[APPLICATION NOTE]

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INCREASING SENSITIVITY OF BIOANALYTICAL ASSAYS UTILIZING MICROBORE UPLC AND TANDEM QUADRUPOLE MASS SPECTROMETRY

Paul D. Rainville and Robert S. Plumb
Waters Corporation, Milford, MA, U.S.

INTRODUCTION

As the pharmaceutical industry develops new chemical entities (NCEs) with increased potency and efficacy, there is increased pressure to develop more sensitive bioanalytical assays for the detection of drug substances and their associated metabolites.

Dealing with the need to detect ever-lower levels of analytes can be addressed by concentration of the sample or by using microbore columns (1 mm internal diameter (I.D.)).

2.1 mm I.D. columns are routinely used for bioanalytical assays and are compatible with systems that have low dispersion volumes, such as the Waters® ACQUITY UPLC® Systems. Moving to 1 mm I.D. columns will provide a four-fold increase in sensitivity in comparison to 2.1 mm I.D. columns due to smaller analyte elution volume, which results in an increase in analyte concentration and subsequent increase in detector response.

A further benefit is gained in the significant reduction in mobile phase solvent consumption due to the lower flow rates.

One challenge in the transition from 2.1 to 1 mm I.D. columns has been that, traditionally, LC system dispersion volume required pre- and post-column optimization to prevent band broadening and a subsequent loss in sensitivity.

In this application note, we demonstrate gains in sensitivity using 1 mm I.D. columns compared to 2.1 mm columns when analyzing ibuprofen in human urine. Furthermore, by using the ACQUITY UPLC System and its low dispersion volume, the full benefits of using 1 mm columns can be realized without having to reconfigure the LC system, and only a simple adjustment of the tandem quadrupole mass spectrometer is required.

EXPERIMENTAL

Acetaminophen, caffeine, hydroxy-coumarin, and tolbutamine where purchased from Sigma-Aldrich (St. Louis Mo., U.S.). The chemicals were dissolved in MeOH and diluted with H_2O to a concentration of 1 ng/mL. Human urine was collected from volunteer individuals eight hours following dosing with 400 mg of ibuprofen. The samples were stored frozen prior to analysis. They were prepared for analysis by centrifugation at 13,000 RCF for 5 min and diluted with H_2O. Samples were then injected onto the UPLC®/MS/MS system.

Figure 1. ACQUITY TQD with the TQ Detector.

LC conditions

LC system:	Waters® ACQUITY® TQD System
Column:	ACQUITY UPLC BEH C_{18} Column
	2.1 x 50 mm, 1.7 µm
	1.0 x 50 mm, 1.7 µm
Column temp.:	40 °C
Flow rate:	600 µL/min for 2.1 mm column
	140 µL/min for 1.0 mm column
Mobile phase A:	0.1 % NH_4OH
Mobile phase B:	MeOH
Gradient:	5 to 95 %B/2 min

MS conditions

MS system:	Waters TQ Detector
Ionization mode:	ESI negative
Capillary voltage:	2000 V
Cone voltage:	15 V
Desolvation temp.:	450 °C
Desolvation gas:	900 L/Hr
Source temp.:	130 °C
Ibuprofen MRM:	205 > 161 m/z
Collision Energy:	10 V

RESULTS

Sensitivity of bioanalytical assays utilizing LC/MS/MS can be increased by the utilization of microbore columns. However, parameters such as flow rate, system volume, and injection volume must be taken into consideration when transitioning to microbore columns.

One parameter that is often overlooked is the reduction in post-column volume when utilizing microbore columns.

To assess extra column band broadening, we analyzed six replicate injections of a test mix that were run with different post-column volumes.

Figure 2 illustrates the effect on peak width when a standard probe capillary (120 µm I.D.) and narrowbore capillary (65 µm I.D.) are utilized in the MS probe assembly.

Here we see that for all the compounds tested, the relative peak width doubled when the standard MS probe capillary was utilized with the microbore column.

A second experiment was then performed in which replicate injections were made on a standard 2.1 mm I.D. narrowbore column with the standard MS probe capillary, and on a 1.0 mm I.D. microbore column with a narrow MS probe (65 µm) capillary. The results of this experiment are shown in Figure 3.

Here we observe that the peaks widths for the test mix analytes are very similar, therefore this optimized configuration was utilized to analyze a model pharmaceutical compound and associated metabolites in urine.

Scaled chromatographic separations were then carried out to assess the capability of the microbore configuration in a complex biological matrix.

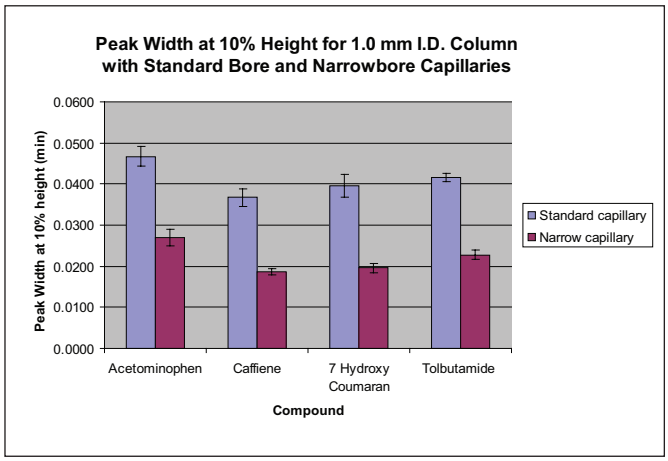

Figure 2. Effect of MS probe capillary diameter of peak width.

