

Nota applicativa

## ACQUITY UPLC M-Class System: Small Molecule Chromatographic Performance with 1.0-mm Diameter Microscale Columns

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This application brief demonstrates chromatographic performance of the ACQUITY UPLC M-Class System for small molecule separations on 1.0-mm diameter columns packed with sub-2- $\mu\text{m}$  particles.

### Benefits

The flexible ACQUITY UPLC M-Class System is optimized for use with 0.075- to 1.0-mm I.D. columns using optical or mass spectral detection.

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## Introduction

Greater sensitivity, in the form of increased detector response or signal-to-noise, is a goal of many laboratories. Migrating an analytical-scale separation performed on 2.1-mm columns to a 1.0-mm diameter microscale column is one tactic to increase this response. These lower-volume columns, packed with sub-2- $\mu\text{m}$  particles, can deliver smaller-volume peaks with increased analyte response. Making effective use of these columns, however, requires a system that is optimized for the task. An optimized system for microscale analyses must not only address the solvent delivery, sample handling, and dispersion management challenges encountered in microscale analyses, but must reproduce the chromatographic performance of the original analytical scale separation. Robust, repeatable microscale chromatographic performance is then a fundamental requirement for successful methods migration. High-quality, repeatable chromatographic performance at the microscale can only be produced by a holistically-designed, nano- to microscale system such as the ACQUITY UPLC M-Class System.

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## Results and Discussion

A standard mixture of small molecules was analyzed using gradient elution with an ACQUITY UPLC M-Class System configured for direct injection with 1.0-mm columns. The ACQUITY UPLC M-Class System consisted of a  $\mu\text{Binary}$  Solvent Manager ( $\mu\text{BSM}$ ), a  $\mu\text{Sample}$  Manager ( $\mu\text{SM}$ ), an ACQUITY UPLC TUV Detector with 100 nL/10 mm path length microscale flow cell, and a Waters Xevo TQ-S configured with a low-flow ESI

probe with a 50- $\mu\text{m}$  internal diameter (I.D.) capillary. A Trap Valve Manager (TVM) provided column temperature control. The column used was an HSS T3 1.0 x 150 mm column, packed with 1.8- $\mu\text{m}$  particles.

A series of five 1- $\mu\text{L}$  standard injections was analyzed using UV detection. Water with 0.1% formic acid was the A solvent, and acetonitrile with 0.1% formic acid was the B solvent. The gradient was 5 to 95% acetonitrile over 25 minutes. The standard diluent was 30% acetonitrile in water. The repeatability of the UV analysis is shown in Figure 1. Retention times and peak widths, within the 25 minute gradient, were all highly repeatable indicating excellent solvent delivery at the 90- $\mu\text{L}/\text{minute}$  flow rate. Average peak widths for each component and area %RSD values are given in Table 1 for the UV analysis. Peak widths below 0.1 minutes (6 sec) and area %RSD values well below 1% demonstrate that the ACQUITY UPLC M-Class System produces high-quality results with UV detection.

