[APPLICATION NOTE]

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ADDRESSING THE CHALLENGES OF FAST DATA MRM COLLECTION WITH THE XEVO TQ MS

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INTRODUCTION

Liquid chromatography combined with mass spectrometry (LC/MS) has become the technique of choice for both quantitative and qualitative analysis in the pharmaceutical industry. ACQUITY UltraPerformance Liquid Chromatography® (UPLC®) has become the technique of choice for fast, high resolution, high sensitivity LC analysis. The sub-2-µm particles employed in UPLC give rise to sharper and more intense peaks, affording greater peak capacity separations and superior assay sensitivity. This increased performance has resulted in a three- to eight-fold increase in LC/MS/MS assay sensitivity for bioanalysis[1] and a three- to five-fold reduction in analysis time.

The accurate quantification of chromatographic peaks requires the collection of 12 to 20 points across the peak. The narrow peaks produced by UPLC, typically 1 to 3 seconds, places a challenge on conventional mass spectrometers. As the data collection dwell time is reduced, the signal-to-noise typically decreases, reducing the overall assay sensitivity. While the measured signal can remain constant at short dwell times, the rapid switching between multiple reaction monitoring (MRM) transitions can generate increased electronic noise on some instruments, leading to a lower signal-to-noise ratio.

The Xevo™ TQ MS is a tandem quadrupole mass spectrometer which is equipped with a novel collision cell that is continuously filled with collision gas, allowing for the simultaneous collection of MRM and full scan MS data.[2] The collision cell incorporates the Waters Traveling Wave (T-Wave™) design rather than quadrupole rods.[3]

The T-Wave is constructed from a series of charged plates with the ion path through the collision cell controlled by a combination of DC and RF signals. T-Wave collision cell technology minimizes source-to-detector ion transit times for high-speed data acquisition. Unlike some collision cell designs, the T-Wave collision cell ensures that signal intensity does not fall as acquisition speed increases.

In this application note, we demonstrate the capabilities of the Xevo TQ MS equipped with this new collision cell, allowing fast MRM switching to acquire data with low dwell times without compromising data quality.

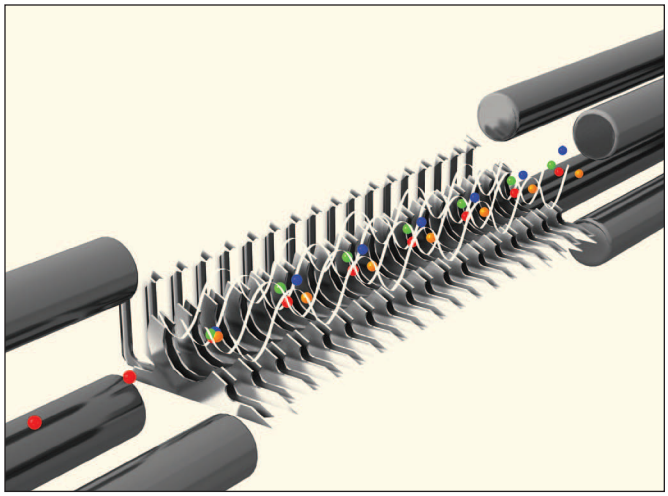

Figure 1. The Xevo TQ MS's T-Wave collision cell.

EXPERIMENTAL

LC /MS conditions

LC system:	Waters® ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temp:	45 °C
Flow rate:	600 µL/min
Mobile phase A:	0.1% Formic acid
Mobile phase B:	Acetonitrile
Gradient:	5 to 95% B over 2 min
MS system:	Waters Xevo TQ MS
MS/MS transition:	260 → 183
Dwell time:	10 and 100 ms
Ionization:	Positive ion ESI
Capillary voltage:	1.0 KV
Collision energy:	25 eV
Cone voltage:	40 V

RESULTS

Multiple reaction monitoring is the technique of choice for quantitative analysis, with sensitivity levels in the pg/mL range. The MRM dwell time is dependent on the peak width and the number of peaks to be monitored. The narrower the peaks and the greater the number of the peaks to be monitored, the lower the dwell time required. Thus for a 1.5-second wide peak collecting two MRM channels, the required dwell time for 15 points across the LC peak is 45 milliseconds assuming a 5-millisecond interscan delay. Increasing the number of analytes monitored to 4 or 5 would reduce the dwell time to just 10 milliseconds. The data below shows the effect on the signal-to-noise of reducing the MRM dwell time from 100 milliseconds to 10 milliseconds on a peak that is 3 seconds wide at the base.

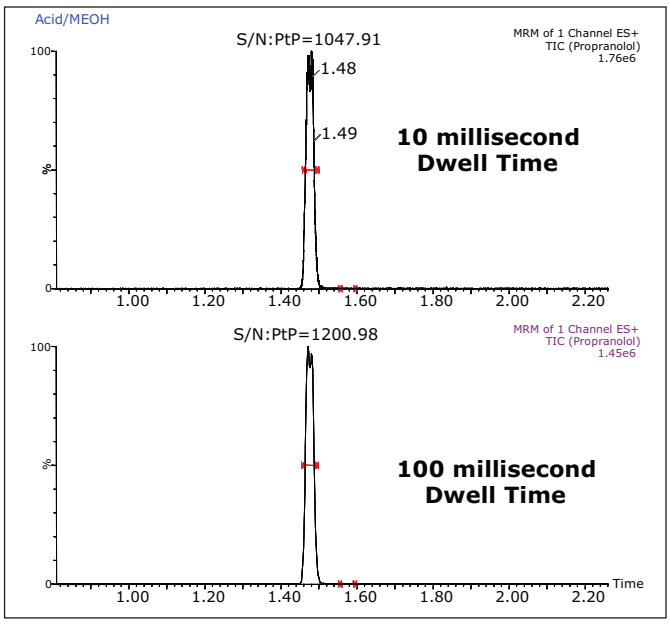

Figure 2. Comparison of MS sensitivity with 100 msec and 10 msec dwell time.

While it is possible to operate a standard tandem quadrupole instrument at low acquisition rates, 10 to 20 milliseconds, this usually results in a higher noise value compared to the signal. This data demonstrates that with the T-Wave collision cell, no reduction in signal intensity was observed when the dwell time was reduced from 100 to 10 milliseconds. In this data we can clearly see that the signal-to-noise value from the 100 msec dwell time is 1200, while with the signal-to-noise value is only reduced by just 13%, to 1047.

CONCLUSION

- The reduction in peak width associated with fast, sub-2-µm UPLC requires that the mass spectrometer collect data at an increased rate to accurately define the LC peaks.
- With conventional tandem mass spectrometers, this reduction in MRM dwell time results in degradation of signal-to-noise.
- The Waters Xevo TQ MS is equipped with a novel T-Wave collision cell that allows for rapid data acquisition.
- The use of the T-Wave collision cell allows the dwell time to be reduced from 100 to 10 milliseconds with limited reduction of signal to noise.

References

1. Shen JX, Wang H, Tadros S, Hayes RN. J Pharm Biomed Anal. 2006 Feb 24; 40(3): 689-706.
2. Twohig M, Alden P, Fujimoto G, Kenny D, Plumb RS. Improving MS/MS Sensitivity using Xevo TQ MS with ScanWave. Waters Corporation. 2008; 720002828en.
3. The traveling wave device is similar to that described by Kirchner in U.S. Patent 5,206,506 (1993).

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