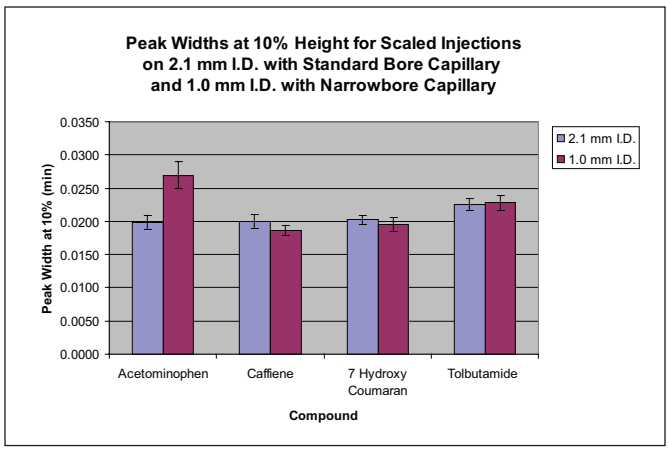

Figure 3. Scaled injection of ibuprofen and related metabolites in human urine.

Figure 4 shows the separation of ibuprofen and some associated metabolites on each system configuration. Here we observe identical resolution, sensitivity, and peak shape between the two chromatograms. This is accomplished with a four-fold decreased amount of sample on the microbore separation.

This can be of particular use if one is faced with small amounts of sample to analyze, for instance, pediatric studies.

We then injected the same amount onto each column, shown in Figure 5. Excellent peak shape along with a four-fold increase in sensitivity was observed. This experimental value was close to the theoretical sensitivity increase of 4.4.

It should be noted that the flow rates have been scaled for the difference in internal diameter and, as a consequence, we observed longer retention times for analytes run on the microbore configuration. This is caused by the delay volume of the ACQUITY UPLC System, but we also noted that the quality of data was not significantly affected.

Nevertheless, the amount of solvent required for each microbore run is substantially reduced compared to the narrowbore run.

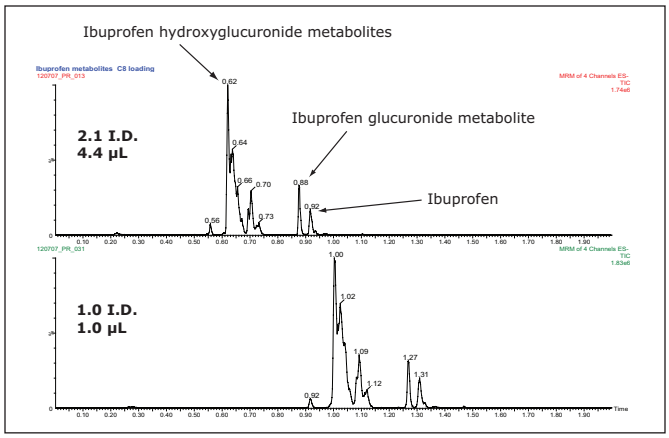

Figure 4. Scaled injection of ibuprofen and related metabolites in human urine.

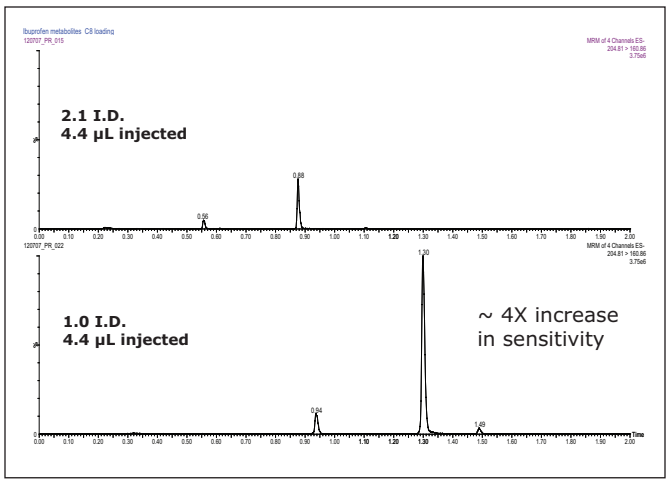

Figure 5. Increase in sensitivity obtained by microbore UPLC.

CONCLUSION

Utilization of microbore ACQUITY UPLC Columns is a viable method for increasing the sensitivity of a bioanalytical assay. However, scaling of chromatographic parameters and control of post-column band broadening are essential in the successful application of this technique.

In the analysis illustrated here, geometrically-scaled chromatographic separations maintained peak shape and resolution in a complex biological matrix. A further four-fold increase in sensitivity for ibuprofen in diluted urine was achieved using a microbore column as compared to the sensitivity yielded with the narrowbore column UPLC/MS/MS assay.

The ACQUITY UPLC System is optimized for both narrowbore and microbore columns. As demonstrated, use of a microbore column for a separation provides gains in sensitivity, is suitable when sample size is limited, and also results in reduced solvent consumption.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com