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應用手冊

Simultaneous Quantification of Chemically Diverse Compounds Using ESCi Multi-Mode Ionization with ACQUITY UPLC and Quattro Premier

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Abstract

This application note demonstrates the feasibility and performance of the ESCi Multi-Mode Ionization Source on a Waters ACQUITY UPLC-Quattro Premier System.

Introduction

The progress of modern drug discovery and development relies heavily on the application of LC-MS/MS technology. In drug discovery, LC-MS/MS is commonly used for various ADME tests; in drug development, it is typically used for quantitative analysis in biological matrices. Experiments are typically run on an atmospheric pressure ionization source (API) with the choice of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Generally, about 80% of compounds can be analyzed by ESI.[1] APCI is the common secondary option for the analysis of compounds that are not ionized by ESI. To determine which ionization mode is more applicable to a particular compound (in terms of ionization and sensitivity), the compound needs to be evaluated in both ESI and APCI. Traditionally, changing from ESI to APCI required the analyst to physically change the ionization source and re-optimize the ion source parameters. This results in lost analysis time, significantly reducing the analytical throughput.

Multi-mode ionization has drawn attention in the field of applied liquid chromatography/mass spectrometry (LC-MS) for its versatility in the analysis of chemically diverse compounds. The expectation is that multimode ionization should improve analytical throughput as well as make LC-MS technology more readily adaptable to lab automation. To run an experiment using the ESCi Multi-Mode Ionization Source, the mass spectrometer switches rapidly between ESI and APCI during the same analysis, eliminating the need to physically change the ionization source and to repeat injections for ESI failed samples. The ESCi source is also capable of performing single mode ionization per analysis if required.

This application note demonstrates the feasibility and performance of the ESCi Multi-Mode Ionization Source on a Waters ACQUITY UPLC-Quattro Premier System. In this work, a quantitative LC-MS/MS analysis of a four compound mixture, Figure 1, was performed.

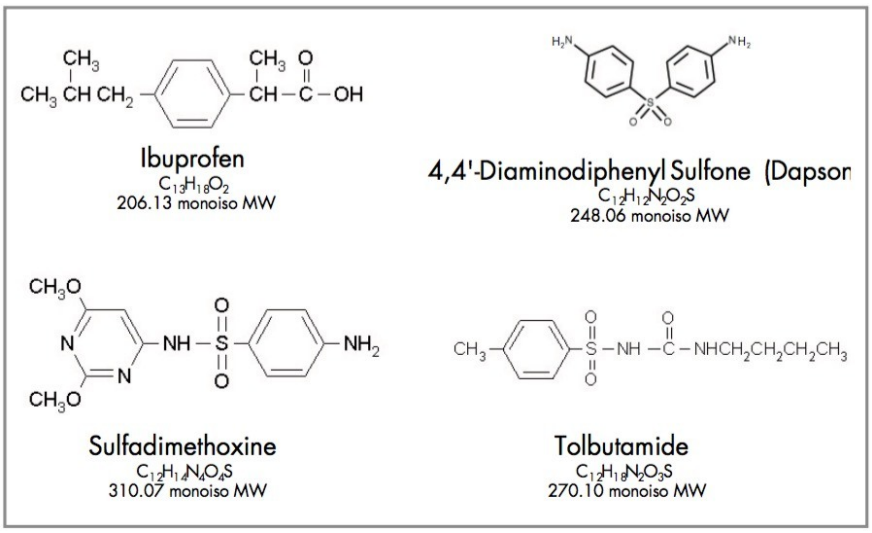

Figure 1. Chemical structures of test standards.

