

High Sensitivity Bioanalysis using the Waters™ Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

A reproducible, high sensitivity, ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) bioanalytical methodology is demonstrated using the Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer. A simple protein precipitation sample preparation was used for the detection of midazolam and imipramine in rat plasma at the 250 ag on column level. Precision and accuracy data analysis demonstrated that the limit of quantification for midazolam and imipramine in rat plasma was 0.2 pg/mL.

Benefits

- Robust, reproducible, highly sensitive bioanalysis
 - Simple sample preparation
 - Use of market-leading Tandem Quadrupole Mass Spectrometer along with the latest in UPLC technology
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Introduction

Bioanalysis forms a critical part of the drug discovery and development process, providing information on pharmacokinetics and dose response for new chemical entities. Lower doses of more potent drug candidates, along with reduced sample sizes from more ethically driven studies, 3R's (reduce, replace, refine), mean that liquid chromatography-mass spectrometry (LC-MS) method sensitivity is increasingly important. Concurrent with this requirement for increased sensitivity is the need to seamlessly transfer assays between laboratories, either within an organization or to a contract analysis organization. Thus, not only is MS sensitivity important, so too is instrument to instrument reproducibility.

In this application note we demonstrate the reproducible, robust sensitivity achievable using the Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer as part of a UPLC-MS/MS system for bioanalytical studies. Sensitivity limits as low as 250 ag (0.25 femtograms) of midazolam and mipramine were detected on-column from a matrix sample (rat plasma) using a rudimentary, generic sample preparation technique (protein precipitation). This level of sensitivity was demonstrated to be reproducible and robust with limits of quantification determined to be 0.2 g/mL for both compounds.

Experimental

Control rat plasma was used to create calibration curves and quality control (QC) samples containing both midazolam and imipramine at various concentrations. A combined 10 ng/mL stock solution was prepared in 50:50 methanol:water by diluting methanolic 1 mg/mL solutions of each of the compounds, these solutions were used as spiking solutions to create the calibration lines and QCs and were purchased from Sigma Aldrich (UK). A freshly prepared calibration line (0.2–100 pg/mL) and 12 QC samples (4 x 0.2 pg/mL, 4 x 0.4 pg/mL, and 4 x 1.0 pg/mL) were prepared and analysed using the Xevo TQ Absolute. These samples were prepared using protein precipitation as follows; 100 μ L of sample was precipitated using 300 μ L of acetonitrile via vortex mixing. Following centrifugation at 25000 g, the supernatant was diluted 1:1 using deionized water. 10 μ L of this was then injected onto the UPLC-MS/MS system. The samples were separated on an ACQUITY UPLC 2.1 x 50 mm BEH C₁₈ 1.7 μ m Column using a reversed-phase gradient over 1.9 minutes using 0.1% formic acid in water with 10 mM ammonium formate as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. The mobile phase

effluent was monitored by positive ion ESI mass spectrometry operated in multi reaction monitoring mode (MRM).

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS System
Detection:	Xevo TQ Absolute
Vials:	1 mL 96-Well Plate
Column(s):	ACQUITY UPLC BEH C ₁₈ Column, 130 Å, 1.7 µm, 2.1 mm x 50 mm, 1/pk
Column temp.:	55 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.45 mL/min
Mobile phase A:	0.1% Formic acid in water with 10 mM ammonium formate
Mobile phase B:	0.1% Formic acid in acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.45	75	25	6
1.9	0.45	55	45	6
2.0	0.45	2	98	6
2.6	0.45	2	98	6
2.7	0.45	75	25	6
3.2	0.45	75	25	6

MS Conditions

MS system:	Xevo TQ Absolute
Ionization mode:	ESI+
Acquisition range:	MRM Transitions: Midazolam – 326.1>291.0 (CV=60, CE=26) Imipramine – 281.1>86.0 (CV=30, CE=16)
Capillary voltage:	0.5 kV
Collision energy:	See above
Cone voltage:	See above

Data Management

Chromatography software:	MassLynx™ v4.2
MS software:	MassLynx v4.2
Informatics:	TargetLynx™ XS v4.2

