

Xevo TQ Absolute XR: Maximum Robustness and Sensitivity For High-Throughput Bioanalysis

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Abstract

The accurate LC-MS/MS quantification of candidate pharmaceuticals, their metabolites and biomarkers in biofluids derived from preclinical and clinical studies is essential to successful drug development. The use of simple sample preparation approaches, such as protein precipitation, can affect instrument performance over time requiring the extensive cleaning of the mass spectrometer ion optics to restore performance. In this study, the novel StepWave™ XR Ion Guide within the Xevo™ TQ Absolute XR Mass Spectrometer was evaluated for long-term reliability across 10000 injections and 3 weeks with no unscheduled downtime. The peak area responses of several pharmaceutical compounds were consistently monitored with %RSDs <20%, demonstrating excellent system uptime and measurement robustness.

Benefits

- Xevo TQ Absolute XR Mass Spectrometer delivers maximum robustness for high-throughput bioanalysis studies
 - Long term instrument stability for large batch analysis and maximum uptime demonstrated by more than 10,000 LC-MS/MS injections of protein precipitated plasma with no loss in signal response
 - Simple user maintenance, simple source and cone cleaning with no need to “break instrument vacuum”
 - Simple at-a-glance system performance monitoring with waters_connect™ for Quantitation Software
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Introduction

Tandem quadrupole LC-MS/MS is the technology of choice for quantitative bioanalysis in support of compound discovery, preclinical compound development and human efficacy trials.¹ Enabling the accurate quantification of candidate drugs and their metabolites in the low pg/mL range from a few microlites of biofluid sample.² The combination of electrospray ionization and multiple reaction monitoring (MRM) acquisition provides excellent specificity, selectivity, wide dynamic range of quantification and analysis times in the 1–5 min per sample time range.^{3,4} The selectivity and specificity of MRM acquisition combined with the exquisite sensitivity of modern mass spectrometers has allowed sample preparation to be significantly simplified, compared to LC-optical analysis, with protein precipitation/removal being the preferred approach. These simplified sample clean-up approaches result in an increased amount of matrix-related material entering the MS source, the neutral and high mass components of which can, if not eliminated, accumulate on the first quadrupole, reducing signal response and detection sensitivity over time. Restoring MS response for this type of contamination will require breaking the vacuum and cleaning of the quadrupole. This becomes increasingly important when batch sizes are large and polarity switching or short dwell times are employed.

The Xevo TQ Absolute XR Mass Spectrometer is equipped with a novel slotted bandpass ion guide that effectively mitigates Q1 quadrupole contamination (Figure 1). This new ion guide combines resolving DC and an axial field to create a bandpass filter, protecting downstream ion optics from contamination by preventing unwanted high mass ion transmission into the MS1 quadrupole. Filtering out high m/z ions from biological matrices prevents quadrupole charging and associated losses in sensitivity observed from the accumulation of these ions on the MS1 quadrupole rods. In this application we demonstrate the signal stability of this new tandem quadrupole mass spectrometer.

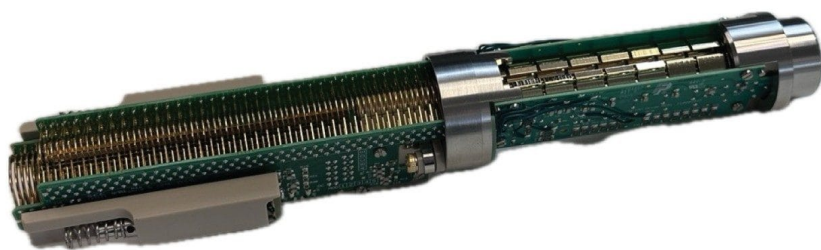


Figure 1. StepWave XR resolving ion guide.

Experimental

Sample Description

Rat plasma samples (100 μ L) were prepared by protein precipitation with cold acetonitrile (200 μ L) in 1.5 mL plastic Eppendorf tubes. The samples were vortex mixed and refrigerated at -20 °C for 1 h. The samples were then allowed to return to room temperature and centrifuged at 14,000 g for 5 mins. The supernatant layer was removed and diluted with water (1:1) containing nefazodone, verapamil, amodiaquine, propafenone, chlorpromazine, dextromethorphan, nifedipine, phenacetin, gefitinib, O-desmethyl metabolite of gefitinib, ibuprofen, and gefitinib D6 to give a final concentration of 5 ng/mL. The resulting samples were transferred to glass autosampler vials for analysis.