Experimental

Experimental Conditions

LC Conditions

Instrument:	Waters ACQUITY UPLC System
Column:	Waters ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Flow rate:	1 mL/minute (400 mL/minute into the MS)
Mobile phase:	A: 10 mM NH_4OAc in ACN/Water 10/90, pH 5.0 B: 10 mM NH_4OAc in ACN/Water 95/5, pH 5.0
Injection Volume:	5 µL

Gradient

Time	A%	Curve
0.0	90%	6
0.4	5%	6
0.5	5%	1
1.0	90%	1

MS Conditions

Instrument:	Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer
Software:	MassLynx 4.0 SP4 with ESCi enabled
Data collection:	MRM with simultaneous ESI+/ESI-/APCI-/APCI+ on-the-fly during each injection
Inter-scan delay:	20 ms
Inter-channel delay:	5 ms
Dwell times:	ESI+: 5 ms APCI-: 10 ms ESI-: 20 ms APCI+: 10 ms

Tune Page Parameters

ESCi Multi-mode ionization enabled from tune page	
ESI capillary voltage:	3.0 kV

APCI corona current:	4.0 µA
Source temp.:	130 ˚C
Desolvation temp.:	420 ˚C
Desolvation gas flow:	980 L/Hr
Cone gas flow:	50 L/Hr

Results and Discussion

ESCi Multi-Mode Ionization is easily applied on the Quattro Premier using the standard electrospray probe together with an APCI corona needle and enabling the ESCi option from the MassLynx tune page. The Quattro Premier mass spectrometer switches between polarities and/or ionization modes on-the-fly with only 20 ms delay. For the MRM data collection, the minimum inter-channel delay and dwell time was 5 ms. This enables ESCi technology to be easily applied to rapid chromatographic separations.

The MS optimization results[2] for the 4 standards and the ionization mode chosen for the quantitative analysis of each compound are listed in Table 1. For Tolbutamide, the best ionization mode is ESI^+. However, for demonstration purpose in this work, we have chosen the $APCI^-$ mode for this compound so that simultaneous quantification was performed in all four ionization modes during a single injection.

	ESI+	ESI-	APCI+	APCI-	Ionization Mode	MRM Transition
Daspone	+++	-	+++	-	ESI+	248.9>156.0
Ibuprofen	-	++	-	+	ESI-	205.2>161.0
Sulfadimethoxine	+++	+	+++	+	APCI+	311.2>155.9
Tolbutamide	+++	+++	++	++	APCI-	269.0>170.0

Table 1. Ionization mode results for standards.

Figure 2 shows the multiple reaction monitoring (MRM) chromatograms of the four standards. The chromatograms were acquired using the optimal ionization modes shown in Table 1. LC separation was obtained by the ACQUITY UPLC System with a 1 minute run time. The injection to injection cycle time was 1.5 minutes. The peak width for each analyte was approximately 1.8 seconds at the base.

