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應用手冊

Data-Directed Detection and Confirmation of Drug Metabolites in Bioanalytical Studies

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Abstract

In this application note, we illustrate the ability of the Xevo TQ MS, in Survey Scan mode, to detect drug

metabolites “on the fly” using a common diagnostic fragment ion.

Introduction

LC-MS/MS analysis has become the analytical method of choice for the accurate quantification of pharmaceutical compounds or active metabolites in biological fluids. The specificity and selectivity provided by tandem quadrupole MS in multiple reaction monitoring (MRM) mode allows for rapid high-sensitivity analysis, often in the pg/mL range. The data produced by LC-MS/MS analysis provides drug concentration data that is critical to successful drug discovery and development.

Recent U.S. FDA Guidance, “Industry Safety Testing of Drug Metabolites,” provides recommendations to industry on when and how to identify and characterize drug metabolites whose non-clinical toxicity needs to be evaluated. The aim of these guidelines is to ensure that variations in metabolic profiles across species are both quantitatively and qualitatively measured.[1]

The Waters Xevo TQ Mass Spectrometer is capable of operating at acquisition speeds up to 10,000 Da/sec, which aids in the adequate characterization of very sharp chromatographic peaks produced by the ACQUITY UltraPerformance LC (UPLC) System. The Xevo TQ MS is equipped with a novel collision cell design that is continuously filled with collision gas, allowing rapid switching between MS and MS/MS modes in a single analytical run.

This new collision cell is capable of enhanced high-sensitivity operation in MS/MS mode. In this Scanwave mode of operation, ions are constrained in the final third of the collision cell using both a DC and RF barrier. 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.

In this application note, we illustrate the ability of the Xevo TQ MS, in Survey Scan mode, to detect drug metabolites “on the fly” using a common diagnostic fragment ion.

Figure 1. Xevo TQ Mass Spectrometer.

Experimental

Rat plasma was spiked with ibuprofen and related major metabolites. Samples were then precipitated using 2:1 acetonitrile to sample (v/v). The sample was evaporated to dryness and reconstituted in 9:1 water/methanol (v/v). The sample was then injected onto the UPLC-MS/MS System.

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Column temp.:	40.0 °C

Flow rate:	600 µL/min
Mobile phase A:	0.1% NH_4OH
Mobile phase B:	Acetonitrile
Gradient:	5% to 95% B/2 min

MS Conditions

MS system:	Waters Xevo TQ MS
Ionization mode:	ESI negative
Capillary voltage:	2000 V
Cone voltage:	15 V
Collision energy:	7 eV

Results and Discussion

The superior efficiency of the ACQUITY UPLC System produces extremely narrow peaks, 2 seconds or less at the base. These narrow peaks require a fast data capture rate mass spectrometer to accurately define the peak. Figure 2 shows the MRM peak for ibuprofen using the transition *m/z* 205 to 161. The peak is 1.2 seconds wide at the base, and the high data capture rate of the Xevo TQ MS allows for more than 60 scans across the peak. This facilitates the accurate definition of the chromatographic peak, even if several MRM transitions are employed during analysis.

