Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Rapid, Simple Impurity Characterization with the Xevo TQ Mass Spectrometer

Robert Plumb, Michael D. Jones, and Marian Twohig
Waters Corporation, Milford, MA, USA

INTRODUCTION

The detection and characterization of impurities and degradation products of an active pharmaceutical ingredient (API) are regulatory filing requirements. The detection and identification of impurities not only ensures medicine safety but can also be used as a fingerprint for patent protection and counterfeit drug analysis.

Impurity characterization and identification is normally carried out using information-rich analytical techniques such as NMR and LC/MS. Analysis by LC/MS provides parent ion mass from full-scan MS and structural information from the fragments generated in MS/MS experiments. With traditional tandem quadrupole instrumentation, the generation of this data requires multiple experiments to obtain MS and MS/MS information.

Modern Linear Ion Trap (LIT) mass spectrometers allow the collection of MS, multiple reaction monitoring (MRM), and MS/MS data in the same analytical run, allowing quantitative and qualitative data to be obtained simultaneously. However, the duty cycle of these instruments when switching between MS and MS/MS modes is typically 2 to 3 seconds. With modern high-resolution, sub-2-µm column particle chromatography such as UPLC,® peak widths of 2 to 3 seconds are now commonplace.

With these LIT MS systems, this would result in just 1 to 2 points across the peak, with the peaks either poorly defined or missed completely; thus slower, lower-resolution LC systems must be used, resulting in reduced throughput and lower data quality.

The Waters® Xevo® TQ Mass Spectrometer is equipped with a novel collision cell design that is continuously filled with collision gas, allowing rapid switching between MS and MS/MS modes. The Xevo TQ MS is capable of operating at up to 10,000 Da/sec and can correctly define the very sharp peaks produced by UPLC, with more than 10 points across a 2-second-wide peak, even on a multi-scan experiment.

This collision cell is capable of enhanced high-sensitivity operation in MS/MS mode. In this mode of operation, ions are constrained in the final third section of the collision cell using both DC and RF barriers. These ions are then ejected from the collision cell, in a controlled manner, from high to low *m/z* in synchronization with the scanning of the final resolving quadrupole. This increases the duty cycle of the instrument, resulting in enhanced sensitivity that is ideal for the detection and characterization of low-concentration impurities that may result in toxic effects.

Figure 1. Xevo TQ Mass Spectrometer with the ACQUITY UPLC® System.

EXPERIMENTAL

To evaluate the performance of this system, the impurities of the common pharmaceutical drug quetiapine, used to treat biopolar disorder, was investigated using UPLC/MS/MS.

LC /MS conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	65 °C
Flow rate:	800 µL/min
Mobile phase A:	20 mM ammonium bicarbonate pH 10
Mobile phase B:	Acetonitrile
Gradient:	15% to 95% B/18 min
MS system:	Xevo TQ MS
Ionization mode:	ESI positive ion mode
Capillary voltage:	30 V
Collision energy:	15 eV

RESULTS

The unique collision cell design allows the Xevo TQ MS to be operated in several different modes of operation: full scan MS, MRM, as well as MS/MS mode. As the collision cell is continuously filled with collision gas, the instrument can rapidly switch between MS and MS/MS in the same analytical run. This allows MRM and MS scans to be performed in the same run. Combined with the high scan rate, this allows for rapid survey scans to be performed, such as MS neutral loss or parent ion, before switching to MS/MS.

This high data-capture rate allows for the accurate definition of the peak, even with the very narrow peaks produced by UPLC. Figure 2 shows the UPLC/MS chromatogram produced in the analysis of an API batch of quetiapine at a concentration of 1 µg/mL. Here we can see that impurity peaks are 2 to 4 seconds wide at the base. The data shown in Figure 3 illustrates the number of scans achieved in MS and MS/MS modes.

Maximizing LC peak definition

In this example, the Xevo TQ MS was operated in ScanWave™ MS mode, switching to ScanWave DS (daughter ion scan) mode when a peak was detected above a user-defined threshold. In this mode of operation, the instrument selects the most intense peak in the MS spectrum and acquires MS/MS data on this peak before returning to MS mode. Since the collision cell is continuous filled with collision gas, there is a no delay in switching between MS and MS/MS modes.

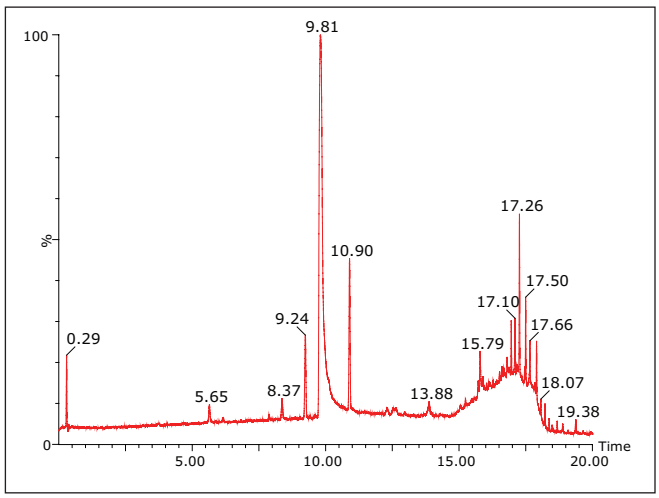

Figure 2. UPLC/MS analysis of quetiapine at 1 µg/mL.