Method Conditions

The plasma extracts (2 μ L) were analyzed using an ACQUITY™ Premier UPLC™ System connected to a Xevo TQ Absolute XR Mass Spectrometer. Chromatographic separations were performed using a 2.1 x 50 mm ACQUITY Premier HSS T3 1.7 μ m Column maintained at 60 °C with a solvent flow rate of 600 μ L/min. eluted with a linear reversed-phase gradient over 1 minute. The initial mobile phase composition was 5% solvent B, which was increased linearly to 95% solvent B over 1 min. This mobile phase composition was maintained for 0.3 min before being returned to the initial starting conditions at 1.3 min, and the column was then re-equilibrated for 0.5 min prior to the next injection. Mobile phase A = 0.1% formic acid (v/v) in 10 mM aqueous ammonium acetate and B = 0.1% formic acid, 10 mM aqueous ammonium acetate (v/v) in acetonitrile. The column effluent was monitored using +ve ESI / -ve ESI switching. MRM transitions were monitored for the MS detection of nefazodone (470.26 \Rightarrow 83.00), verapamil (455.31 \Rightarrow 165.09), amodiaquine (356.14 \Rightarrow 283.16), propafenone

(342.19 => 116.0), chlorpromazine (319.08 => 85.99), dextromethorphan (272.19 =>171.08), nifedipine (347.11 => 315.19), phenacetin (180.04 => 109.96), gefitinib (447.18 => 128.1), O-desmethyl metabolite (447.18 => 100.2), ibuprofen (205.0 => 161.0), d6-gefitinib-d6 [IS] (453.16 => 134.2).

LC Conditions

LC system:	ACQUITY Premier System with Binary Solvent Manager and Flow Through Needle
Detection:	MRM +ve/-ve ESI
Vials:	Maximum Recovery Vials (p/n: 186005661CV)
Column(s):	ACQUITY Premier BEH C ₁₈ 2.1 x 50 mm 1.7 µm (p/n: 186009455)
Column temperature:	60 °C
Sample temperature:	10 °C
Injection volume:	5 µL
Flow rate:	600 µL/min
Mobile phase A:	0.1% formic acid (v/v) in 10 mM aqueous ammonium acetate
Mobile phase B:	0.1% formic acid, 10 mM aqueous ammonium acetate (v/v) in acetonitrile
Gradient:	See Table

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.6	95	5	Linear
1	0.6	5	95	Linear
1.3	0.6	5	95	Linear
1.31	0.6	95	5	Linear

MS Conditions

MS system:	Xevo TQ Absolute XR Mass Spectrometer
Ionization mode:	+ve / -ve ESI
Acquisition range:	MRM
Capillary voltage:	1 KV
Collision energy:	Compound dependent
Cone voltage:	Compound dependent

Data Management

MS software:	waters_connect
Informatics:	waters_connect for Quantitation

Results and Discussion

Robustness

Instrument robustness was determined by the comparison of the analyte peak area for each individual rat plasma sample analyzed. The analysis was made in ten separate batches of 1,000 injections performed consecutively without interruption. The samples were prepared as a single batch, which was then divided into separate aliquots in Maximum Recovery Glass Vials. In total, more than 8.3 mL of rat plasma was loaded onto the system over the course of the 10,000-sample study.

The data in Figures 2 and 3 illustrates the variation in nifedipine and propafenone peak area responses observed across the duration of the 10,000 extracted rat plasma samples. The coefficient of variation (%CV) for nifedipine and propafenone was determined to be 13.1 and 14.9% respectively. The data in Figures 2 and 3 illustrate that there was no significant temporal trend in the peak area responses, suggesting there was no detrimental contamination occurring in the Q1 quadrupole region of the MS. The coefficient in variation of signal response for nefazodone, verapamil, amodiaquine, propafenone, chlorpromazine, dextromethorphan, nifedipine, phenacetin, gefitinib, O-desmethyl metabolite, ibuprofen, d6-gefitinib-d6 [IS], ranged from 13.1–20.4%.

The variation in analyte peak area ratios over the course of the 10,000-sample batch for gefitinib and the O-desmethyl metabolite of gefitinib are given in Figure 4. The internal standard corrected peak area ratio showed no discernible trend across the course of the study with a %CV of 2.3 and 2.7% for gefitinib and O-desmethyl metabolite, respectively.

