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應用手冊

Comparison of 1- and 2-Millimeter ACQUITY UPLC Columns for LC-MS

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Abstract

This application note demonstrates that the ACQUITY UPLC BEH 1 mm and 2.1 mm ID Columns both generate high quality chromatographic separations.

Introduction

The emerging area of metabonomics relies upon the detection and characterization of biomarkers. In this application, assay sensitivity and chromatographic resolution are critical as these biomarkers are often present at very low levels and/or the sample volumes are often limited (e.g., small rodent studies). The application of microbore columns (1 mm internal diameter) offers a significant sensitivity advantage over tradition analytical columns (4.6 mm, 3.9 mm, and 2.1 mm internal diameter) as the peaks eluting from the column are contained within a smaller volume, assuming the same chromatographic performance, and are thus more concentrated for a given sample load. However, the implementation of microbore chromatography has not been well-received in practice, due to the complexities of controlling peak dispersioncause by extra-column volumes and instrument delay volumes. Here we show the Waters ACQUITY UPLC System design facilitates the use of 1-and 2 mm columns without performance compromises.

Experimental

Urine samples were collected from male animal following the oraladministration of either Pravastatin at 10 mg/kg or vehicle alone, over the time period from 8 to 24 hours post dose, and stored frozen prior to analysis. The urine samples were centrifuged at 13,000 rpm for 5 minutes at 5 °C, and a 25 µL aliquot of urine from each sample was diluted with 100 µL of distilled water and transferred to an autosampler vial for analysis.

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 100 mm or 1.0 x 100 mm, 1.7 µm
Mobile phase A:	0.1% aqueous formic acid

Mobile phase B:	Acetonitrile with 0.1% formic acid
Flow rate:	0.5 mL/min. (for 2.1 mm), 0.136 mL/min. (for 1.0 mm)
Gradient:	0–95% B over 20 min.
Injection volume:	10 µL (for 2.1 mm), 2.5 µL (for 1.0 mm)
Sample temperature:	10 °C
Column temperature:	40 °C

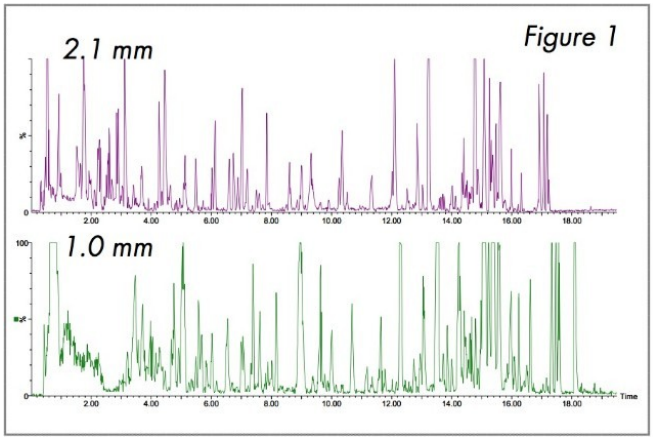
MS Conditions

MS system:	Waters Micromass Q-Tof micro MS
Ionization mode:	ESI+
Source gas:	300 L/hr at 250 °C
Acquisition mass range:	100–800 *m/z*
Cone voltage:	35 V
Collision energy:	10 eV
Dwell:	0.1 sec.
Collision gas	Argon

Results and Discussion

The injection volumes were scaled such that the same injection mass load was delivered to each column and the mobile phase flow rate was scaled so that each column was operated with the same linear velocity. The data displayed in Figure 1 shows that the chromatography produced by the 1 mm ACQUITY UPLC BEH Column (bottom) is of similar quality to that generated by the 2.1 mm column (top), yet the 1 mm column has a 10% increase in peak width. This is attributable to post-column dispersion in the MS source and connecting tubing.

The signal-to-noise ratio of a typical peak in the urine sample (*m/z* =355) foreach column is given in Figure 2. Here, the 2.1 mm column (top) produces a peak with a S/N value of 70:1 while the 1 mm column (bottom) produces a peak with a S/N value of 117:1. The expectation is that these values would be similar, as the concentration of peaks eluting from each column should be identical. However, the extra sensitivity observed from the 1 mm column is likely due to the reduction in solvent volumeentering the MS interface, thus significantly enhancing ionization efficiency.



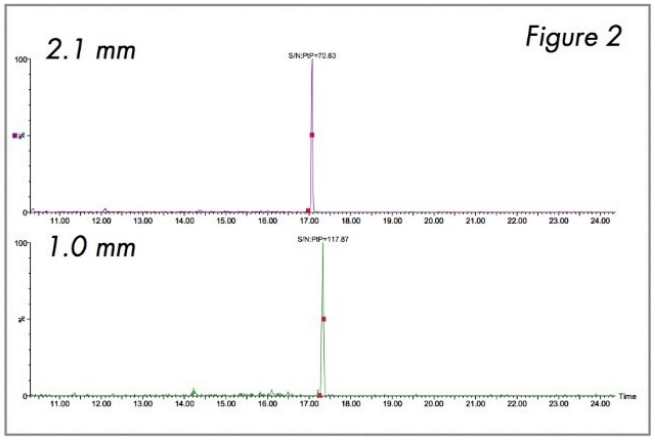


Figure 1 and 2. UPLC-MS analysis of rat urine following the oral administration of Pravastatin.

Conclusion

The work shown here demonstrates that the ACQUITY UPLC BEH 1 mm and 2.1 mm ID Columns both generate high quality chromatographic separations. The advantage of using 1 mm columns gives rise to a more sensitive assay with a reduction in solvent and sample consumption.

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