

## Dual Scan MRM Mode: A Powerful Tool for Bioanalytical LC-MS/MS Method Development

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### Abstract

This application note describes the use of dual scan MRM for the development of an assay for alprazolam in rat plasma.

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### Introduction

The inherent sensitivity and specificity of LC-MS/MS has made it the technique of choice for bioanalysis. However, as compounds become more potent and are dosed at lower levels the resultant circulatory levels also fall. This requires accurate and precise methods capable of analyzing analyte concentrations in the pg/mL range in plasma.

The development of a bioanalytical method at these low levels can be a time-consuming process, requiring the careful balance of mass spectrometry, chromatographic separation, and sample preparation. In early discovery

the development of such a method may not present too much of an issue, due to the higher dosing levels and reduced assay validation needs. However, as compounds progress into development, the need for a more sensitive, robust assay increases and method development becomes critical.

The development of a bioanalytical method is normally an iterative process. It is often necessary to adjust one part of the method to take account or address a finding from other stages. For example, if analyte response in the mass spectrometer is significantly greater at one pH or in one organic solvent than another, then it is appropriate to perform the chromatography using these conditions. Additionally, if there is an interference that cannot be removed by solid-phase extraction (SPE) or liquid/liquid extraction, then it may be necessary to modify the chromatography conditions or employ a different MS multiple reaction monitoring (MRM) transition.

All of these individual components in a bioanalytical assay have to be evaluated and often require both MRM and full-scan MS data to identify and resolve issues. With a conventional tandem quadrupole mass spectrometer, this often requires two or more separate analytical runs as the collision cell must be emptied for full-scan MS data acquisition and to be filled with collision gas for MRM analysis, and thus reducing productivity.

The Waters Xevo TQ MS is a tandem quadrupole mass spectrometer 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.<sup>1</sup> This novel capability can be used to simplify the bioanalysis method development process, allowing both the analyte's response to changes in methodology to be monitored, and also any background interferences to be detected and identified. The rapid data acquisition rates of the Xevo TQ MS allow full-scan MS data to be acquired while still collecting a sufficient number of points across the analyte peak, in MRM mode, for accurate quantification. This application note describes the use of dual scan MRM for the development of an assay for alprazolam in rat plasma.

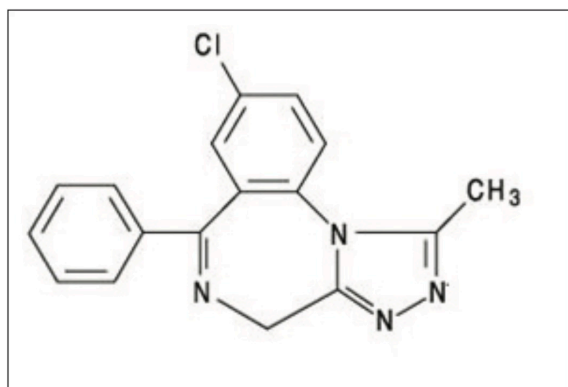


Figure 1. Structure of alprazolam.

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## Experimental

### LC-MS Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C <sub>18</sub> Column 2.1 x 50 mm, 1.7 $\mu$ m
Column temp.:	45 °C
Flow rate:	500 $\mu$ L/min
Mobile phase A:	0.1% ammonium hydroxide
Mobile phase B:	Methanol
Gradient:	5 to 95% B/2 min

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MS system:	Waters Xevo TQ MS
MS/MS transition:	309 $\Rightarrow$ 281
Full scan:	150 to 800 <i>m/z</i>
Ionization:	Positive ion ESI
Capillary voltage:	1.0 KV
Collision energy:	18 eV
Cone voltage:	40 V

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## Results and Discussion

Alprazolam is a triazolo analog of the 1,4 benzodiazepine class of central nervous system-active compounds. It is indicated for the management of anxiety disorders or the short-term relief of symptoms of anxiety. A typical 2 mg daily dose results in peak plasma levels of 8 ng/mL with a half life of 11 hours. The major metabolites formed are the p450 3A mediated 4-hydroxyalprazolam and  $\alpha$ -hydroxyalprazolam. Due to the low circulating levels of alprazolam, an assay in the pg/mL range is necessary to accurately define the compound's pharmacokinetics. Initial evaluation showed that the use of a basic aqueous/methanol gradient gave the maximum MS signal response in positive ion mode using the MRM transition 309  $\Rightarrow$  281.