Results and Discussion

To evaluate the sensitivity and reproducibility of the methodology, a calibration curve and QC samples were successfully analyzed using a UPLC-MS/MS system consisting of an ACQUITY UPLC I-Class and a Xevo TQ Absolute. The batch and injection order were as follows, the calibration line (0.2, 0.4, 1, 5, 10, 50, and 100 pg/mL) with plasma sample blanks before and after, were followed by the QC samples (n=4 @ 0.2, 0.4, and 1.0 pg/mL) in order of increasing concentration, with plasma sample blanks before and after. The two target analytes were well retained on the reversed-phase chromatography system and were baseline resolved. The retention times for midazolam and imipramine were 1.1 and 1.5 minutes respectively. Calibration lines for both analytes were linear over the full range of 0.2–100 pg/mL for both midazolam and imipramine, with r^2 values of above 0.99 using 1/x weighting. The limit of detection was determined to be 0.2 pg/mL for both analytes, with the lowest level calibrator and all four QC samples at that level being detectable in all cases (see figure 1). Precision (%CV) for both analytes at 0.2, 0.4, and 1.0 pg/mL was calculated to determine a limit of quantification for both analytes. The mean calculated concentration for all QC levels were accurate to within 15% of the nominal value for both analyses. Lower limits of quantification (LLOQ) were determined to be 0.2 pg/mL for imipramine and midazolam with precision in both cases <11%. Figure 2 shows the coefficient of variation observed for imipramine and midazolam at the 0.2, 0.4, and 1.0 pg/mL concentration levels.

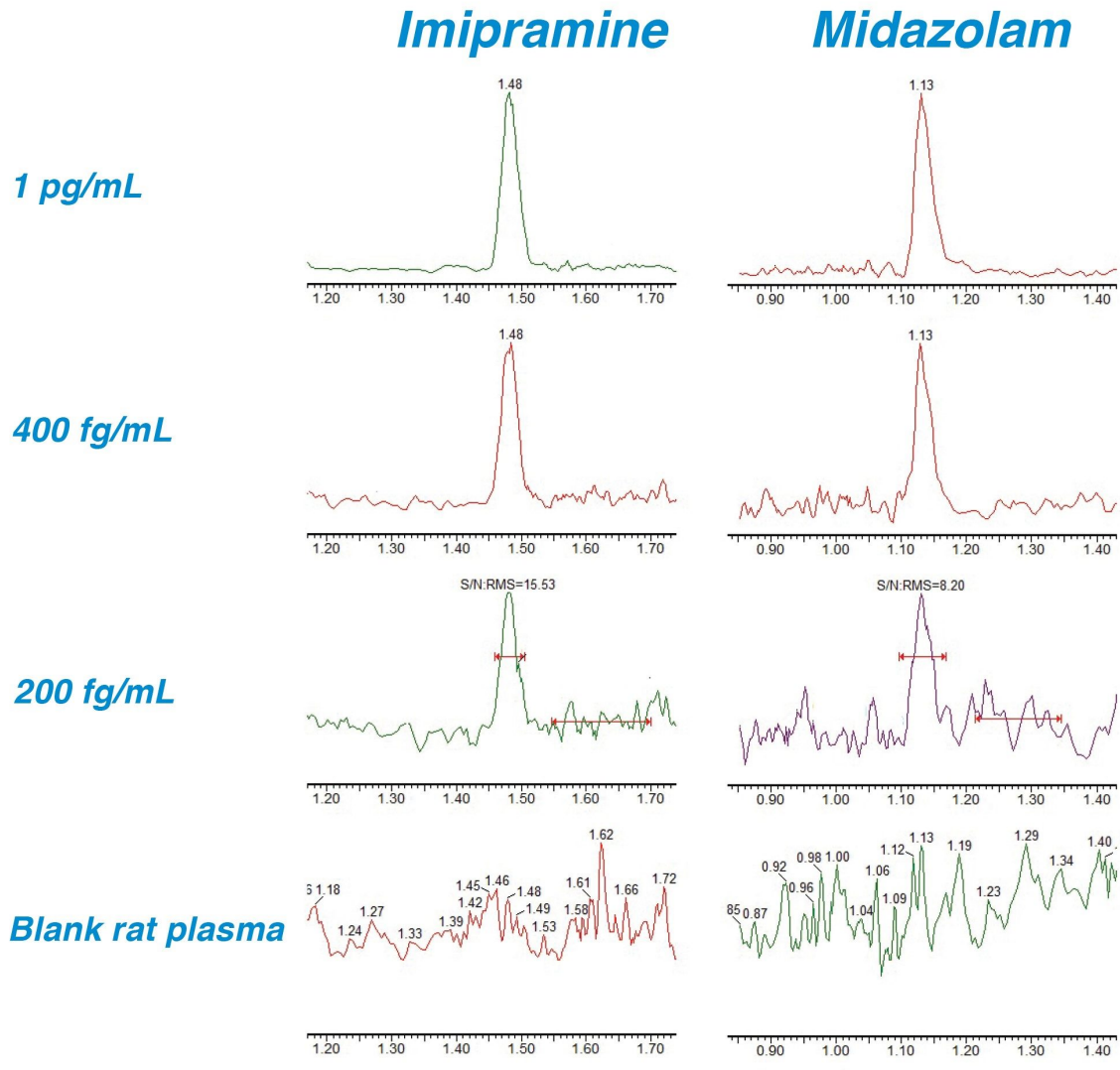


Figure 1.

