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Applikationsbericht

Simultaneous Confirmation and Quantification using Xevo TQ MS: Product Ion Confirmation (PIC)

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Abstract

This application note demonstrates simultaneous confirmation and quantification using Xevo TQ MS.

Introduction

Tandem quadrupole mass spectrometers are used extensively in the pharmaceutical industry for analyte quantification. This is primarily performed by multiple reaction monitoring (MRM) as the matrices are complex and the specificity of MRM gives the best signal-to-noise ratios.

As well as performing quantification, these instruments are often used for initial qualitative information, with the instrument operated in scan mode. This information is used to confirm the identity of the peak of interest that is being quantified.

In complex matrices, situations can arise where closely-related compounds, e.g., metabolites or matrix interferences, can give rise to signals even in MRM mode. This can lead to ambiguity and may require a second qualitative experiment. Product ion confirmation provides a means of verifying that the signal from the MRM peak is from the compound of interest.

With conventional instrumentation, these experiments require separate full-scan analyses. Many conventional tandem quadrupole MS instruments are unable to perform MRM and scan experiments simultaneously, in the timeframe of an LC peak, while maintaining data quality. The Waters Xevo TQ Mass Spectrometer is equipped with a novel collision cell design. The collision gas is always on, allowing both quantification (MRM) and characterization to be performed simultaneously on the peak as it elutes from the LC or UPLC Column while maintaining good data quality.

The new ScanWave mode of operation allows ions within the collision cell to be accumulated and then separated according to their mass-to-charge (m/z) ratio. Synchronizing the release of these ions with the scanning of the second quadrupole mass analyzer greatly improves duty cycle, which significantly enhances the signal intensity of full-scan spectra for both MS and product ions.

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

Results and Discussion

Product Ion Confirmation on Xevo TQ MS

The Xevo TQ MS can simultaneously acquire a product ion confirmation (PIC) scan along with an MRM chromatogram to obtain additional information about an eluting peak. A PIC scan is enabled in the MRM method, where a scan is used to collect either:

- MS scan
- Enhanced MS scan using ScanWave mode
- Product ion scan
- Enhanced product ion scan using ScanWave DS mode

In PIC mode, the Xevo TQ MS will switch from MRM to scan after the apex of an LC peak as long as a minimum intensity threshold is achieved. The trigger to start will occur after four consecutive downward scans have been detected. If the minimum intensity criteria is met, an MS or MS/MS spectrum is acquired using the final resolving quadrupole (MS2) to perform the scan before switching back to MRM mode (Figure 2). The threshold ensures that the PIC scan is of sufficient quality to be beneficial to the user.

