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应用纪要

Confirming Peak Identification in Bioanalytical Studies Utilizing Xevo TQ MS Product Ion Confirmation

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Introduction

The accurate quantification of candidate pharmaceuticals in bioanalysis relies on the use of sensitive, specific methodology. The advent of atmospheric pressure ionization sources, in the late 1980s, allowed the simple interfacing of liquid chromatography with tandem quadrupole mass spectrometry. The selectivity and sensitivity provided by LC/MS/MS has made it the technique of choice for bioanalytical studies.

Bioanalytical scientists have relied on the specificity and sensitivity of the multiple reaction monitoring (MRM) MS acquisition mode to ensure that the correct analyte is quantified. However, even with the specificity of MRM analysis and modern sample preparation techniques, such as solid phase extraction, extraneous peaks can interfere during sample analysis. The appearance of an extra peak during MRM analysis could be due to chromatographic peak splitting, associated drug metabolites, or matrix interferences.

The occurrence of extra peaks during analysis would require additional experiments to correctly identify the analyte of interest. With a conventional tandem quadrupole mass spectrometer, this would require

separate analysis to acquire full scan MS or full scan MS/MS spectra to confirm the identity of the peaks in question. With the introduction of the Waters® Xevo™ TQ MS, both MRM and full scan MS or product scan MS/MS can now be acquired during a single injection.

Figure 1. The ACQUITY UPLC System with the Xevo TQ MS.

Experimental

Rat plasma was spiked with fluticasone propionate, a corticosteroid used for the treatment of asthma. The sample was then prepared by solid phase extraction utilizing an Oasis® HLB µElution plate. The sample was analyzed using UltraPerformance LC® (UPLC®) coupled with tandem mass spectrometry.

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temperature:	45 °C

Flow rate:	500 µL/min
Mobile phase A:	0.1% NH4OH
Mobile phase B:	Methanol
Gradient:	15 to 95 %B/1 min

MS Conditions

MS system:	Waters Xevo TQ MS
Ionization mode:	ESI positive
Capillary voltage:	3000 V
Cone voltage:	30 V
Desolvation temperature:	600 °C
Source temperature:	150 °C
Desolvation flow:	1000 L/Hr
Collision energy:	17 V
MRM transition:	m/z 501 > 293

Product Ion Confirmation (PIC)

The novel collision cell design of the Xevo TQ MS allows for simultaneous acquisition of MRM and full scan MS data. Product Ion Confirmation (PIC) takes advantage of this capability to collect a high-quality full scan MS or MS/MS spectrum during MRM acquisition.[1] This MS or MS/MS spectrum is acquired after

the apex of the MRM peak and before the return to baseline, Figure 2.

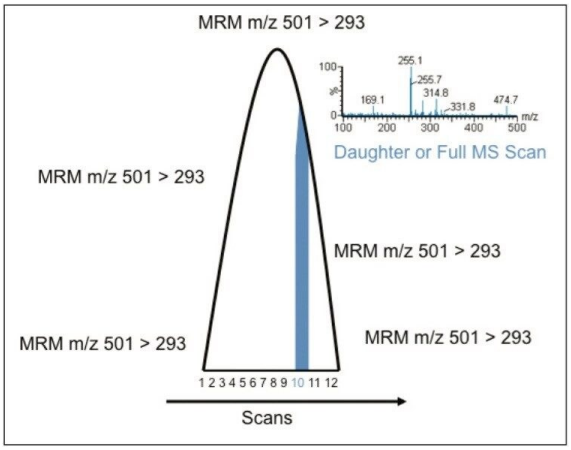

Figure 2. Schematic illustrating PIC data acquisition.

Furthermore, the fast data capture rate of the Xevo TQ MS allows for multiple full scan MS or MS/MS spectra to be acquired across the narrow, 2- to 3-second-wide chromatographic peaks that are typically generated during analysis when coupled with UPLC.

Results and Discussion

During the development of an LC/MS/MS method for fluticasone propionate, two peaks with the same MRM transitions were detected, Figure 3.

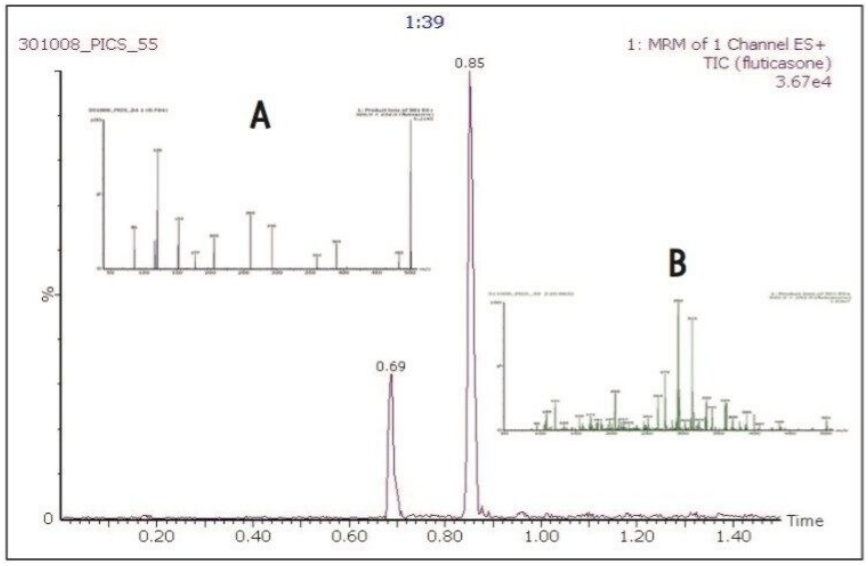

Figure 3. Analysis of rat plasma spiked with 100 ng/mL fluticasone propionate.

