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OBTAINING ULTIMATE SPEED AND SENSITIVITY FOR QUANTITATIVE BIOANALYSIS USING UPLC/MS/MS

Kate Yu and Rob Plumb
Waters Corporation, Milford, MA, USA

INTRODUCTION

Ultra Performance LC™ (UPLC™) coupled with tandem quadrupole mass spectrometry is a powerful tool for quantitative analysis. As previously demonstrated[1], UPLC has added a new dimension to separation science that retains the practicality and principles of HPLC, while improving speed, sensitivity, and resolution. The Waters® ACQUITY UPLC™ System has been holistically designed with low system and gradient dwell volumes to take full advantage of the sub-2 µm particle technology. As a result, the ACQUITY UPLC System can generate chromatographic peak widths of less than a second at the base.

The Waters® Micromass® Quattro Premier™ tandem quadrupole mass spectrometer has been designed to perform high speed MRM data analysis allowing sufficient data points to be collected across these extremely narrow UPLC peaks for reliable quantitation[2]. The combination of the ACQUITY UPLC with the Quattro Premier provides high speed quantification without the loss of sensitivity or precision.

This technical note demonstrates the speed and sensitivity that can be obtained with the ACQUITY UPLC/Quattro Premier platform for the UPLC/MS/MS quantification analysis using a 4 compound test mixture.

EXPERIMENTAL CONDITIONS

LC Conditions

Instrument:	ACQUITY UPLC System		
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm		
Flow Rate:	0.6 mL/min.		
Mobile Phase:	10 mM NH_4OAc at pH 5.0 A: 10% ACN B: 80/20 ACN/MeOH		
Inj. Volume:	5 µL		
Gradient:	Time (min.)	%A	Curve
	0.0	95	–
	0.8	5	6
	1.0	5	6
	3.0	95	1

MS Conditions

Instrument:	Quattro Premier Mass Spectrometer
Capillary Voltage:	0.5 kV
Source Temp:	130 °C
Desolvation Temp:	400 °C
Desolvation Gas:	800 L/Hr
Cone Gas:	50 L/Hr
Data Collection:	ESI^+ MRM
Dwell Time:	10 ms
Inter-channel Delay:	10 ms

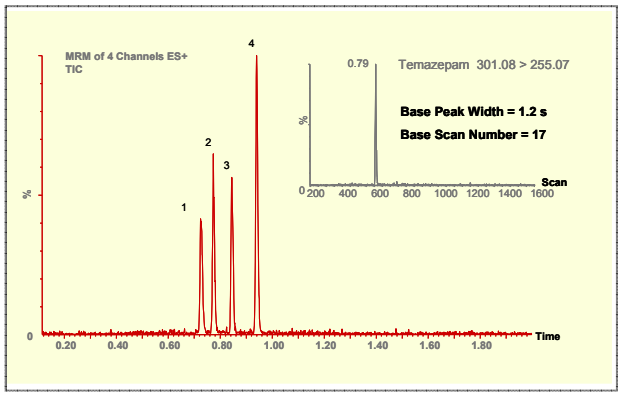

Figure 1. Total Ion Chromatogram of the test mixture with four channels per collection cycle: 1. Alprazolam (309.0 > 281.0), 2. Temazepam (301.0 > 255.0), 3. Diazepam (285.0 > 193.0), 4. Prazepam (325.1 > 271.1)

RESULTS AND DISCUSSION

Figure 1 shows the total ion chromatograms of the sample, demonstrating baseline resolution of all of the analytes contained. The data collection was performed for all four compounds simultaneously, which translates to 4 MRM channels per data collection cycle. Figure 2 shows the individual MRM chromatogram of each compound. A 10 ms second dwell time per channel was employed with a 10 ms inter-channel delay, giving a total cycle time per collection cycle of 70 ms. Also shown in Figure 1 (upper right), is a MRM chromatogram of temazepam using scan number as x-axis. The LC peak width at the base is 1.2 seconds, and the total number of data points collected across the peak is 17.

It is generally accepted that for reproducible peak quantification, the chromatographic peaks should be defined by no less than 15 data points[3]. The results shown in Figure 1 indicate that even with peak widths of 1.2 seconds at the base, the Quattro Premier is still capable of collecting a sufficient number of data points to allow for the accurate quantification of complex mixtures, thus making the Quattro Premier the ideal mass spectrometer to couple to the ACQUITY UPLC system[4].