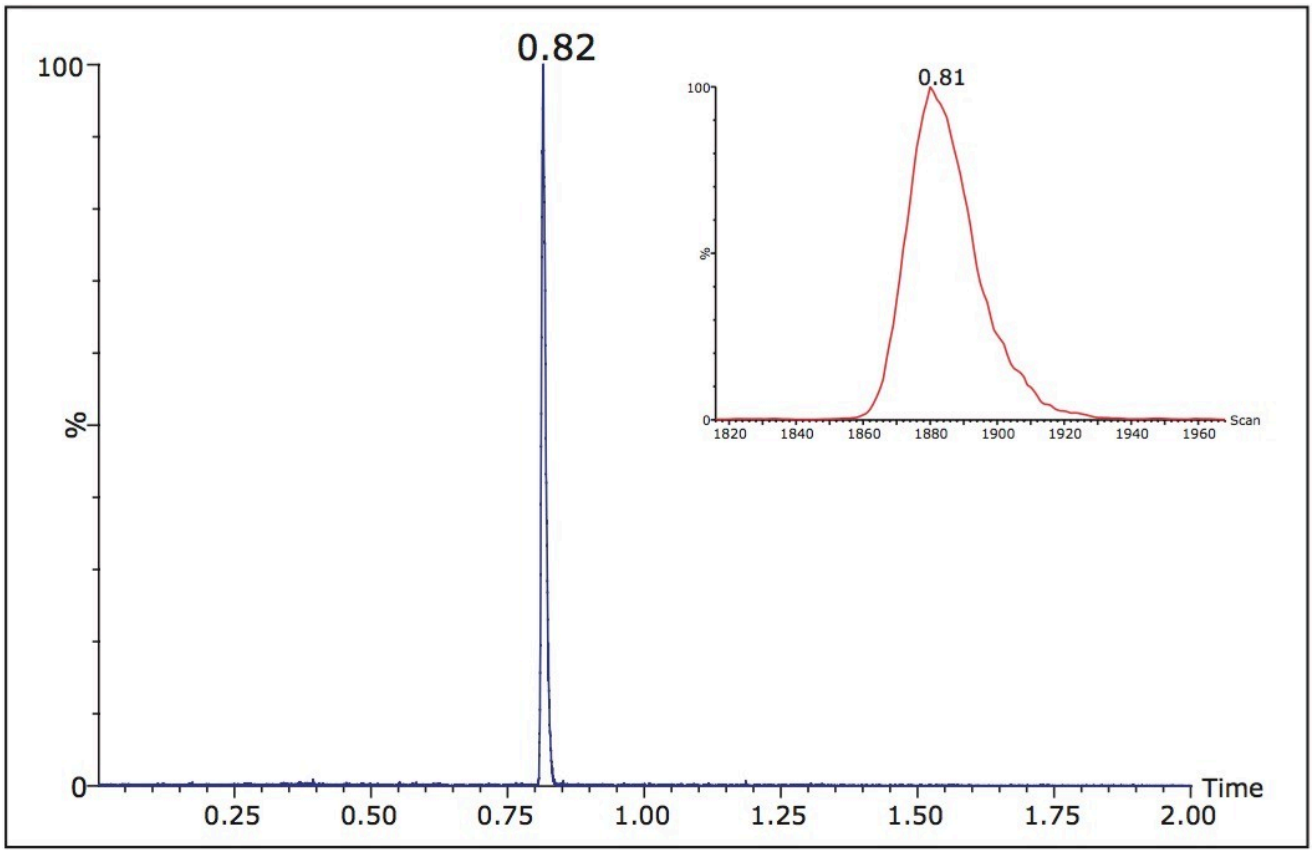

Figure 2. UPLC-MS/MS of ibuprofen using the MRM transition m/z 205 to 161.

Recent FDA guidelines have recommended that, during human clinical trials, the concentration and identity of any metabolites with an exposure of greater than 10% of the dosed compound must be determined. Mass spectrometry can detect and identify drug metabolites by various means. One method is to utilize Survey Scan mode. In this mode of operation, the MS is set to monitor a diagnostic fragment ion from the parent drug compound.

The use of a common fragment ion requires the mass spectrometer to scan the first quadrupole (Q1) while monitoring for a fixed *m/z* with the final resolving quadrupole (Q3). Ibuprofen gives rise to several distinctive product ions, *m/z* 113, 133, and 161.[2] Figure 3 illustrates Xevo TQ MS operation in Survey Scan mode.