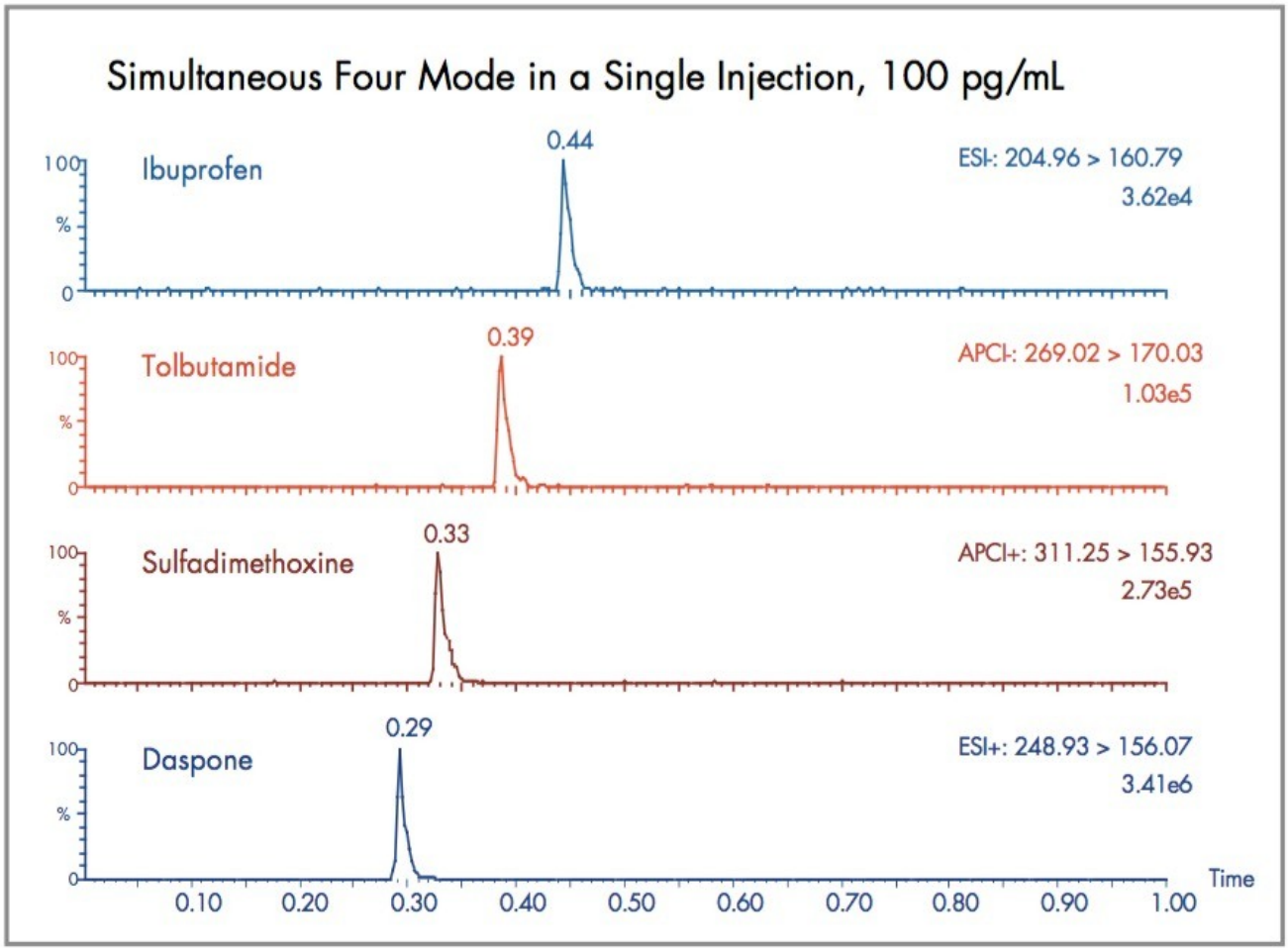

Figure 2. MRM chromatograms of the four standards.

For each of the chromatographic peaks, approximately 15 data points were obtained, which was sufficient for a full quantitative evaluation to be conducted for this ESCi method. Figure 3 shows the calibration curves of the 4 standards, and Table 2 lists their dynamic ranges, the linearities and the limits of detection. The analysis was completed simultaneously in four ionization modes during a single injection (ESI+/ESI-/APCI-/APCI+). Full quantitative evaluation demonstrated up to 4 orders of magnitude of linear range and the limit of detection was as low as 50 fg on column.

	Ionization Mode	Linear Range (ng/mL)	r^2	Limit of Detection (ng/mL, 5 mL Inj)
Daspone	ESI+	0.01 - 200	0.991	0.01
Ibuprofen	ESI-	0.1 - 100	0.984	0.1
Sulfadimethoxine	APCI+	0.1 - 1000	0.992	0.01
Tolbutamide	APCI-	0.1 - 1000	0.992	0.1

Table 2. ESCi quantitative analysis results.