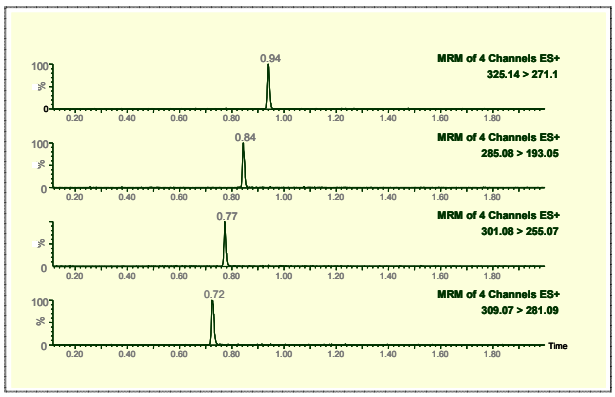

Figure 2. Individual MRM chromatograms of the test compounds

The calibration curves of the sample compounds are displayed in Figure 3. The linear range was 0.02 to 1000 ng/mL (4.5 orders of magnitude) for alprazolam, and 0.05 to 1000 ng/mL (4 orders of magnitude) for diazepam, prazepam and temazepam. The calibration curves displayed in Figure 3 were produced from five replicate injections at each concentration. Each curve was constructed using external calibration with linear curve fittings and 1/x weighting.

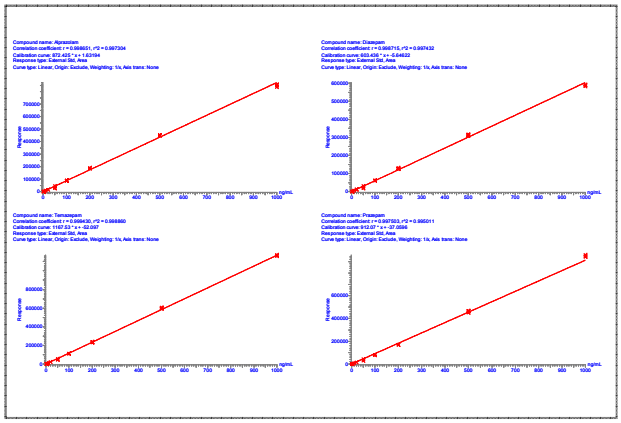

Figure 3. Calibration curves for test compounds

The results from the quantitative analysis each of the test compounds are summarized in Table 1. The r^2 values reported here were determined using a 15 point calibration for alprazolam and a 14 point calibration for diazepam, prazepam and temazepam.

Compound Name	MRM Transition	Cone Voltage (V)	Collision Energy (V)	Linear Range (ng/mL)	* R^2
Alprazolam	309.0 > 281.0	43	27	0.02 - 1000	0.997
Diazepam	285.1 > 193.0	36	32	0.05 - 1000	0.997
Prazepam	325.1 > 271.1	36	24	0.05 - 1000	0.995
Temazepam	301.1 > 255.0	26	20	0.05 - 1000	0.999

* R^2 was determined based on 15 point calibration for Alprazolam and 14 points calibration for Diazepam, Prazepam and Temazepam. Five replicates were made for each concentration point.

Table 1. Summary of quantitative analysis

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CONCLUSION

UPLC/MS/MS quantification was performed on a four compound mixture using the Waters ACQUITY UPLC System/Quattro Premier MS platform in just one minute using four simultaneous MRM transitions. This work demonstrates how UPLC/MS/MS can produce a high throughput, high quality assay with ultimate sensitivity. The ACQUITY UPLC produced an average LC peak width of 1.2 seconds at the base, and due to the high speed MRM capabilities of the Quattro Premier, it was possible to obtain approximately 17 data points collected across each peak, ensuring accurate peak characterization. Thus, the system is ideal for multi-component complex sample analysis. All analytes showed a linear response within the measured concentration range (0.02 – 1000 ng/mL for alprazolam, and 0.05 – 1000 ng/mL for diazepam, prazepam and temazepam).

REFERENCES

1. Ultra Performance LC (UPLC): New Boundaries for the Chromatography Laboratory, White Paper, Waters Corporation, May 2004, 720000819EN.

2. N. Kirchner "Ion Processing : Control and Analysis", US Patent No. 5,206,506, April 27th, 1993.

3. Application note, "Quattro Premier™: MRM Inter-Channel Crosstalk", Waters Corporation, June 2004, 720000917EN.

4. K. Yu, et al. "HT Quantification Analysis for a 5 Drug Mixture in Rat Plasma – A Comparison of HPLC/MS/MS and UPLC™/MS/MS", Presented at the 52nd ASMS Conference, Nashville, TN, June 2004.

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

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