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アプリケーションノート

Maintaining High Spectrum Resolution with Increasing Scan Speeds for Metabolite ID using UPLC with the Q-Tof Premier

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Abstract

This application note shows the consistent high spectral resolution obtained from the Q-Tof Premier with increasing data acquisition speeds. This is demonstrated using *in vitro* microsomal incubations of buspirone. All analyses were performed with the ACQUITY UPLC and Q-Tof Premier.

Introduction

The detection and identification of candidate pharmaceuticals and their metabolites in biological fluids is an essential part of the drug discovery and development process. UltraPerformance LC (UPLC) coupled with mass spectrometry (MS) sets a new standard in the discipline of metabolite identification, producing superior chromatographic separations, high throughput, and increased sensitivity.

The performance of the Q-Tof Premier mass spectrometer is ideal for the challenging requirements of a metabolite ID study. It offers high spectral resolution with exact mass measurement for both MS and MS/MS analyses. As a result, positive confirmation of analyte identity is obtained in complex matrices from a single injection.

Reliable accurate mass measurement is critically dependent on the spectral resolution, which brings operational parameters into consideration when coupled with the ACQUITY UPLC System. Typical peak widths generated from UPLC separations are in the range of 1–3 seconds at the base. High acquisition speeds are required to ensure good peak definition. However, fast data acquisition often results in reduced spectral quality and reduced mass accuracy with many mass spectrometers.

This application note shows the consistent high spectral resolution obtained from the Q-Tof Premier with increasing data acquisition speeds. This is demonstrated using *in vitro* microsomal incubations of buspirone. All analyses were performed with the ACQUITY UPLC and Q-Tof Premier.

Figure 1. The ACQUITY UPLC system with the Q-Tof Premier for metabolite ID.

Experimental

In vitro Microsome Incubation

The parent drug buspirone was incubated with human and rat liver microsomes at a 100 µM level. The incubation was carried out at 37 °C, in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing the appropriate co-factors. The reaction was terminated after 90 minutes with two volumes of cold acetonitrile to one volume of sample. The samples were stored frozen at -20 °C and diluted with water in a one-to-one ratio prior to UPLC-MS analysis.

UPLC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm 2.1 mm x 100

	mm, 65 °C
Mobile phase:	A: Water + 0.1% Formic acid B: Acetonitrile + 0.1% Formic acid

Gradient

Time (min)	Flow (mL/min)	%A	Curve
0.00	0.600	98.0	-
3.50	0.600	30.0	6
4.00	0.600	0.0	1
7.00	0.600	98.0	1

MS Conditions

MS system:	Q-Tof Premier mass spectrometer
Ionization mode:	ESI+
Cone voltage:	30 V
Capillary voltage:	3 kV
MS mode:	MS full scan in W-mode
Desolvation temp.:	450 °C
Source temp.:	120 °C
Collision energy:	5 eV

Results and Discussion

Buspirone (MW 385) is an anti-anxiety drug with hydroxylation as one of its major metabolic pathways.[1] Figure 2 shows the extracted ion chromatograms for the M+16 buspirone metabolites at *m/z* 402.