We can see from this data that the instrument has acquired 9 points across the peak in MS mode, and 15 points across the peak in MS/MS mode – despite the fact that the peak is only 2 seconds wide at the base. This high data-capture rate enables the Xevo TQ MS to perform high quality, data-dependent MS-to-MS/MS experiments in a UPLC timeframe with sufficient data points to accurately define the peak. This dual mode of operation can also be used to acquire full-scan MS data simultaneously with MRM data, or to detect a peak with precursor ion scanning before switching to MS/MS mode.

Precursor ion scanning

The detection of new impurities, degradation products, or, in a DMPK study, drug metabolites, is often confounded by the signal from the matrix. To detect and visualize these analytes, the analytical chemist can use the specificity of the mass spectrometer.

Since compounds can undergo fragmentation as a result of the degradation or metabolism process, the use of simple, predicted MRM transitions for common degradation/metabolism pathways may result in the non-detection of a potentially toxic impurity, degradation product, or metabolite. A more comprehensive way to detect these compounds is to monitor for the common fragment ions of the molecule of interest. The Xevo TQ MS's Survey Scan functionality utilizes the fast data-capture rate of the instrument to facilitate the collection of precursor ion data as well an MS/MS spectrum of the peaks detected.

This functionality was used to evaluate a commercially-purchased API sample of quetiapine. The MS/MS spectra of quetiapine revealed that it gave rise to three major product ions having *m/z* values 221, 253, and 279. This data was used to detect drug-related impurities in the API batch by performing a Survey Scan analysis on each of these ions.

The data collected for the parent ion chromatogram of *m/z* 279 is displayed in Figure 4. Here, we can see the presence of seven major peaks, six impurities and the quetiapine active peak, eluting with a retention time of 9.86 minutes. A similar analysis using the common fragment ion *m/z* 221 to trigger data collection produced the chromatogram shown in Figure 5. With this fragment ion, a total of 12 peaks were detected and MS/MS data acquired.

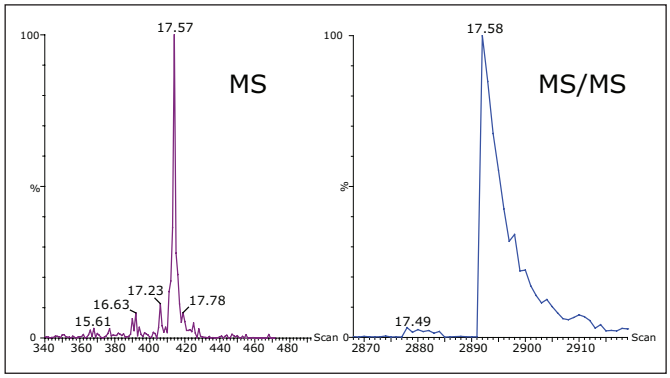

Figure 3. Rapid data collection is performed simultaneously in both MS and MS/MS modes.

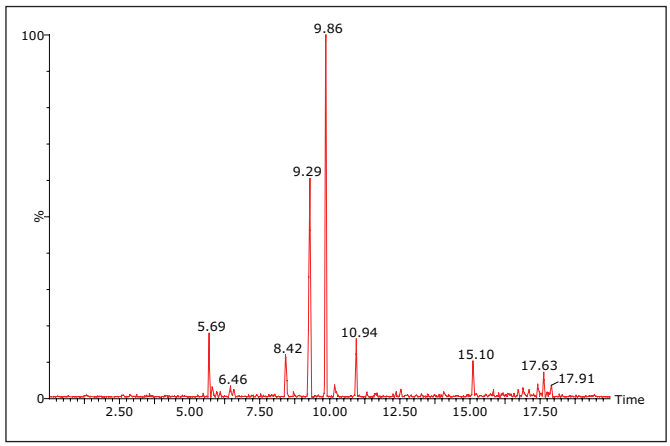

Figure 4. Survey Scan UPLC/MS analysis of quetiapine for ion m/z 279 in positive ion mode.

