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应用纪要

Quantitative Analysis by ACQUITY UPLC - Quattro micro with Polarity Switching

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Abstract

This application note demonstrates the ability of the Waters Micromass Quattro micro Mass Spectrometer coupled with a Waters ACQUITY UPLC System to perform quantitative analysis while operating in polarity switching mode.

Introduction

LC-MS/MS using tandem quadrupole mass spectrometers has become the technique of choice for quantitative analysis due to the wide range of compounds that can be detected and quantified. The most common mode of ionization for MS/MS systems is electrospray (ESI). Although the vast majority of compounds can be detected by the positive ESI, many compounds favor negative ESI. In order to simultaneously detect the widest range of compounds in a mixture, it is desirable to switch polarity during an analysis. The recent advancement of UltraPerformance LC (UPLC) results in much faster analysis times and narrower peak widths (less than 2 seconds), thus placing a significant challenge upon the data acquisition requirements of the MS systems. This application note demonstrates the ability of the Waters Micromass Quattro micro Mass Spectrometer coupled with a Waters ACQUITY UPLC System to perform quantitative analysis while operating in polarity switching mode. The results obtained from the UPLC analysis were compared with that of an HPLC analysis carried out under similar conditions.

Fast, On-The-Fly Polarity Switching

Polarity switching requires a specific period of time to allow the MS electronics to stabilize after the switching event. As a result, the detection cycle time for the mass spectrometer will be increased. Therefore, to perform polarity switching on-the-fly involves a balance between sensitivity and the number of data points that can be obtained across the LC peak. The delay time for the polarity switching for the Quattro micro is 100 milliseconds. Figure 1 demonstrates the MRM chromatograms obtained from an ACQUITY UPLC/Quattro micro System with polarity switching. The peak width obtained was 1.8 seconds, and the scan number collected across each peak was 10.