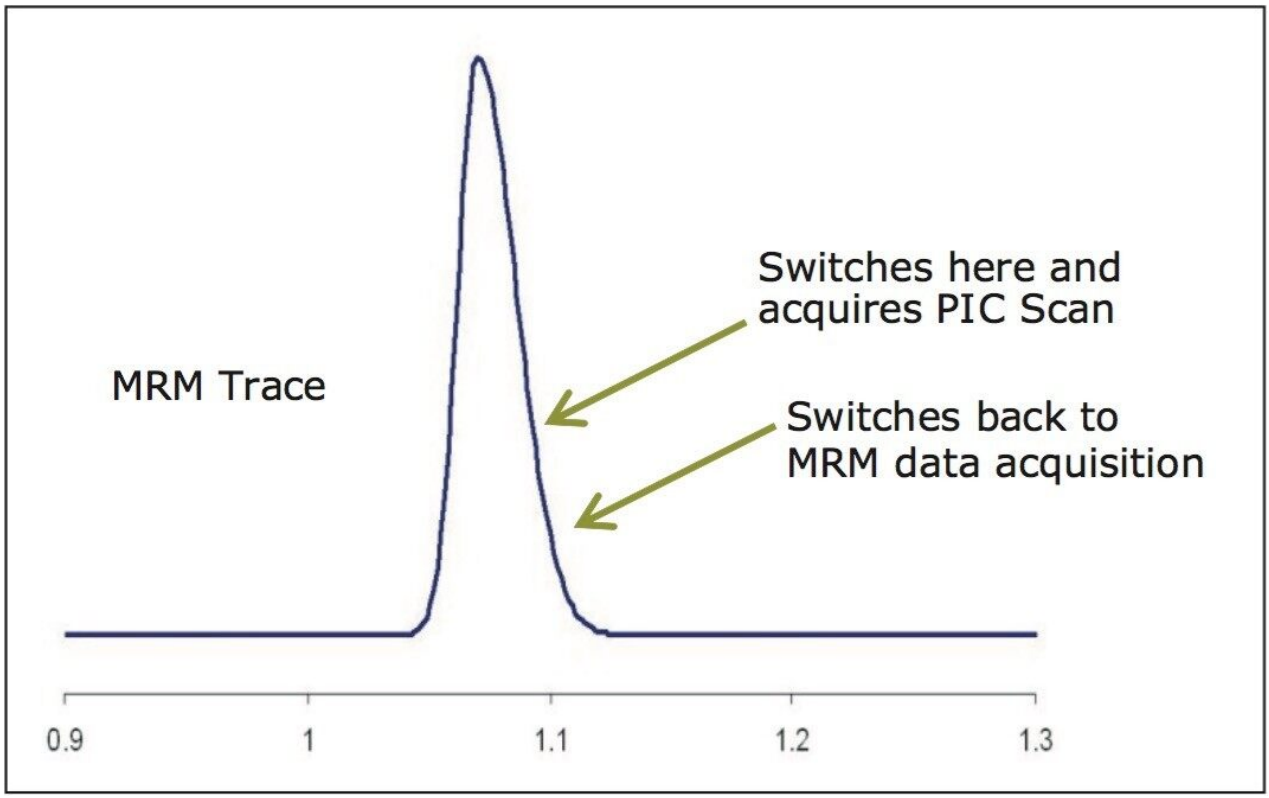

Figure 2. Schematic showing Product Ion Confirmation (PIC) switching after the peak top.

The high data collection rate of the Xevo TQ MS is such that the area of the MRM peak can still be accurately determined, since PIC is triggered after the peak top is detected and the definition of the peak itself is not affected. Consequently, quantitative and qualitative data are acquired simultaneously.

Figure 3 shows an example of an MRM chromatogram (3A) obtained from the quantification of the corticosteroid fluticasone, *m/z* 501. Qualitative confirmation of the peak of interest is provided by the resulting PIC spectrum operated in ScanWave DS mode (3B). The scan range for the PIC is selected by the software, in this case *m/z* 40 to 511.