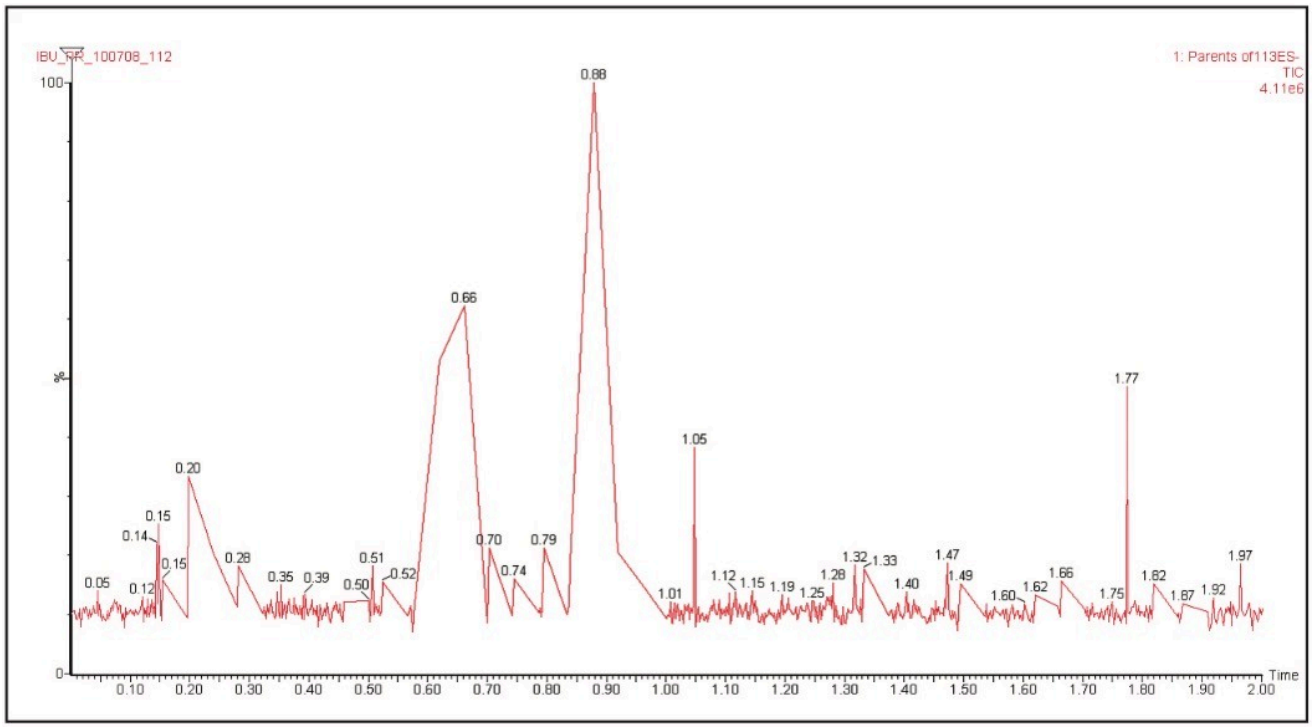

Figure 3. Survey Scan: precursors of m/z 113 switching to product ion scan.

In this example, the common fragment ion of *m/z* 113 was monitored by the resolving quadrupole. When a peak, containing a *m/z* of 113, was detected the MS switched to collect product ion data on the precursor ion containing the *m/z* 113. Peaks that exceed a user-defined detection threshold are used to trigger the acquisition of product ion data.

Figure 4 illustrates the MS/MS spectra obtained for the peak detected at 0.66 minutes. In this example, we can see that the precursor peak *m/z* value is 397. The *m/z* 397 produces major fragment ions at *m/z* 113, 175, 193, and 221.

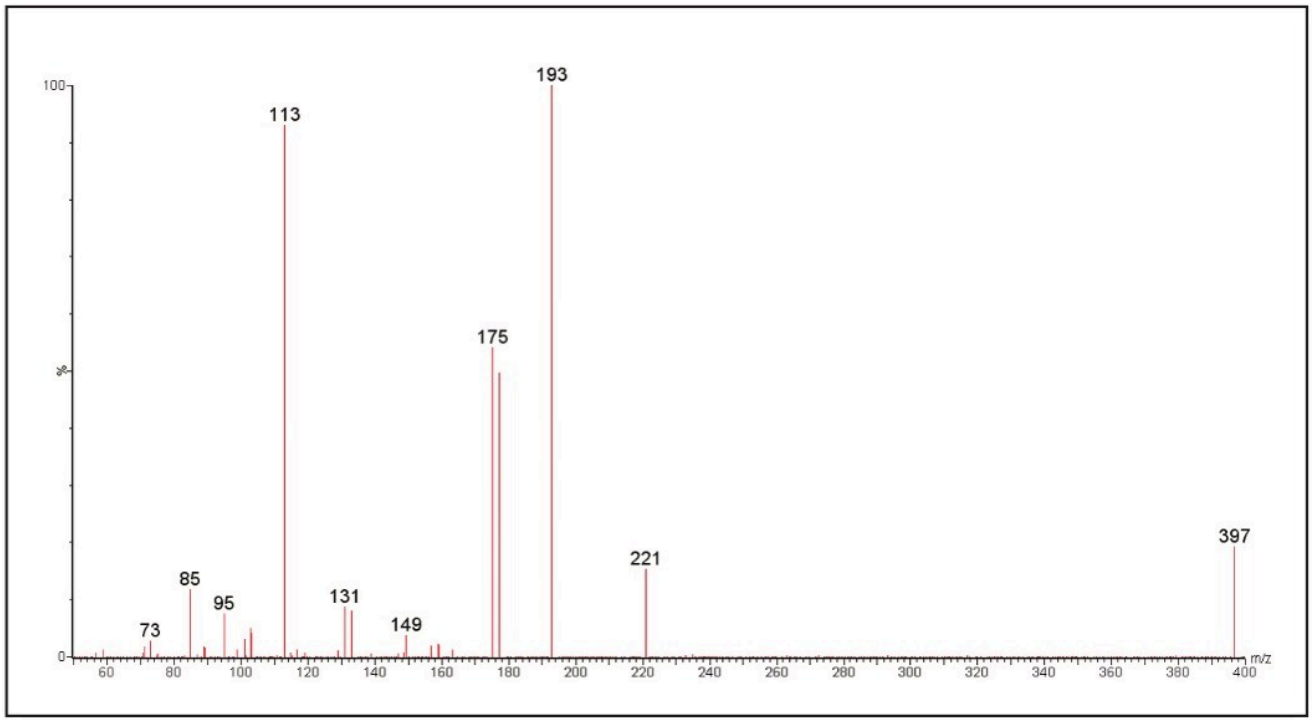

Figure 4. Survey Scan ScanWave DS spectrum of peak eluting at 0.66 minutes.