The simplest form of sample preparation is protein precipitation. The resulting MRM and full-scan data obtained from this sample preparation approach is displayed in Figure 2. In this example, the alprazolam analyte peak is monitored in MRM mode, the plasma phospholipids are monitored using precursors of *m/z* 184, and the remaining endogenous compounds by full-scan MS. Here we can see that the analyte peak is clearly resolved from the lipid fraction and the phospholipids, which are known to cause ion suppression.

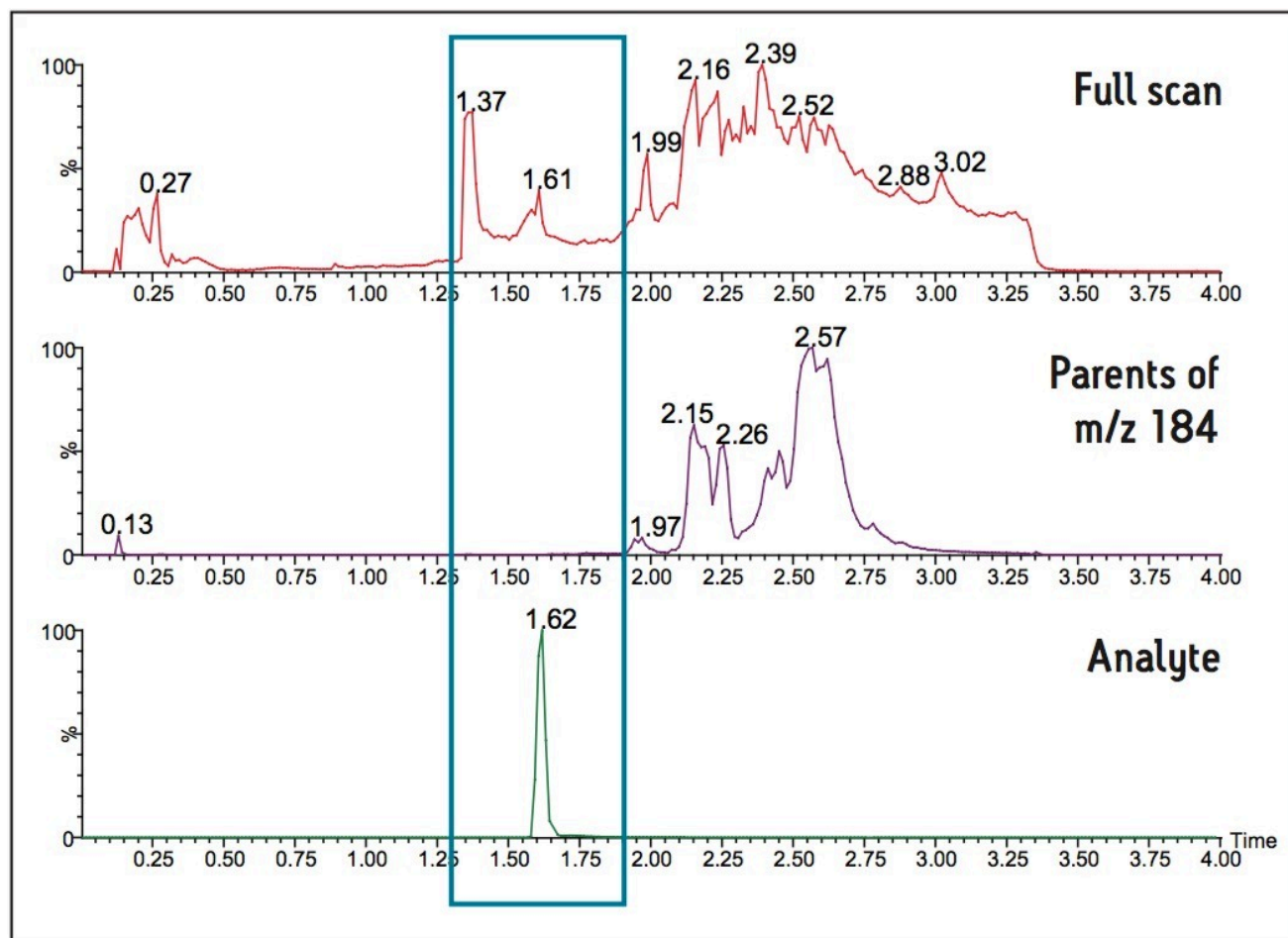


Figure 2. LC-MS/MS chromatogram of alprazolam (bottom) with lipid fraction (middle) and full-scan MS trace of endogenous matrix (top).

However, a careful investigation of the full-scan MS data revealed an endogenous peak eluting at the same retention time as alprazolam. This analyte had a parent mass of  $m/z$  329. An extracted ion chromatogram of  $m/z$  329 clearly shows that this peak coelutes with the analyte peak. This coelution could well be responsible for ion suppression, limiting the assay sensitivity, Figure 3.