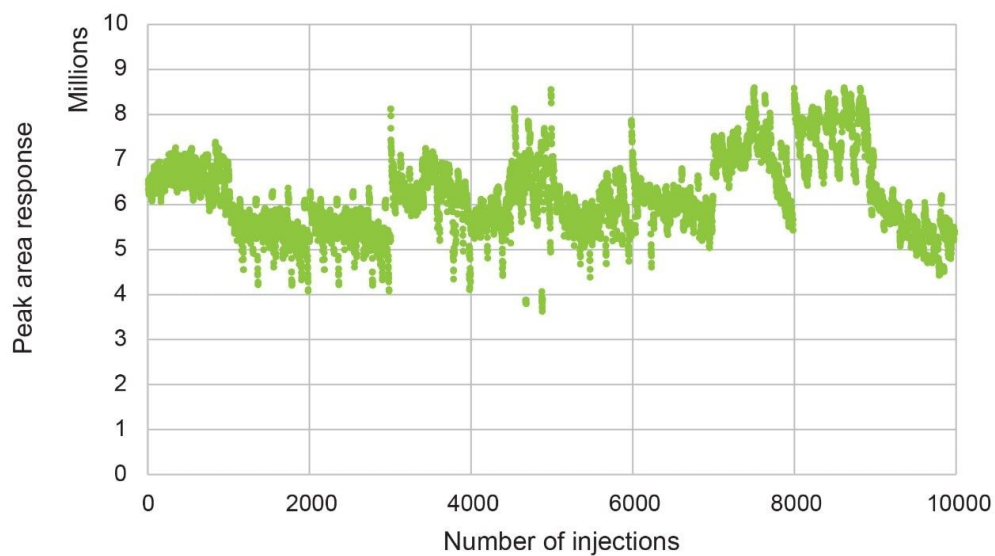


Figure 2. Variation in peak area response for the analysis of nifedipine in rat plasma extract over the course of a 10,000-injection batch using the Xevo TQ Absolute XR Mass Spectrometer.

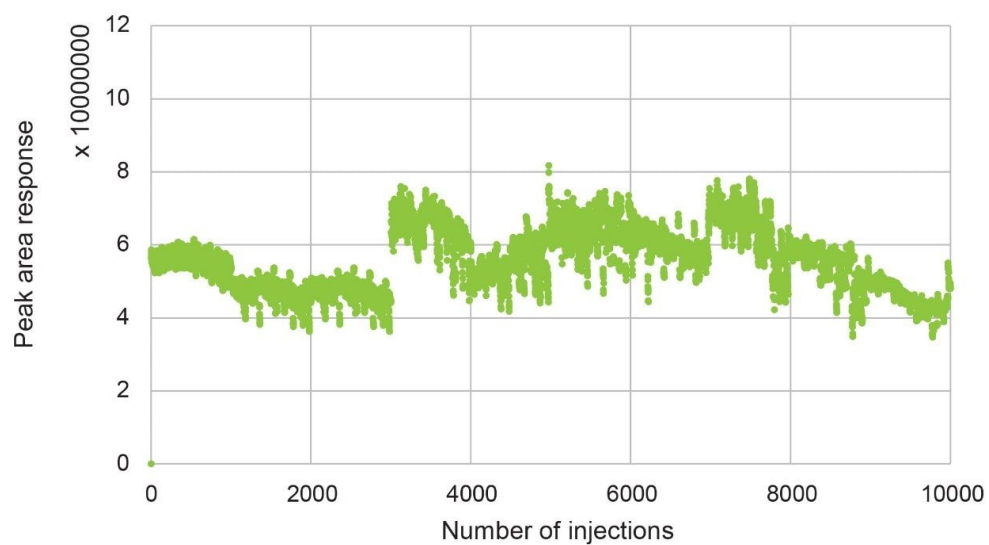


Figure 3. Variation in peak area for the analysis of propafenone in rat plasma extract over the course of a 10,000-injection batch using the Xevo TQ Absolute XR Mass Spectrometer.

