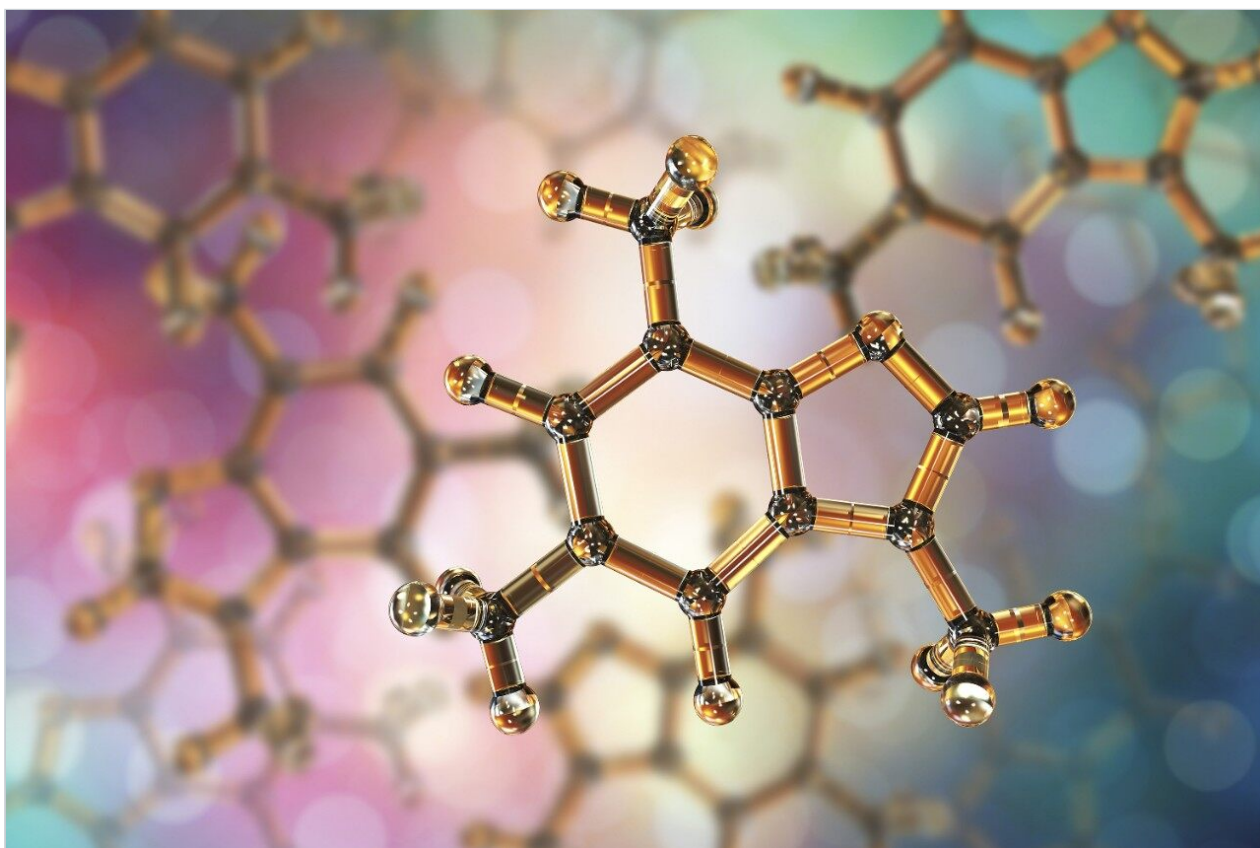


Meeting Analytical Challenges with UPLC-MS

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Abstract

In this applications note, we show the outstanding performance of the ZQ 2000 with narrow peaks in both

an ESI mode (Electrospray Ionization) and in an ESCi multi-mode ionization (both ESI and APCI) in the analysis of a multi-component drug test mixture.