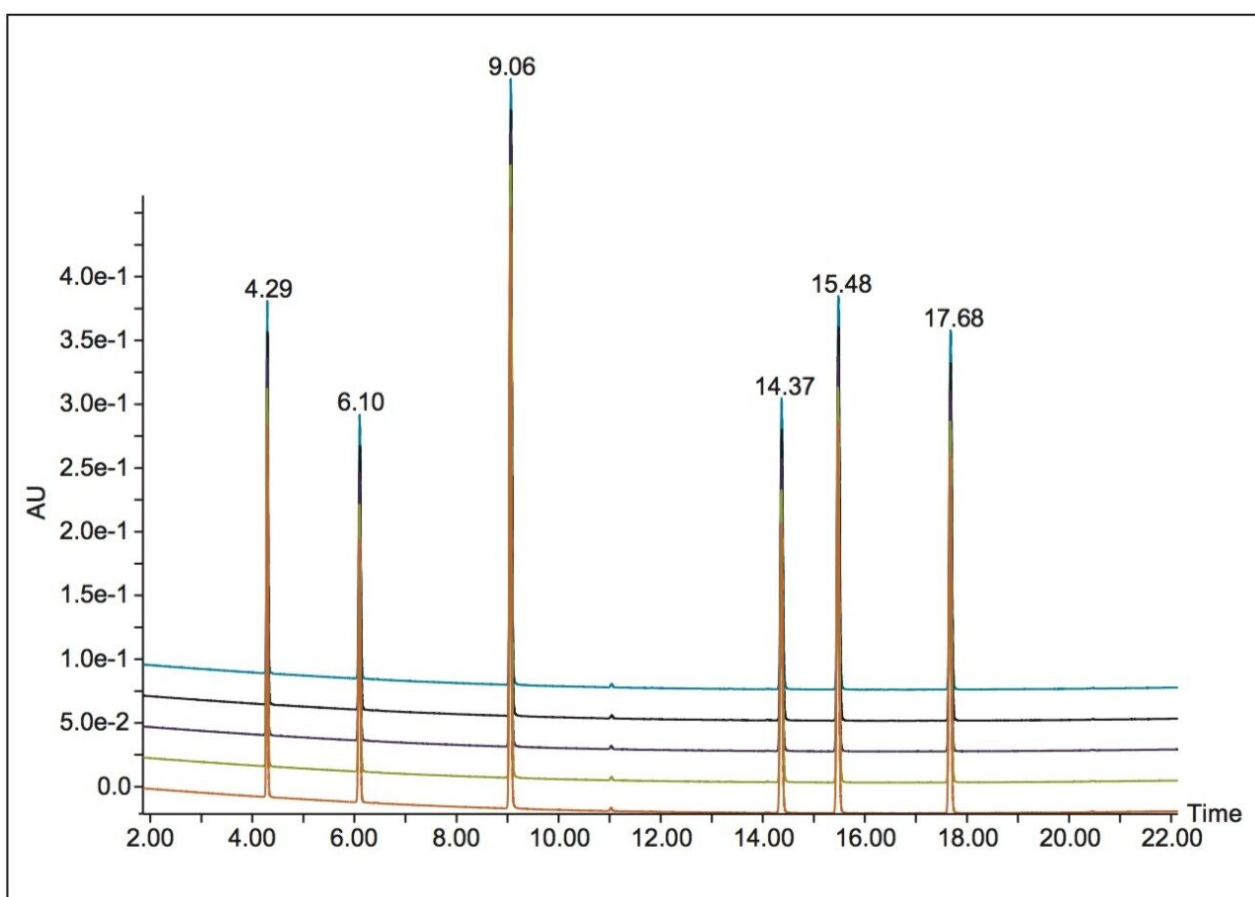


Figure 1. UV repeatability. Overlay of five consecutive 1- $\mu\text{L}$  injections on a Waters HSS T3 1.0 x 150 mm, 1.8  $\mu\text{m}$  Column. Gradient separation of 5 to 95% acetonitrile over 25 minutes. The sample diluent was 30% acetonitrile. Optimized low volume flow cell.

| Retention time (min) | Compound         | Avg peak width (min) | %RSD area |
|----------------------|------------------|----------------------|-----------|
| 4.29                 | Caffeine         | 0.05                 | 0.80      |
| 6.10                 | Acetanalide      | 0.07                 | 0.79      |
| 9.06                 | Sulfadimethoxine | 0.07                 | 0.80      |
| 14.36                | Flavone          | 0.07                 | 0.33      |
| 15.48                | Nabumetone       | 0.08                 | 0.34      |
| 17.67                | Danazol          | 0.08                 | 0.46      |

Table 1. Summarized UV repeatability results from Figure 1.

A second series of five 0.1- $\mu$ L standard injections was analyzed using MS detection. Water with 0.1% formic acid was the A solvent, and acetonitrile with 0.1% formic acid was the B solvent. The gradient was 5 to 95% acetonitrile over 22 minutes. The standard diluent was 30% acetonitrile in water. For the MS analysis, 17OH progesterone was used instead of flavone, and the absolute amounts of the analytes were modified to account for ionization differences. As in the UV analysis, the overlay of MRM TIC chromatograms show highly repeatable retention times within the, in this case, 22-minute gradient (Figure 2). The tabulated results for average peak width, retention time, and area %RSD are in Table 2. With peak widths at or below 0.1 minutes (6 sec), retention time %RSD below 0.10%, and area %RSD values well below 6%, for a 100-nL injection volume, the ACQUITY UPLC M-Class System can also produce high-quality MS results. These results show that the ACQUITY UPLC M-Class System can produce very high-quality, repeatable chromatography for small molecule analysis at the microscale, using 1.0-mm diameter columns.