The product ion scan spectra simultaneously generated by the PIC function during analysis is displayed in Figure 3. Spectra (A) is that obtained from the peak eluting with a retention time of 0.69 minutes. Spectra (B) is that obtained from the peak eluting with a retention time of 0.85 minutes.

Each of the product ion scan spectra generated from the analysis were then compared to the product ion scan spectra obtained during sample tuning of the fluticasone propionate standard, Figure 4.

Here we can see that comparison of the two spectra show good agreement, confirming that the peak with the retention time of 0.85 minutes is the analyte of interest, fluticasone propionate. Thus, in one analytical run, we were able to identify the peak at 0.85 minutes as the analyte of interest without the need for further analysis.

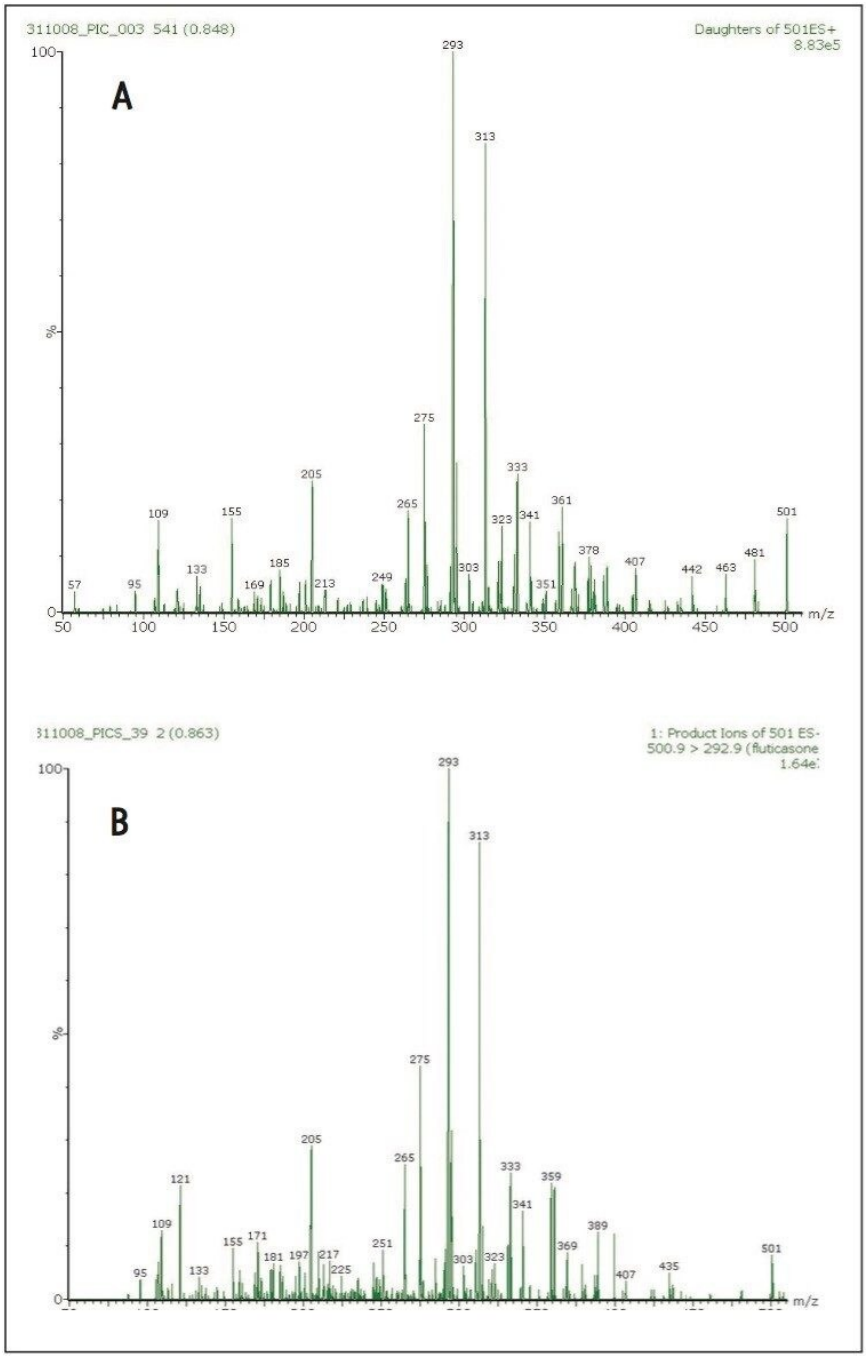

Figure 4. (A) Product scan comparison of fluticasone propionate standard and (B) Product scan acquired by PIC of analyte peak eluting at 0.85 min.

Conclusion

Multiple peaks with the same MRM transition can be observed during method development or routine analysis in bioanalytical studies. These spurious peaks can be generated by chromatographic peak splitting, metabolites, or matrix interferences. Product Ion Confirmation (PIC) functionality enables the confirmation of peak identity during MRM quantitative analysis by obtaining a quality product scan spectra during MRM MS analysis. The ability of the Xevo TQ MS to perform this function results in the reduction of further confirmatory experiments, saving time and money, which ultimately increases laboratory productivity. Furthermore, this function can be carried out with the narrow, 2- to 3-second-wide chromatographic peaks generated by UPLC.

References

1. Twohig M, Fujimoto G, Mather J, Plumb RS. Simultaneous Confirmation and Quantification using Xevo TQ MS: Product Ion Confirmation (PIC). Waters Application Note. 720002829, 2008.

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Xevo TQ Absolute Triple Quadrupole Mass Spectrometer < https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html>

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