Benefits

When comparing UPLC-MS to traditional HPLC-MS analysis, the following high performance benefits can be expected:

- Higher sample throughput
- Enhanced qualitative detection and sensitivity
- Increased selectivity and resolution
- More quality information obtained in less time
- Added capabilities from a single benchtop system
- Improved overall efficiency and productivity

Introduction

The utilization of single quadrupole mass spectrometers for drugdiscovery and development has increased significantly over the past five years, particularly for Open Access walk-up and Mass-Directed Autopurification environments. The ongoing demand for higher throughput and increased speed, sensitivity, and resolution continues to drive chemists to seek out the latest in high performance technologies. Recently, with the introduction of the Water ACQUITY UltraPerformance LC (UPLC) System, many chemists are considering the addition of this breakthrough chromatographic technology to further improve their overall data quality and productivity. To demonstrate, we have utilized the ACQUITY UPLC System with a Waters ZQ 2000 Mass Detector for the analysis of a representative selection of pharmaceutical compounds. The results address a common concern when peaks are very narrow (as with UPLC), and the resulting MS spectral quality from such fast analyses. In this applications note, we show the outstanding performance of the ZQ 2000 with narrow peaks in both an ESI mode (Electrospray Ionization) and in an ESCi multi-mode ionization (both ESI and APCI) in the analysis of a multi-component drug test mixture (Figure 1).

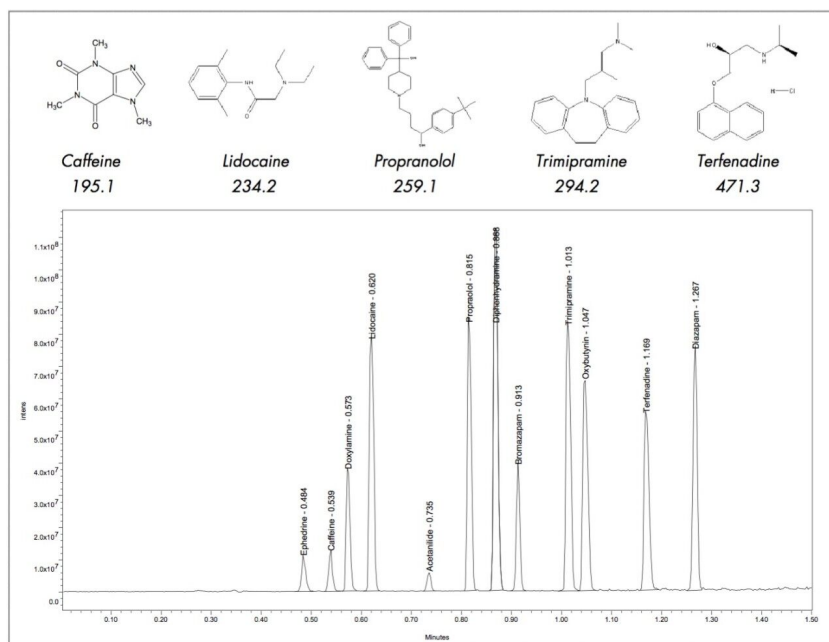


Figure 1. UPLC-MS enables accelerated, high resolution separations with 1.5–2.0 second typical peak widths.

Experimental

Method 1 (See Figures 1–2)

LC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C ₁₈ , 1.7 µm 2.1 mm x 50 mm
Flow rate:	0.75 mL/min
Injection:	2 µL
Mobile phase A:	0.1% Formic acid in H ₂ O
Mobile phase B:	0.1% Formic acid in acetonitrile

Gradient:	5% to 100% B in 0.5 min
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MS Conditions

MS system:	ZQ 2000 Mass Detector
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Scan range:	150–700 Da
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Scan time:	0.11 sec
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Interscan delay:	0.02 sec
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Cycle time:	0.13 sec
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Ionization mode:	ESI+
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Method 2 (See Figures 3–5)

LC Conditions

LC system:	ACQUITY UPLC System
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Column:	ACQUITY UPLC BEH C ₁₈ , 1.7 µm 2.1 mm x 50 mm
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Flow rate:	0.85 mL/min., 400 µL split flow to MS
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Injection:	2 µL
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Mobile phase A:	0.1% Formic acid in H ₂ O
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Mobile phase B:	0.1% Formic acid in acetonitrile
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Gradient:	10% B to 90% B in 3 min
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MS Conditions