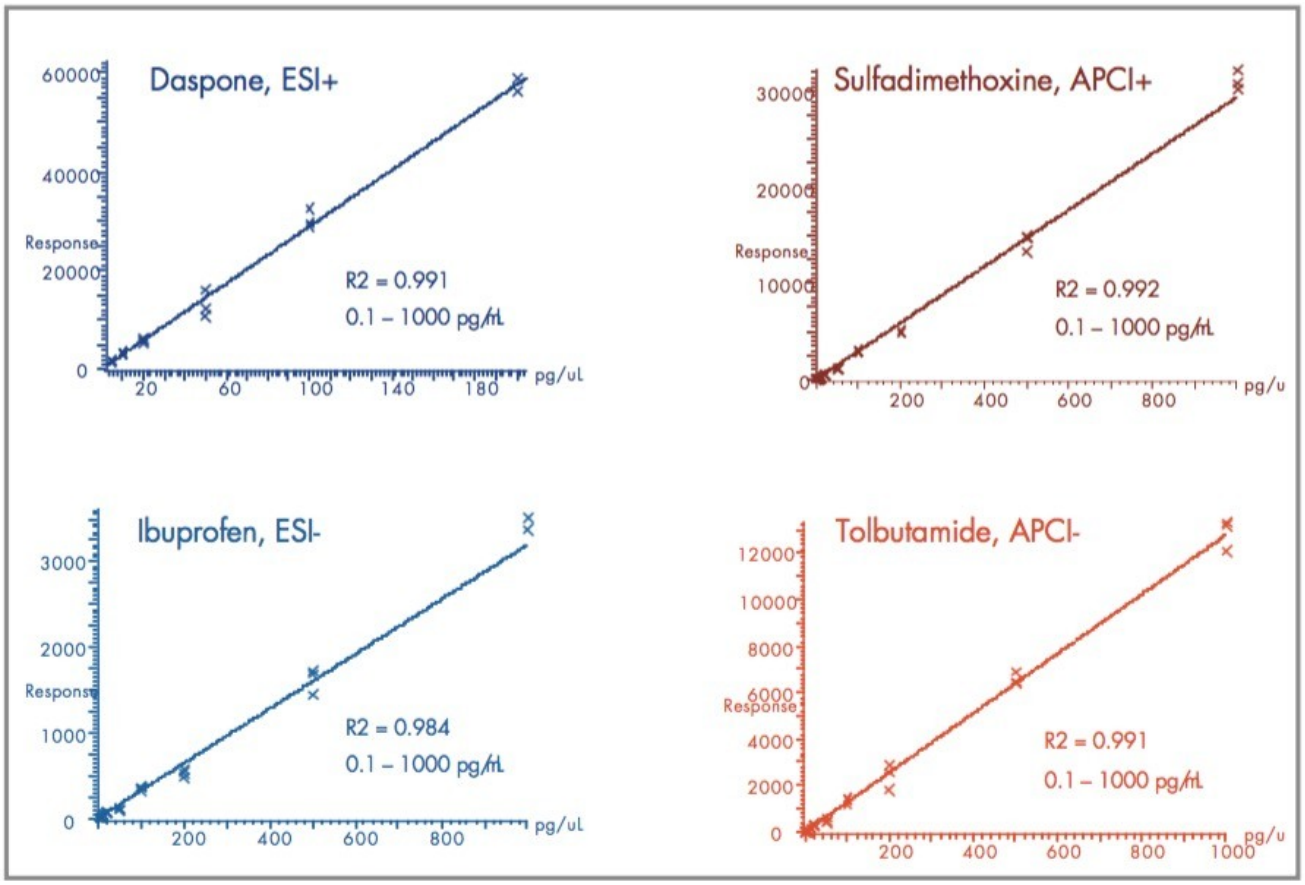

Figure 3. Calibration curves for the four standards.

Conclusion

We have demonstrated a quantitative LC-MS/MS study of a 4 compound mixture using ESCi Multimode

Ionization. As shown in a previous LC-MS study, the "ESCi source could identify about 10% more samples than the standard ESI or APCI sources from the plate analysis"[1]. Our LC-MS/MS results also demonstrated that the ESCi Multi-Mode Ionization offers time-savings in analysis and therefore can further improve analytical throughput. As a result, the ESCi Multi-mode ionization can be applied for the analysis of a large number of compounds with high speed and has the ability to ionize compounds with diverse structures for analysis in drug discovery and development.

References

1. R. Gallagher, M. Balogh, P. Davey, M. Jackson, I. Sinclair, and L. Southern. *Analytical Chemistry*. 75, p 973–977, 2003.
2. K. Yu, P. Alden, L. Di, S. Li, and E. Kern. Applications of ESCi-UPLC-MS/MS in Drug Discovery and Development by ACQUITY UPLC-QuattroPremier. *21st Montreux LC-MS Symposium* on LC-MS and Related Topics, Montreux, Switzerland, November 2004.

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