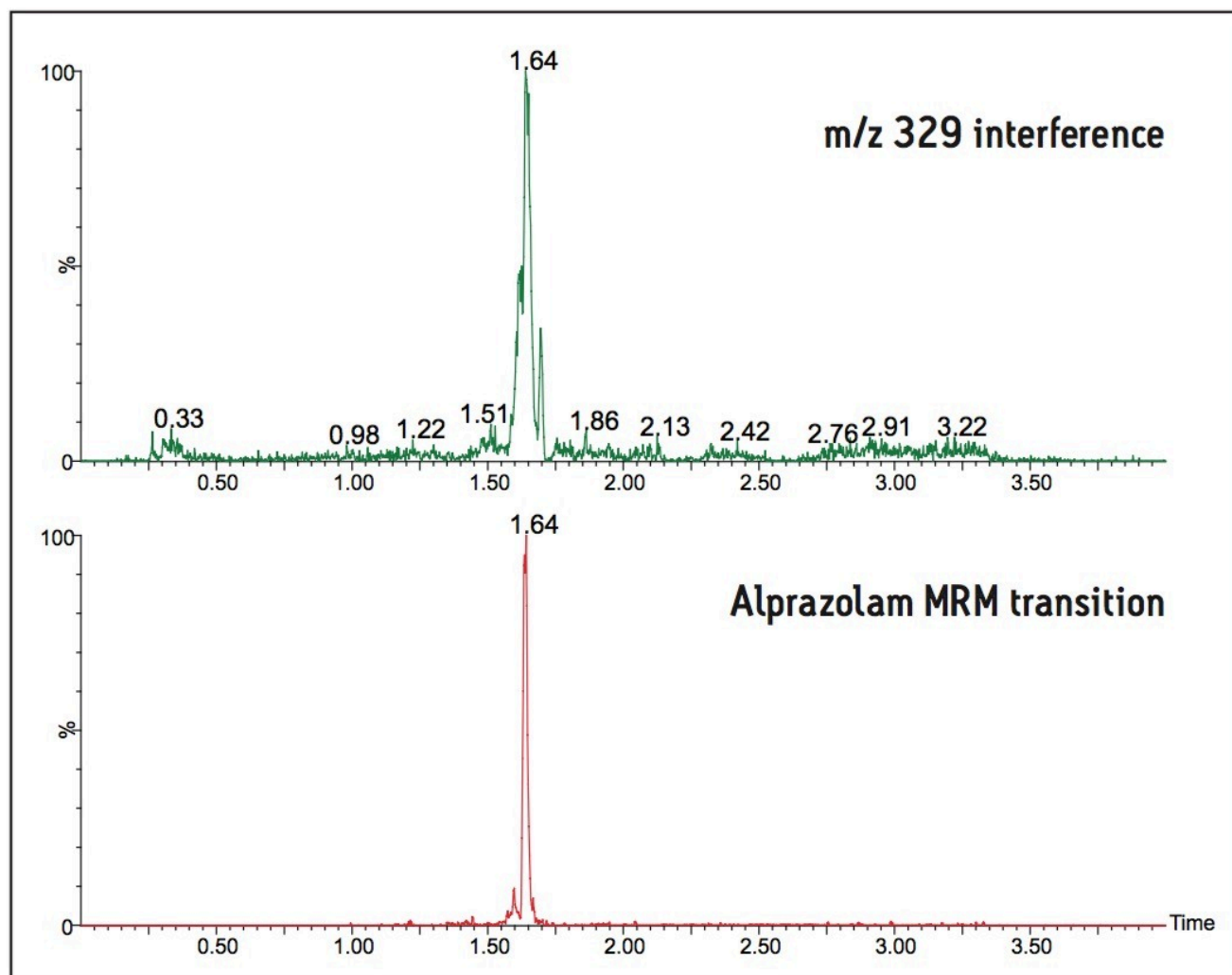


Figure 3. LC-MS/MS of alprazolam (bottom) and extracted ion chromatogram  $m/z$  329 (top) after protein precipitation.

To eliminate this interfering plasma peak, an SPE method was developed using Oasis MCX. The LC-MS chromatogram produced using this approach is displayed in Figure 4. In this data, we observe that the method was successful in removing the coeluting matrix component, reducing the potential for matrix effects. This improved assay allowed for a limit of detection of 5 pg/mL to be achieved.