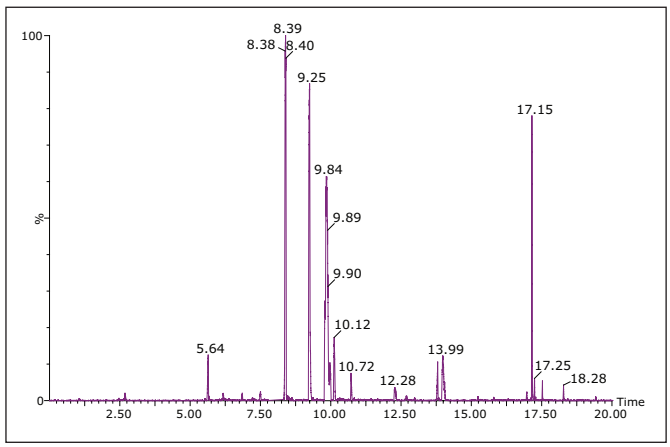

Figure 5. Survey Scan UPLC/MS analysis of quetiapine for ion m/z 221 in positive ion mode.

The MS/MS spectra obtained from the peak eluting with a retention time of 5.6 minutes is displayed in Figure 6. This impurity has a *m/z* value of 400 amu and has been identified as the S-Oxide impurity of quetiapine.

ScanWave Technology

The detection of low-level impurities is becoming increasingly important, especially when monitoring potential genotoxins. Collection of the MS/MS spectrum from Survey Scan experiments, either precursor ion or common neutral loss, can be performed in two modes of operation: standard MS/MS or ScanWave MS/MS.

As described previously, ScanWave Technology allows for increased sensitivity in the collection of MS/MS data. This increase in sensitivity is illustrated by the MS spectra obtained for the desthanol impurity of quetiapine (Figure 7). The top spectrum is obtained in standard MS/MS mode, while the lower spectrum is obtained in ScanWave MS/MS mode. In this example, we can see that the ScanWave MS/MS data is 13 times more sensitive than that in standard MS/MS mode. This increase is essential for the correct confirmation or identification of low-level impurities.

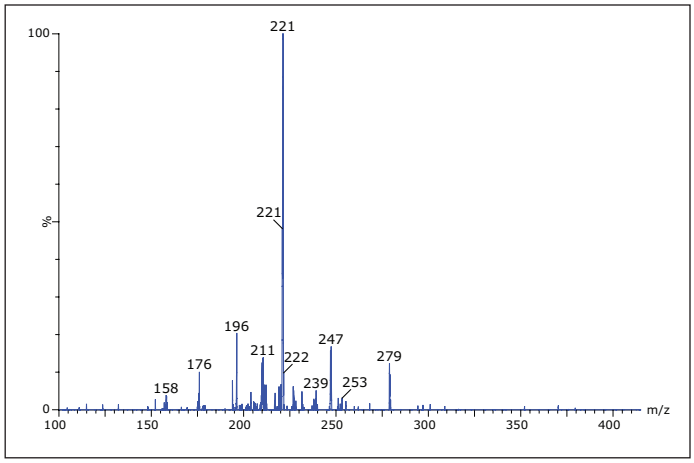

Figure 6. AutoScanWave MS/MS spectrum of S-Oxide of quetiapine eluting at 5.6 minutes.

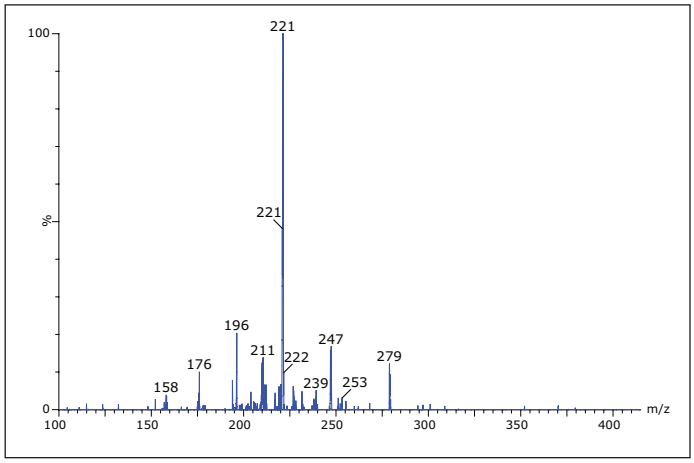

Figure 7. Comparison of standard and ScanWave MS/MS sensitivity.

CONCLUSIONS

- The Xevo TQ MS provides unrivaled levels of sensitivity and functionality.
- The high data-capture rates of the instrument, and its unique collision cell design and ScanWave Technology, allows the maximum amount of data to be collected in one analytical run.
- This reduces the number of experiments needed to make a decision, allowing impurities to be detected and identified quicker, and making maximum use of instrumentation.
- The rapid switching between MS and MS/MS possible with the Xevo TQ MS allows the collection of qualitative data and quantitative data in the same analytical run.
- The instrument's high data-capture rate ensures that, even with the narrow peaks of 2 to 3 seconds produced by today's modern sub-2-µm particle LC systems, sufficient points can be collected across for accurate quantification.
- The use of ScanWave Technology ensures that even the lowest-level peaks are detected and MS/MS spectra acquired, ensuring comprehensive impurity detection.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com