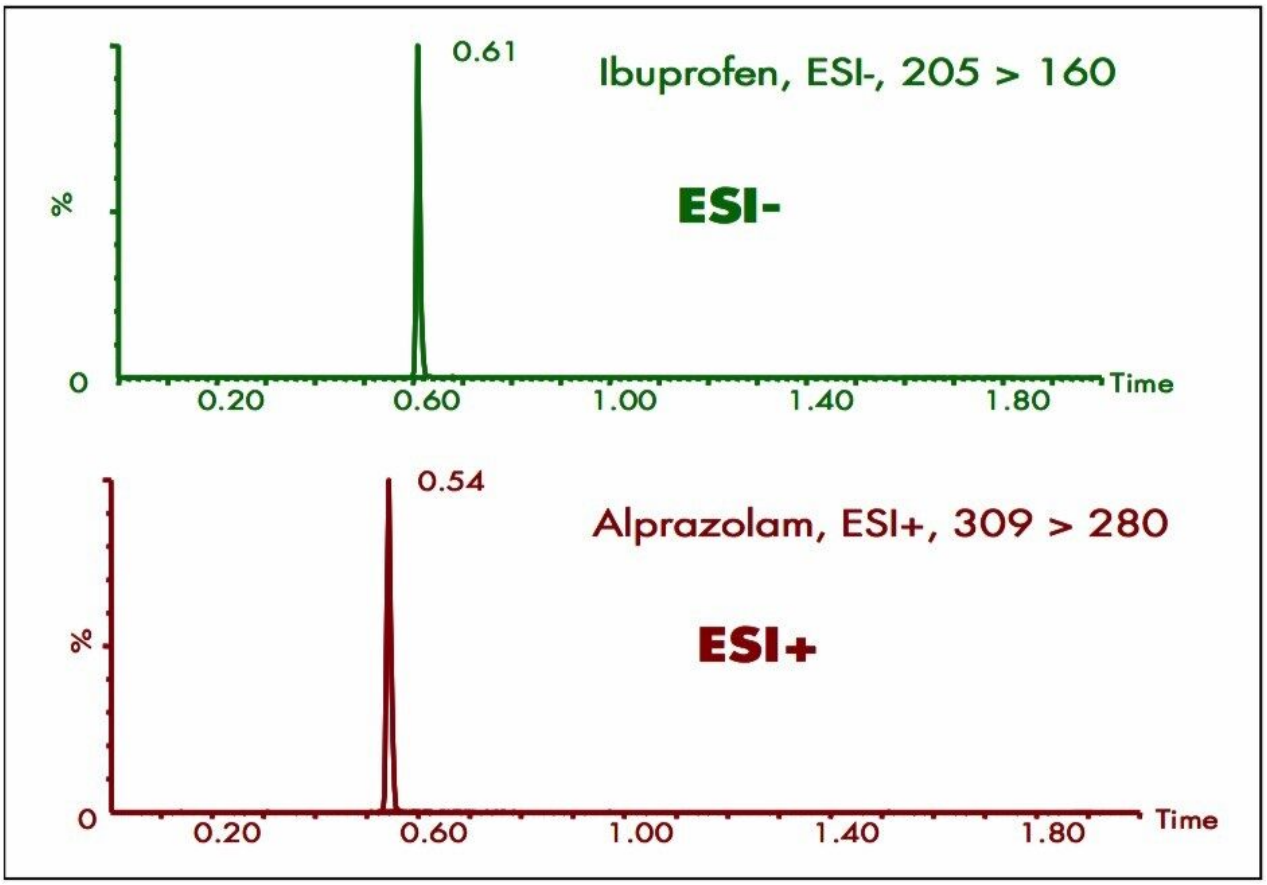

Figure 1. UPLC-MS/MS MRM chromatograms for Ibuprofen (ESI^-) and Alprazolam (ESI^+) obtained during a single injection with rapid polarity switching.

Experimental

LC Conditions

LC systems:	ACQUITY UPLC System(UPLC) Waters Alliance HT System (HPLC)
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm (UPLC)

	Xterra MS RP C_{18}, 2.1 x 50 mm, 3.5 µm (HPLC)
Flow rate:	0.6 mL/min (UPLC and HPLC)
Mobile phase:	10 mM NH_4OAc A:10% ACN, pH 5.0 B: 80/20 ACN/MeOH
Injection vol:	5 µL

Gradient (UPLC)

Time(min)	%A	Curve
0.0	90	1
0.4	10	6
0.5	0	1
2.0	90	1

Gradient (HPLC)

Time(min)	%A	Curve
0.0	80	1
1.5	0	6
3.5	80	1

MS Conditions

MS system:	Quattro micro Tandem Quadrupole Mass Spectrometer
Ionization mode:	ESI^+ and ESI^-
Detection mode:	MRM
Capillary voltage:	0.5 kV
Source temp:	130 °C
Desolvation temp:	350 °C
Desolvation gas:	800 L/hr
Cone gas flow:	50 L/hr
Interscan delay:	100 ms (UPLC and HPLC)
Interchannel delay:	20 ms (UPLC and HPLC)
Dwell time:	10 ms (UPLC), 50 ms (HPLC, ESI^+), 70 ms (HPLC, ESI^-)

Sample Preparation

The samples were prepared using neat standards prepared fresh from 1mg/mL methanol stock solutions purchased from CerilliantCorp. (Round Rock, TX). Calibration standards were prepared by dilution of the stock solutions in 90% water/10% acetonitrile, yielding concentrations ranging from 0.05 to 1000 ng/mL.

Results and Discussion

Figure 2 displays the comparative MRM chromatograms with on-the-fly polarity switching. The alprazolam was analyzed by ESI^+(left), while the ibuprofen was analyzed by ESI^-(right). The top chromatograms are from the HPLC analysis, and the bottom from the UPLC analysis. As demonstrated here, UPLC offered reduced LC run time with narrower peaks and increased signals. As a result, when compared with HPLC, UPLC peak areas increased more than 2-fold, and signal-to-noise ratios increased more than 4-fold.