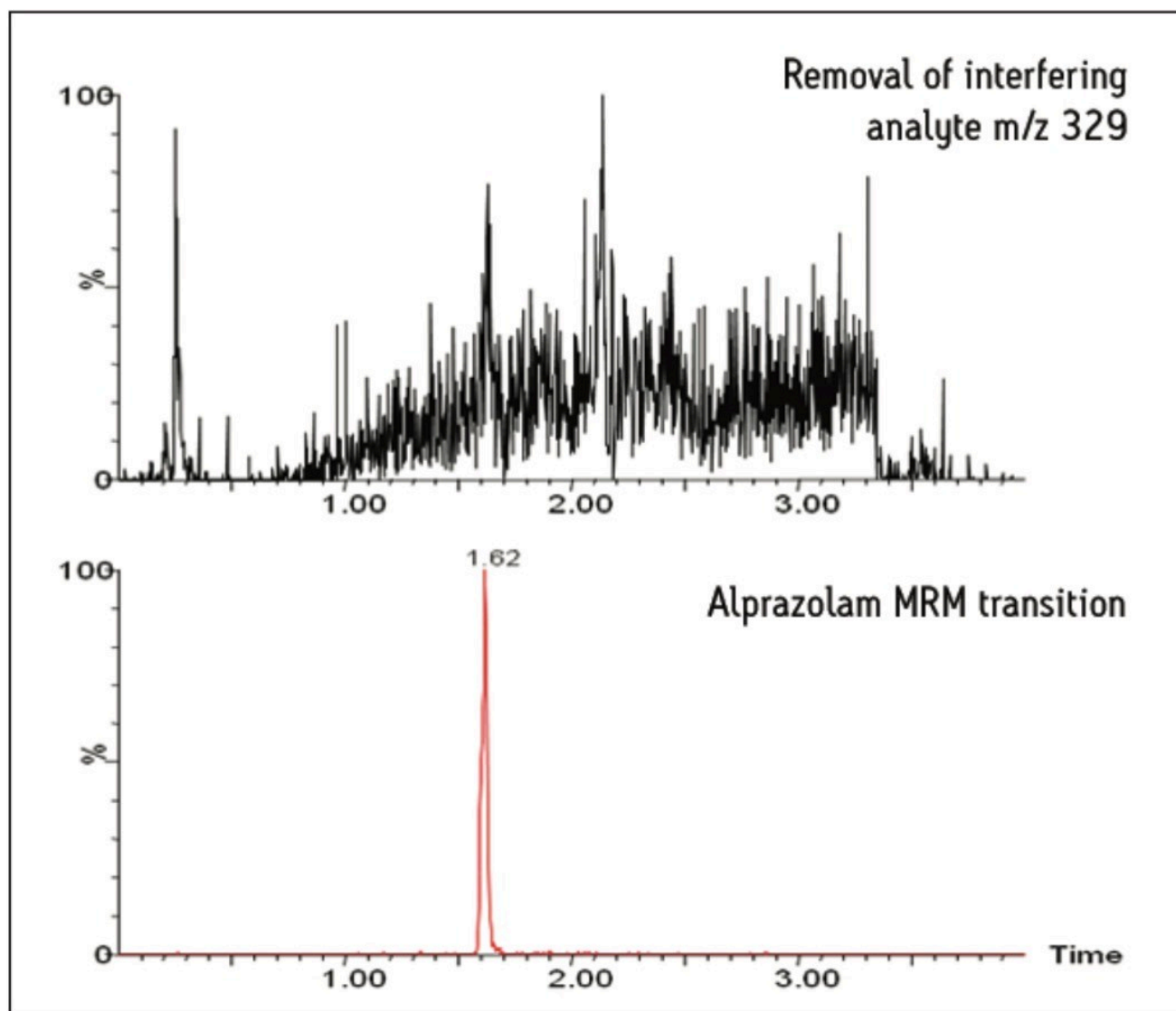


Figure 4. LC-MS/MS chromatogram of alprazolam (bottom) and extracted ion chromatogram m/z 329 (top) following SPE.

To ensure the assay specificity, it is necessary to monitor drug metabolites and ensure that they are resolved from the parent drug. Metabolite detection can be achieved by the use of full-scan MS or common fragment ion detection. The novel collision cell design of the Xevo TQ MS allows for the simultaneous collection of MRM, full-scan MS, and common fragment ion data. The data displayed in Figure 5 shows the simultaneous acquisition of MRM, full-scan MS, and common fragment ion data for the analysis of alprazolam in plasma.