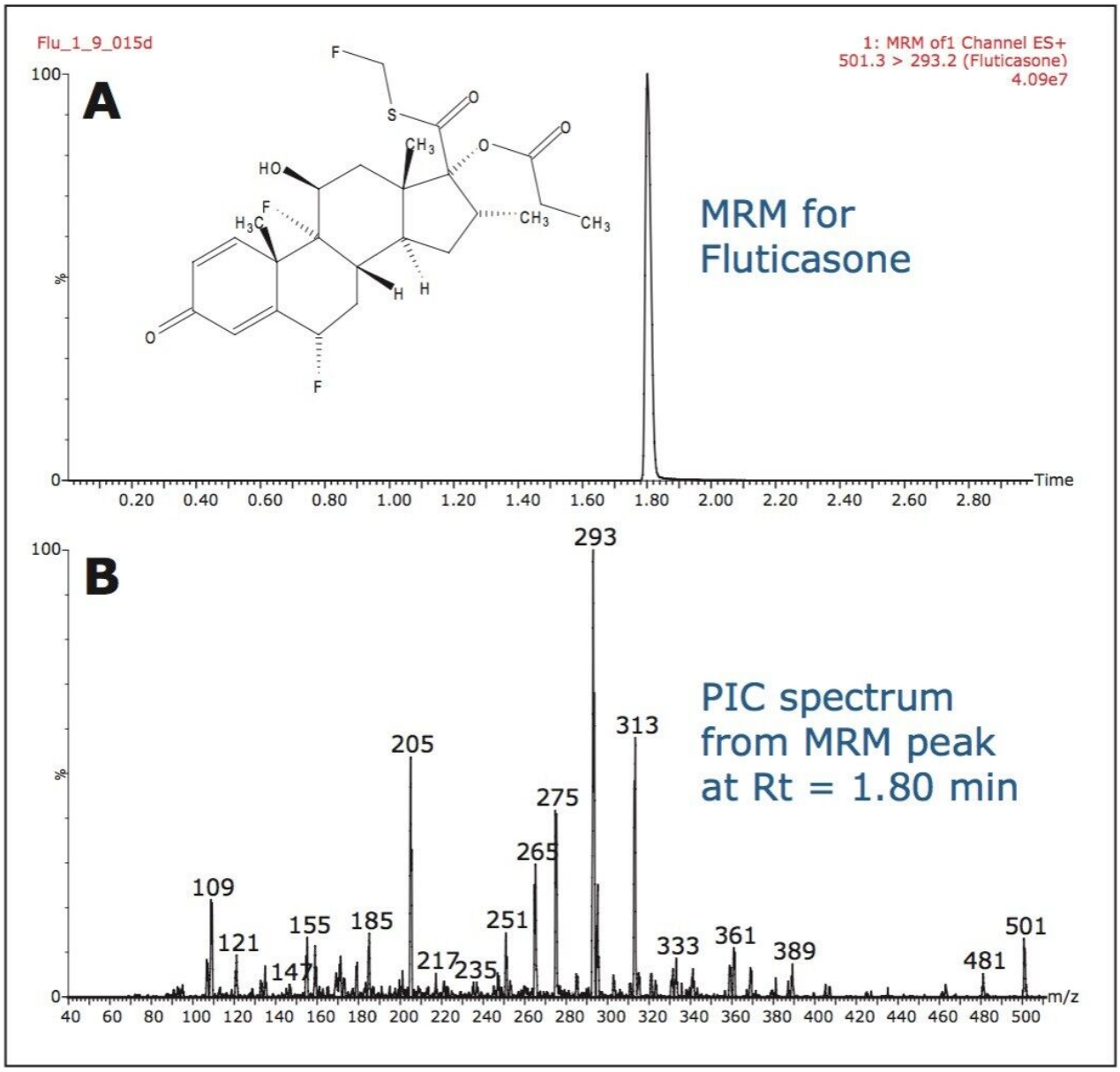

Figure 3. Chromatogram from the analysis of fluticasone, with MRM 501 > 293, and an example of the ScanWave DS PIC spectrum.

A PIC spectrum using ScanWave DS is displayed in Figure 4A. Here it is been compared with a PIC spectrum using conventional product ion scan (DS), 4B, and a combined spectrum (20 scans) from a ScanWave DS of fluticasone, 4C. The spectral quality is maintained when a PIC spectrum in ScanWave DS mode (4A) is compared to a combined ScanWave DS spectrum (4C).