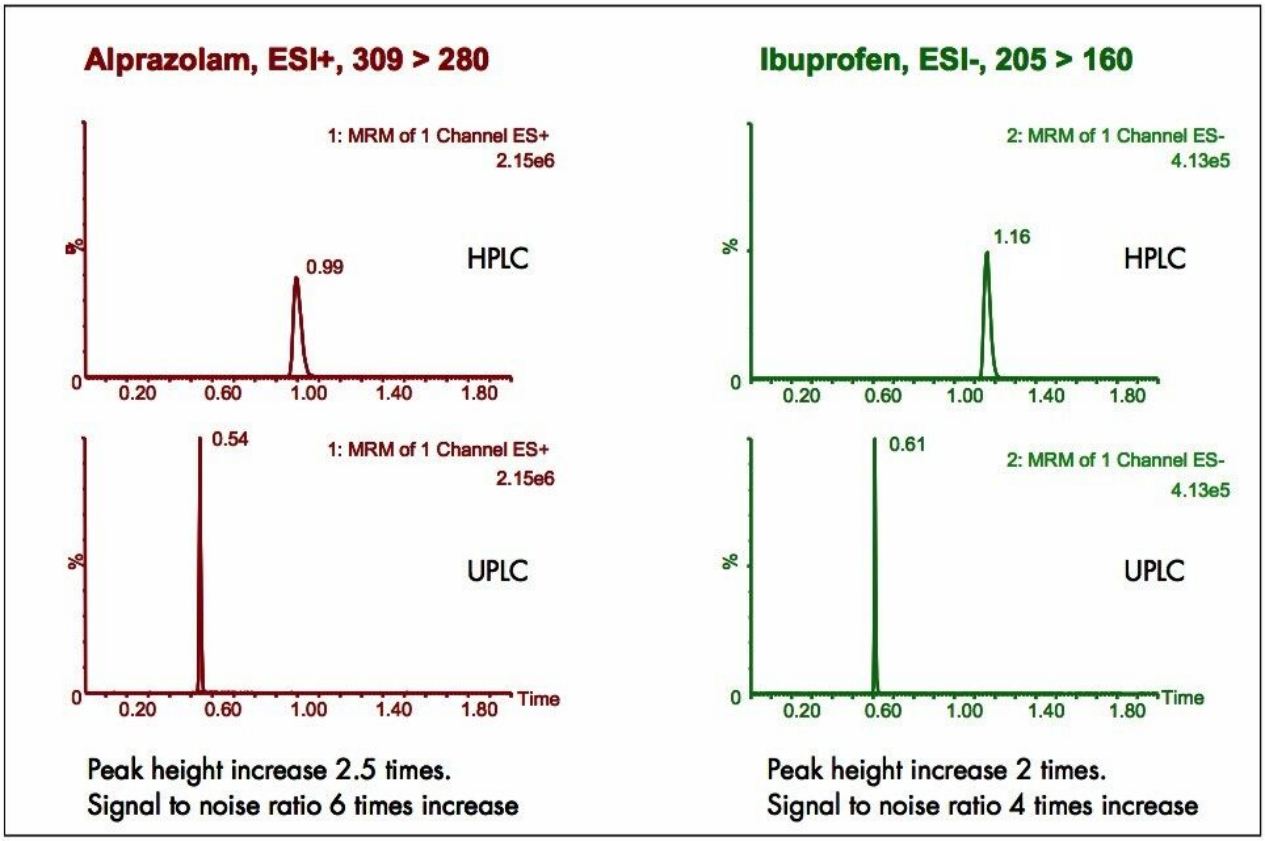

Figure 2. LC-MS/MS MRM chromatograms of alprazolam and ibuprofen: HPLC vs. UPLC.