MS system:	ZQ 2000 Mass Detector
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Scan range:	150–700 Da
Scan time:	0.11 sec
Dwell time:	50 ms
Interchannel delay:	0.02 sec
Interscan delay:	0.02 sec
Cycle time:	0.14 sec
Ionization mode:	ESI+
SIR mode:	$m/z=295$ (for Trimipramine) and $m/z=472$ (for Terfenadine)



Waters ACQUITY UPLC System with the Waters ZQ 2000 Mass Detector and the Waters ACQUITY UPLC Photodiode Array Detector.

Results and Discussion

MS Spectral Quality – Narrow Peaks

Separations were accomplished in less than 30 seconds and MS spectral quality allows easy identification of all compounds. This is an indication of what can be achieved with UPLC and a single quadrupole MS in a high throughput laboratory when faster chromatography is the objective.

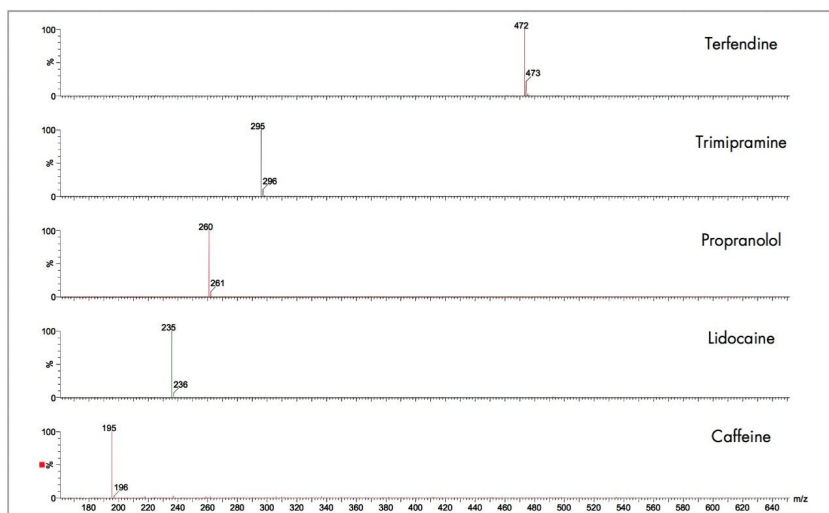


Figure 2. Individual spectra for five compounds in the test mix, where $[M+H]^+$ ions are easily discernable.

Excellent Linearity

Any analytical method requires a linear response relative to analyte concentration. In Figure 3, excellent linearity of response for Terfenadine is demonstrated for the UPLC-MS method.

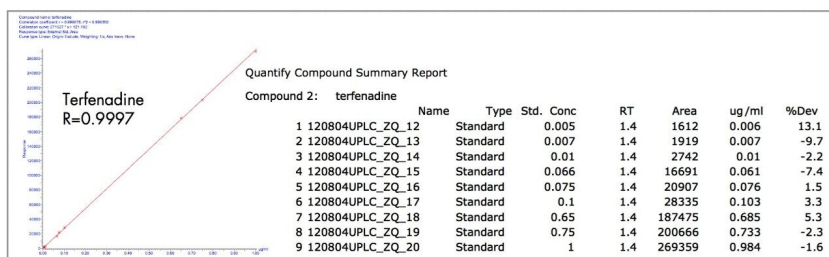


Figure 3. Excellent linearity is demonstrated for Terfenadine.

Lower Limits Of Detection

The detectability of low amounts (pg) is also required. The combination of the enhanced resolution and peak concentration afforded by ACQUITY UPLC separations and the sensitivity and selectivity of the ZQ 2000 mass detection enables the reliable detection of minor constituents (Figure 4).

