient UHPLC methods.

References

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