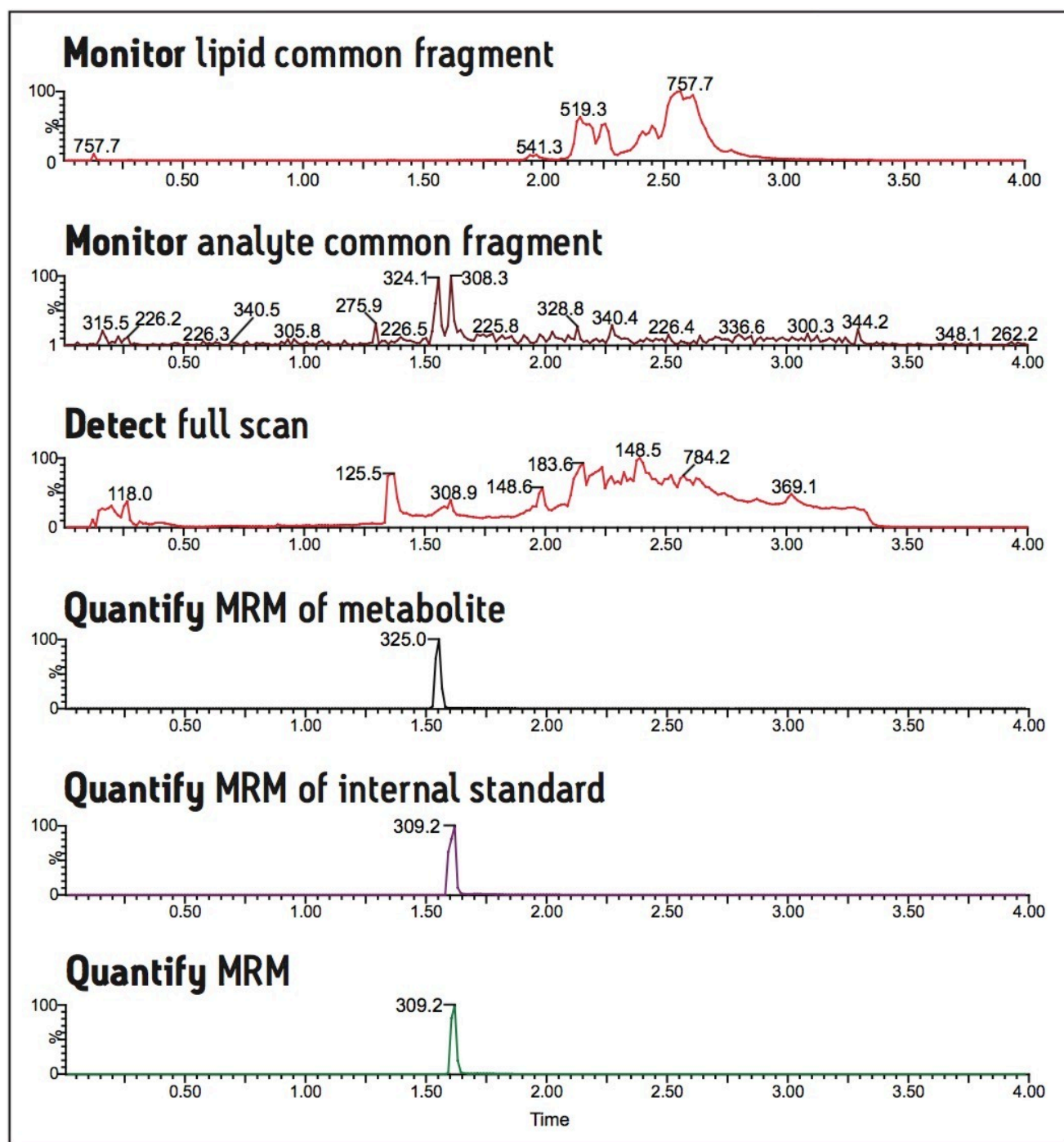


Figure 5. Simultaneous acquisition of MRM, full-scan MS, and common fragment ion data.

Using this approach, the  $\alpha$ -hydroxy metabolite of alprazolam was clearly detected by the MRM with a retention



time of 1.55 minutes. This metabolite was also detected using the common fragment  $m/z$  281. The full-scan MS data was used to confirm the identity of the metabolite. The data in Figure 5 confirms that of the metabolite is well-resolved from the parent drug using a generic UPLC gradient, thus eliminating the possibility of an over-estimation of drug compound.

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## Conclusion

- Utilizing the Xevo TQ MS's dual scan MRM mode allowed for the detection of coeluting analytes from the plasma matrix in a single injection, reducing time and the need for additional analytical runs during the method development process.
- Oasis MCX was employed to effectively isolate alprazolam from interfering components in the plasma matrix.
- Endogenous metabolite information was obtained in a single injection with dual scan MRM mode.
- The major metabolite of alprazolam was well-resolved from the parent drug utilizing a generic UPLC gradient.
- Dual scan MRM allows full scan data to be acquired simultaneously with MRM, which allows matrix monitoring for method development as well as discovery of non-targeted compounds.
- The Xevo TQ MS and its dual scan MRM, used in conjunction with ACQUITY UPLC, improves productivity in bioanalytical method development.

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## References

1. Twohig M, Alden P, Fujimoto G, Kenny D, Plumb RS. Improving MS/MS Sensitivity using Xevo TQ MS with ScanWave. Waters Corporation. 2008; [720002828en](https://www.waters.com/webassets/cms/library/docs/720002828en.pdf) <  
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