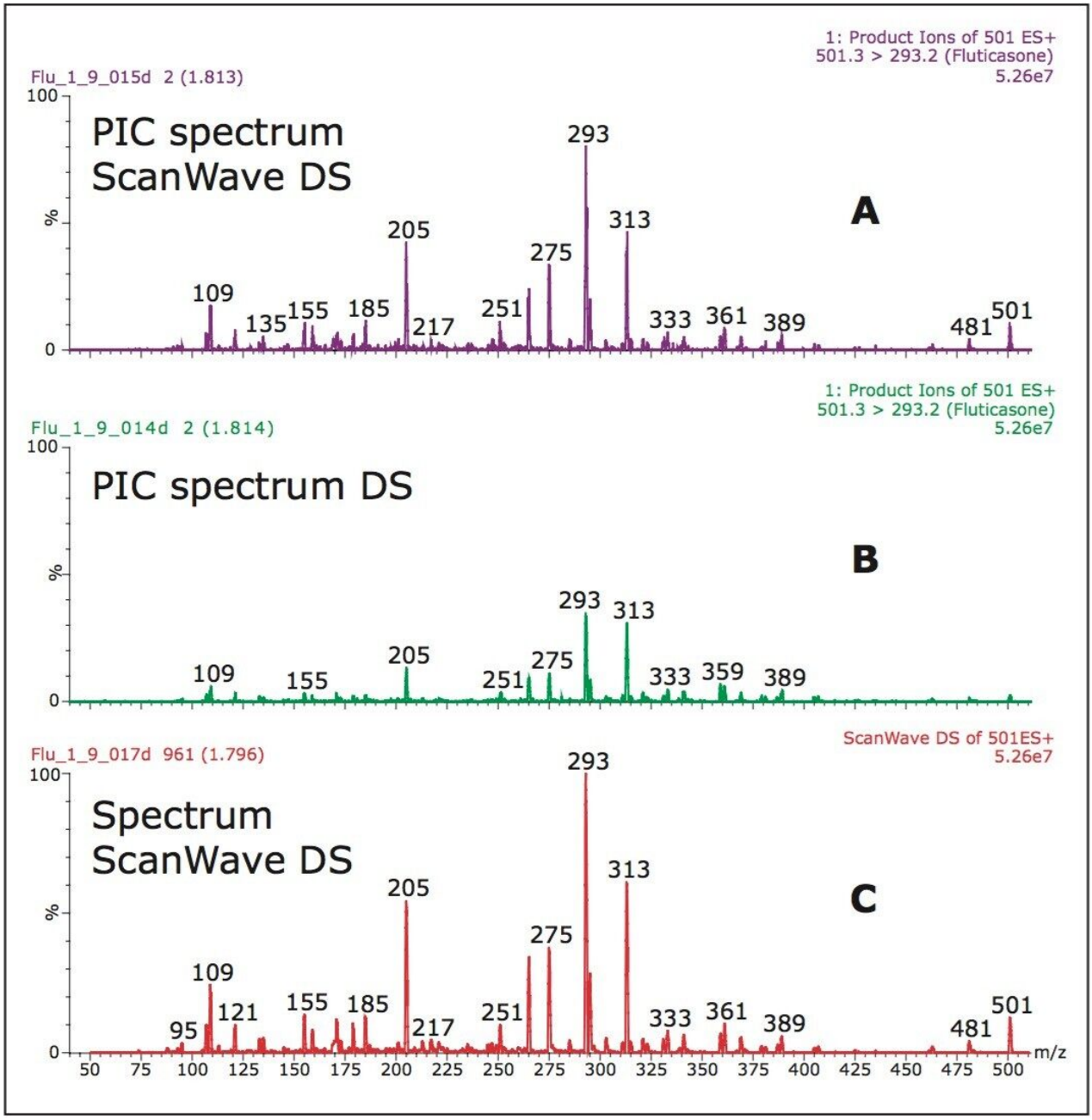

Figure 4. Spectrum shows a comparison of a PIC spectrum for ScanWave DS, a regular product ion PIC spectrum and a combined spectrum acquired by ScanWave DS for fluticasone m/z 501 (Vertical axis linked).

The data show that a four-fold signal enhancement was observed when ScanWave DS mode (4A) is used to collect the PIC spectrum compared to a conventional product ion spectrum (4B). This is due to the more efficient duty cycle that is achieved in ScanWave mode.

This extra sensitivity available with ScanWave mode allows for high quality spectra to be obtained even at

low levels.

Conclusion

The Xevo TQ MS can be used to perform quantification of fluticasone with simultaneous characterization of the MRM peak as it elutes from the chromatographic system. This eliminates the need for separate injections when qualitative confirmation of MRM peaks is required and reduces the total analysis time in these situations. When used routinely, product ion confirmation increases user confidence in qualitative results from complex matrixes, and thus reduces the need for re-analysis.

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