**Precision (%CV) for Midazolam and Imipramine in rat plasma (n=4)
on Xevo TQ Absolute**

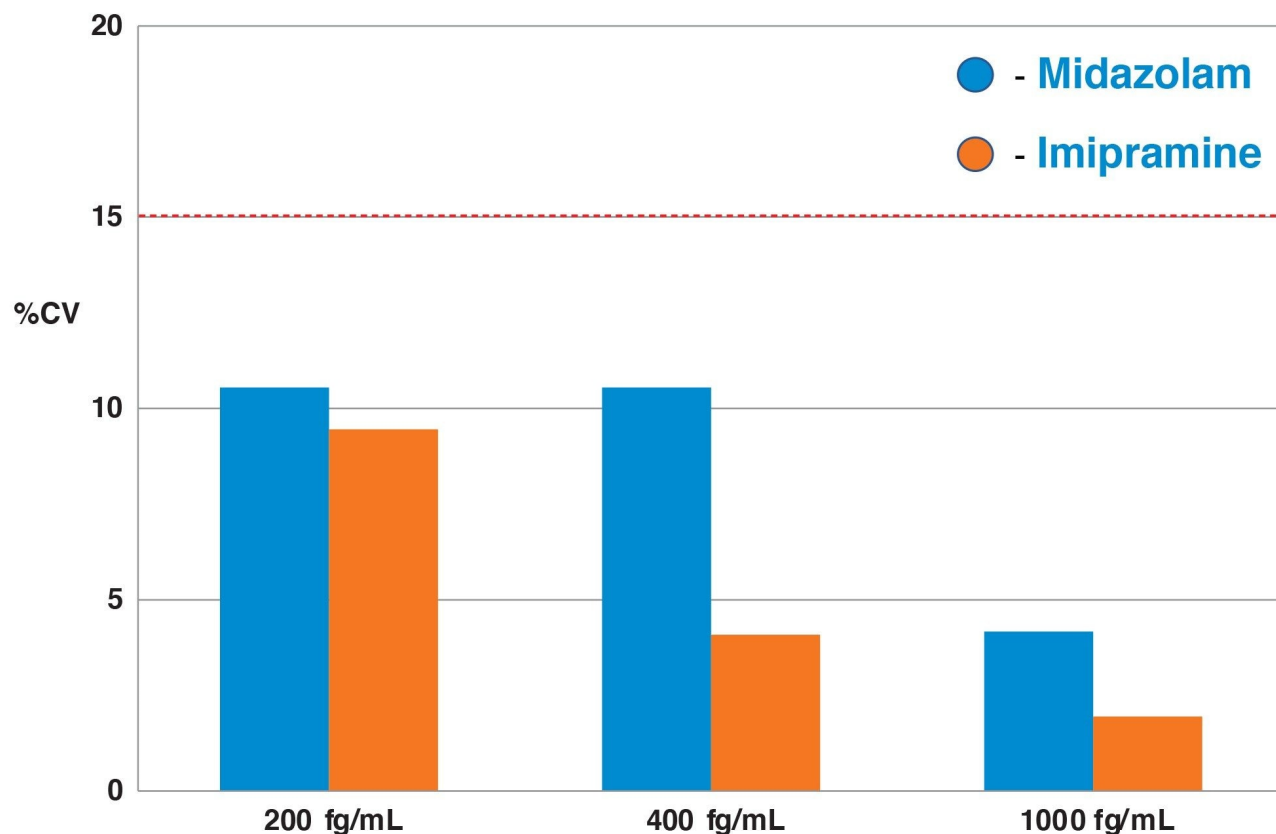


Figure 2.

Conclusion

A high sensitivity UPLC-MS/MS bioanalytical methodology for the quantification of midazolam and imipramine in rat plasma has been demonstrated using the Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer in combination with an ACQUITY UPLC I-Class PLUS System. This method has been shown to have very high levels of sensitivity (250 ag on column), the limits of quantification, linearity, and precision have also been calculated showing that the method is extremely sensitive, linear, and reproducible.

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[Xevo TQ Absolute](#)

[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

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