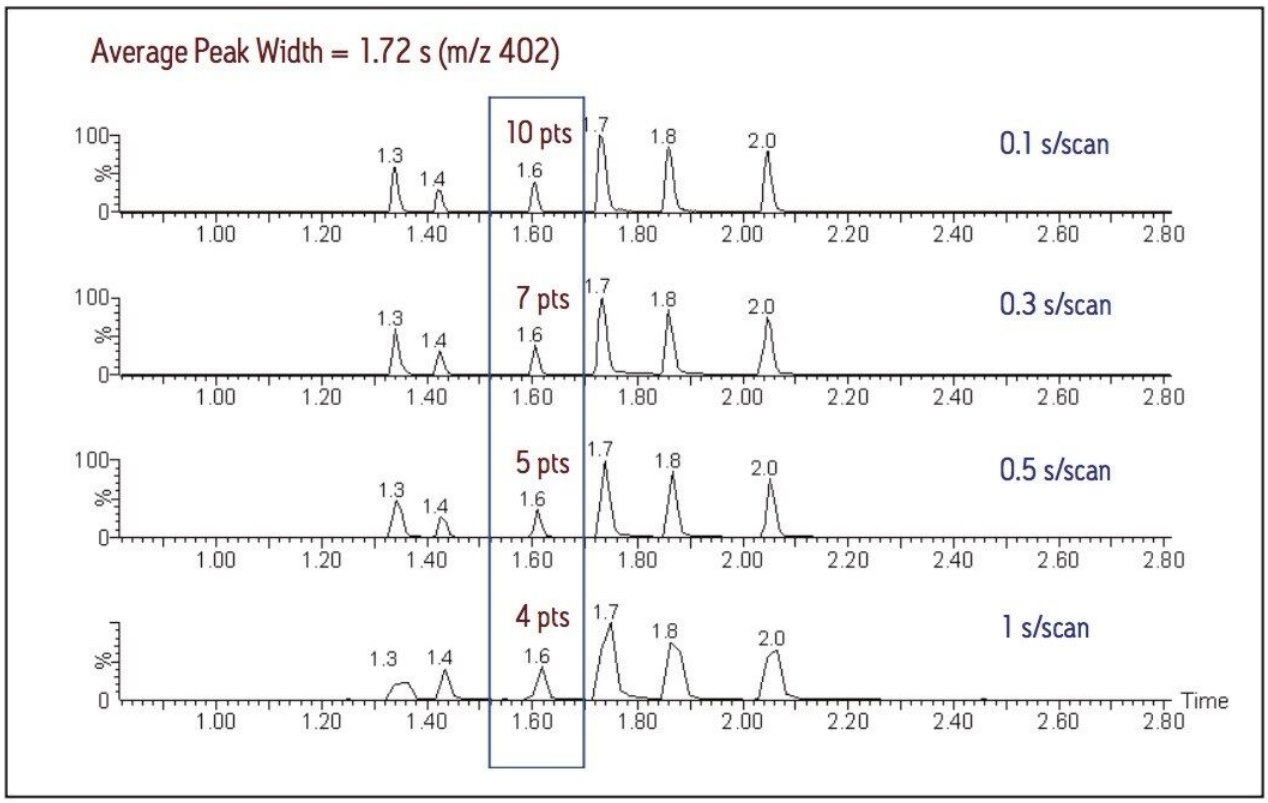

Figure 2. Selected ion chromatograms for the M+16 buspirone metabolites at m/z 402 obtained at different scanning speeds. More data points were collected as the scan speed increased resulting in better peak definition.

In order to study the effect of fast data acquisition speeds on spectral resolution, the same sample was injected multiple times by employing the same UPLC-MS protocol. The data acquisition speed was varied from injection to injection. Figure 2 clearly shows that higher scan speed data capture experiments gave much better peak definitions. Data points are important for analytical precision, especially if quantitative or semi-quantitative results are required for the analysis.

Figure 3 shows the mass spectra obtained for the *m/z* 402 ion eluting at 1.6 minutes. The spectral resolution was maintained at approximately 20,000 FWHM (full width at half maximum) regardless of the data acquisition speed. The consistent high resolution obtained by Q-Tof Premier is essential for high quality exact mass measurement during sample analysis, making confirmation of peak identity a much easier task.

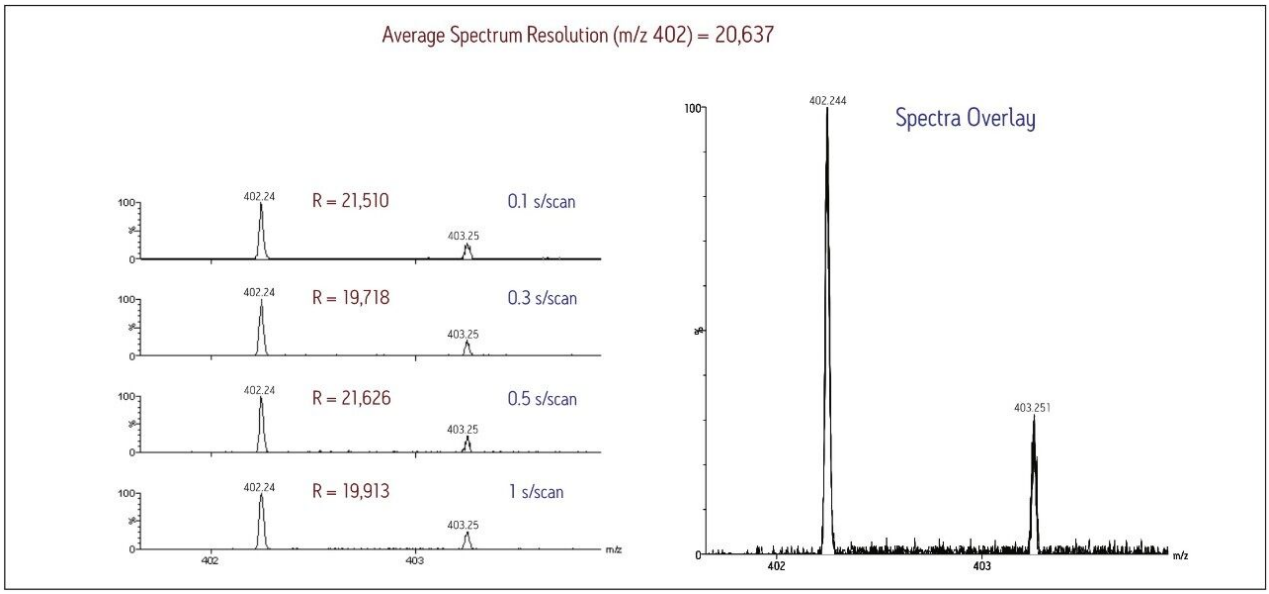

Figure 3. The mass spectra for buspirone metabolites (M+16) showing spectral resolution obtained at different acquisition speeds.

Conclusion

We have demonstrated that the Q-Tof Premier has superior and consistent spectral resolution with 20,000 FWHM at scan speeds ranging from 1 to 0.1 scans/second. This makes the Q-Tof Premier the ideal mass spectrometer for structural analysis. When the Q-Tof Premier is coupled with the ACQUITY UPLC, metabolite ID can be performed rapidly with no loss in data quality.

References

1. Kerns EH, Rourick RA, Volk KJ, Lee MS. *J Chromatogr B Biomed Sci Appl.* 1997 Sep 26; 698 (1–2): 133–45.

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