The *m/z* values and MS fragment pattern confirm the identity of this peak as the O-glucuronide metabolite of ibuprofen.[2] The data acquired for the peak eluting with a retention time of 0.88 minutes are shown below in Figure 5.

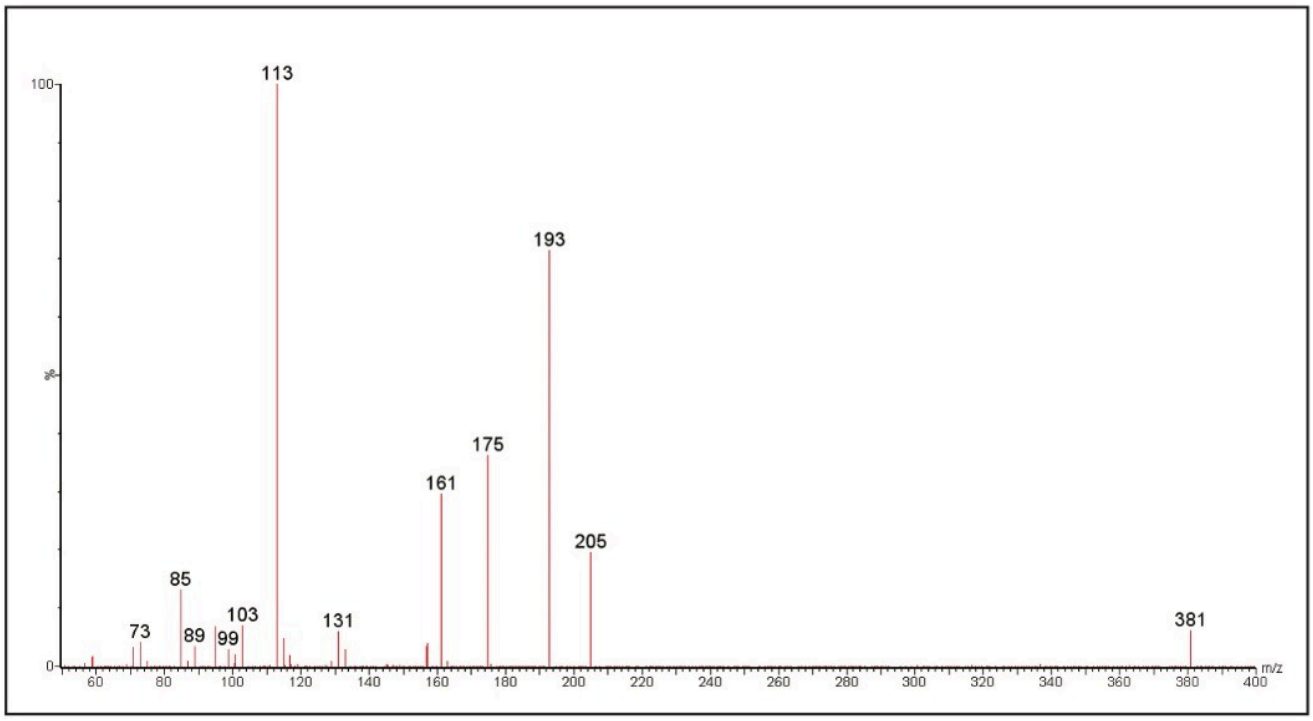

Figure 5. ScanWave DS of peak eluting at 0.88 minutes with a m/z value of 381.

This peak was determined to have a *m/z* value of 381. Resulting fragment ions produced from the product ion MS/MS were *m/z* 113, 161, 175, 193, and 205. This data confirmed that this peak was related to ibuprofen and, with the precursor ion *m/z* value of 381, was confirmed as the glucuronide conjugate of ibuprofen.[2] Thus with one simple analytical experiment, along with the knowledge of the fragmentation pattern of the ibuprofen, the metabolites could be detected and the structure confirmed.

Conclusion

The quantification of pharmaceutical compounds in biological fluids is a regulatory requirement as part of any new drug submission, e.g., IND, CTX. More recently, these regulations have required that drug metabolites with an exposure greater than 10% of the active pharmaceutical be quantified and characterized. The Xevo TQ MS can perform data-directed MS/MS experiments, allowing metabolite structural confirmation using common fragment ions within a UPLC peak timeframe.

References

1. U.S.FDA. Guidance for Industry, “Safety Testing for Metabolites.” http://www.fda.gov/CDER/GUIDANCE/6897fnl.pdf

2. Plumb R., Rainville P, *et al. Rapid Communications in Mass Spectrometry*. 2007; 21: 4079–85.

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