As can be seen in Figure 1, approximately 10 data points were collected across each of the UPLC peaks. Figure 3 shows the calibration curves for alprazolam obtained by ESI^+ and ibuprofen obtained by ESI^- with on-the-fly polarity switching. Both calibration curves demonstrated excellent linearity, with correlation coefficients greater than 0.993 over more than 4 orders of magnitude.

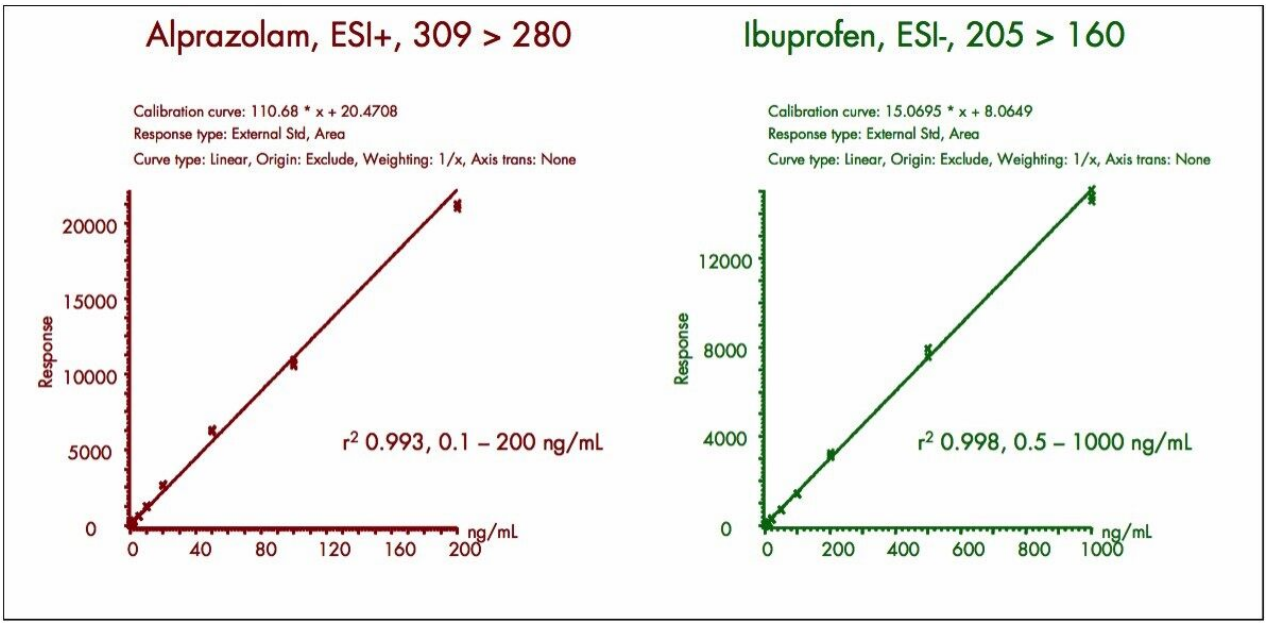

Figure 3. UPLC-MS/MS Calibration curves for alprazolam (ESI^+) and ibuprofen (ESI^-) run with polarity switching. Both demonstrated good linearity and dynamic range.

MS dwell time was optimized for both UPLC and HPLC to give approximately 20 data points across peaks. It is well accepted that for quadrupole (not T-Wave) instruments, the sensitivity is reduced as the dwell time is shortened. Therefore, as we have only a 10 millisecond dwell for UPLC and a 30 millisecond dwell for HPLC, we are underestimating the actual increase in sensitivity for UPLC. But, in this experiment, we are aiming to maximize the response for both modes. Even under these somewhat unfavorable conditions, UPLC is proven to be significantly more sensitive than HPLC.

Conclusion

The Quattro micro tandem quadrupole mass spectrometer provides the speed and sensitivity required for on-the-fly polarity switching –whether combined with the superior chromatographic performance of the ACQUITY UPLC System or with a traditional HPLC system. As shown, additional benefits can be realized with the use of UPLC, providing significant increases in sensitivity and throughput when compared to HPLC.

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