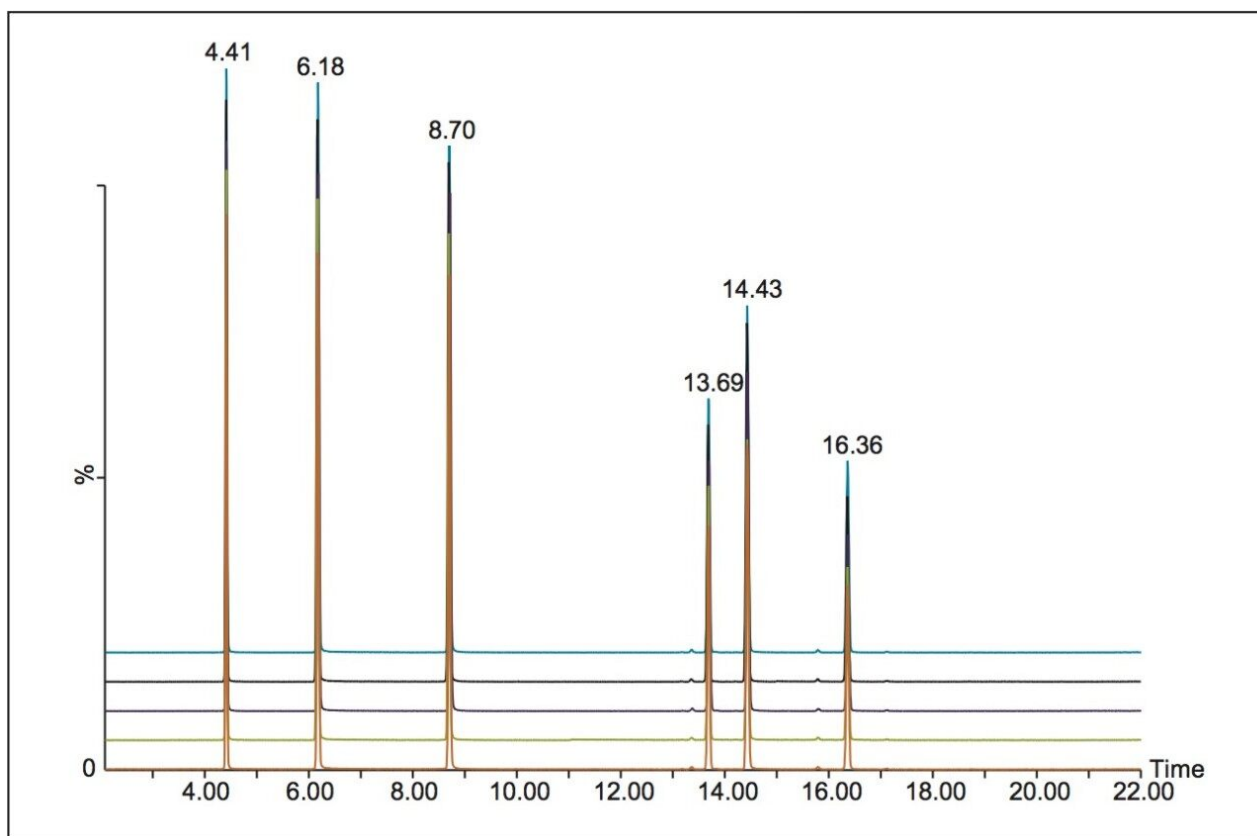


Figure 2. MS repeatability. Overlay of five consecutive 0.1-  $\mu$ L injections on a Waters HSS T3 1.0 x 150 mm, 1.8  $\mu$ m Column. Gradient separation of 5 to 95% acetonitrile over 22 minutes. The sample diluent was 30% acetonitrile. Optimized low flow ESI probe.

| Retention time (min) | Compound          | Avg peak width (min) | %RSD area |
|----------------------|-------------------|----------------------|-----------|
| 4.41                 | Caffeine          | 0.06                 | 2.85      |
| 6.18                 | Acetanalide       | 0.08                 | 3.43      |
| 8.71                 | Sulfadimethoxine  | 0.09                 | 3.11      |
| 13.70                | 17OH-progesterone | 0.01                 | 2.21      |
| 14.44                | Nabumetone        | 0.11                 | 6.18      |
| 16.37                | Danazol           | 0.11                 | 3.42      |

Table 2. Summarized MS repeatability results from Figure 2. N=5.

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## Conclusion

To satisfy the desire of many laboratories to enhance the sensitivity of their analyses, the ACQUITY UPLC M-Class System was designed as a flexible system for nano- to-microscale separations. Highly repeatable, accurate solvent delivery and sample introduction, as well as appropriately scaled detection, enable the ACQUITY UPLC M-Class System to deliver repeatable and reliable results from 1.0-mm diameter microscale separations.

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## Featured Products

[ACQUITY UPLC M-Class System <https://www.waters.com/134776759>](https://www.waters.com/134776759)

[ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>](https://www.waters.com/514228)

[Xevo TQ-S <https://www.waters.com/10160596>](https://www.waters.com/10160596)

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