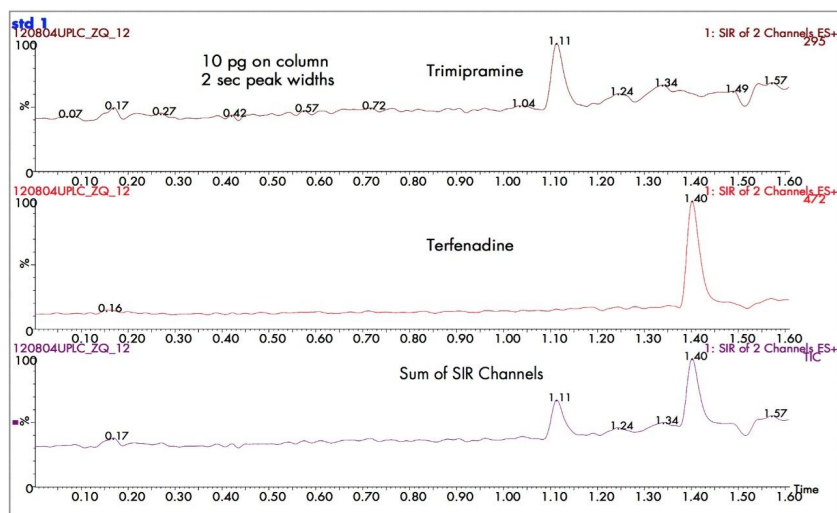


Figure 4. UPLC-MS limits of detection demonstrated with 10 pg on column for the analysis of Trimipramine and Terfenadine.

Precision Of Quantitation

The quality of UPLC separations coupled with the high scan rate capabilities of the ZQ yield exceptional analysis precision.

Compound 1: trimipramine									
	#	Name	Sample Text	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	1	120804UPLC_ZQ_21std 7		Standard	0.65	1.12	108959	0.649	-0.1
2	2	120804UPLC_ZQ_22std 7		Standard	0.65	1.12	109058	0.65	0
3	3	120804UPLC_ZQ_23std 7		Standard	0.65	1.12	108932	0.649	-0.1
4	4	120804UPLC_ZQ_24std 7		Standard	0.65	1.12	107243	0.639	-1.7
5	5	120804UPLC_ZQ_25std 7		Standard	0.65	1.12	112014	0.668	2.7
6	6	120804UPLC_ZQ_26std 7		Standard	0.65	1.12	108068	0.644	-0.9
							Ave:	109046	0.650
							Std dev:	1615	0.010
							%RSD:	1.5	1.5
Compound 2: terfenadine									
	#	Name	Sample Text	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	1	120804UPLC_ZQ_21std 7		Standard	0.65	1.4	191001	0.655	0.7
2	2	120804UPLC_ZQ_22std 7		Standard	0.65	1.41	189382	0.649	-0.2
3	3	120804UPLC_ZQ_23std 7		Standard	0.65	1.41	190987	0.655	0.7
4	4	120804UPLC_ZQ_24std 7		Standard	0.65	1.41	187998	0.644	-0.9
5	5	120804UPLC_ZQ_25std 7		Standard	0.65	1.41	193832	0.664	2.2
6	6	120804UPLC_ZQ_26std 7		Standard	0.65	1.41	184803	0.633	-2.6
							Ave:	189667	0.650
							Std dev:	3079	0.011
							%RSD:	1.6	1.6

Figure 5. UPLC-MS reproducibility for Trimipramine and Terfenadine.

Conclusion

The robust UPLC-MS platform yields exceptional throughput, speed, and sensitivity for routine analyses. As

the expectation to produce more quality data in less time becomes standard, the unique combined capabilities of the ACQUITY UPLC System with the ZQ 2000 Mass Detector provide more resolution faster, thus allowing overall throughput to be increased without compromising data quality. This superior data is achieved without the need to resort to very high analytical flow rates, which compromises sensitivity. As a result of this optimized performance, this system solution delivers the uptime, throughput and productivity necessary for any high throughput analytical laboratory.

Featured Products

ACQUITY UPLC System <<https://www.waters.com/1000396>>

ACQUITY UPLC PDA Detector <<https://www.waters.com/514225>>

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