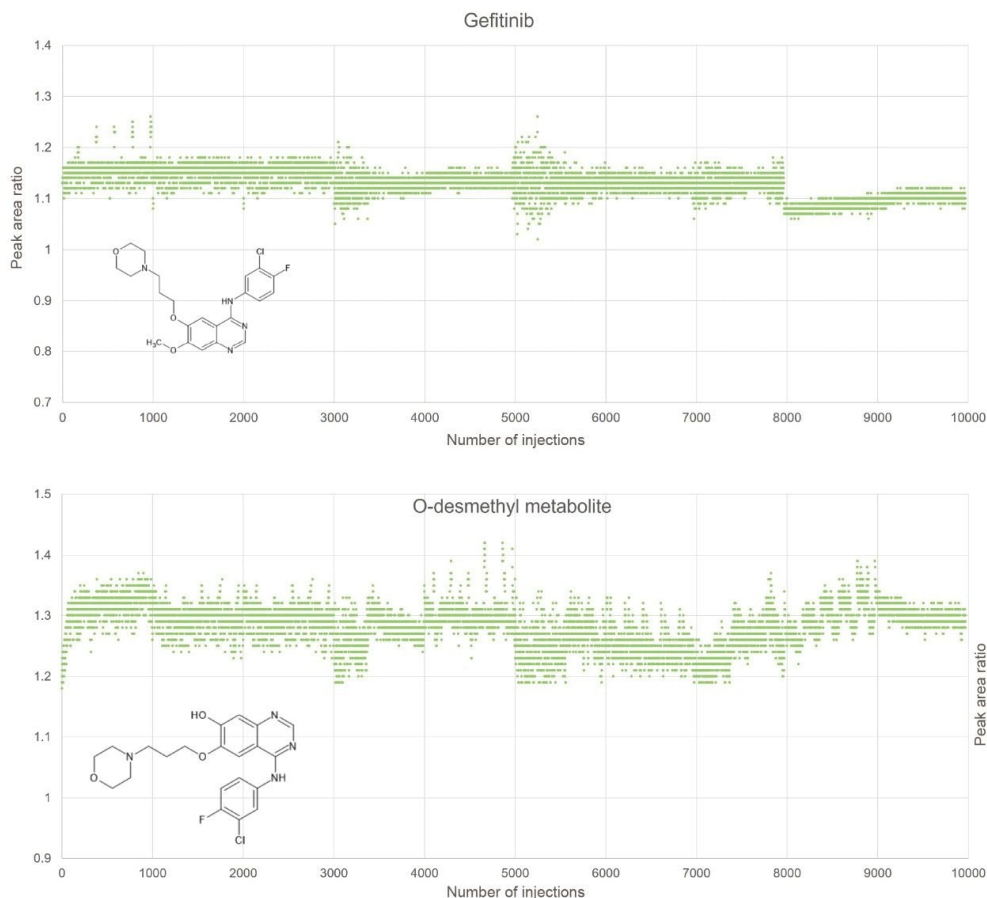


Figure 4. Variation in peak area ratio response for gefitinib and O-desmethyl metabolite of gefitinib in rat plasma matrix over the course of a 10,000-injection batch.

Source Maintenance

Notwithstanding the exemplary performance of the StepWave XR Ion Guide, routine cleaning of the MS source and sampling orifice is highly recommended to ensure the highest MS sensitivity and reproducibility. To simplify this process, the source region of the Xevo TQ Absolute XR Mass Spectrometer is designed to allow for the source and sampling cone to be removed and cleaned without specialist tools, or needing to break the MS vacuum. Thus, the whole process can be completed in just a few minutes.

MS Performance Monitoring

Monitoring MS instrument performance during the course of a batch or study is important to maximize system utilization. This can be quickly achieved via the waters_connect for Quantitation Software (Figure 5). As stable-

isotope-labeled (SIL) internal standards and QCs are routinely used in LC-MS/MS bioanalysis to ensure and confirm the bioanalytical assay's performance, the IS and QC data can be used to visualize trends in signal response and confirm performance of the instrument. The waters_connect for Quantitation Software allows for performance limits to be automatically set, *e.g.*, minimum values and RSD, and flagged when the assay exceeds these limits.

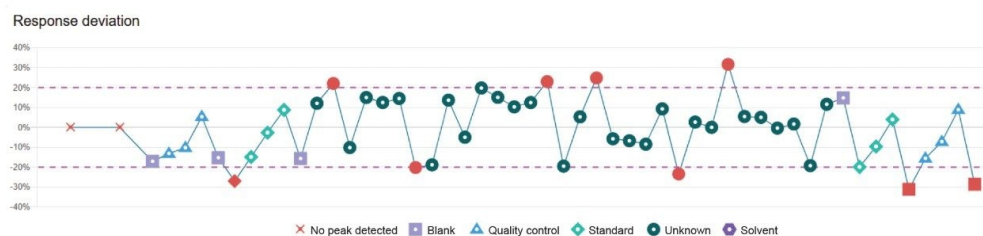


Figure 5. Illustration of waters_connect for Quantitation Internal Standard response summary plot obtained from the data Overview feature.

Conclusion

LC-MS/MS based quantitative bioanalytical assays provide critical concentration data to support discovery, preclinical, and human trial DMPK studies. Advances in LC-MS/MS instrumentation has enabled the analysis of several hundred samples per day. MS instrument robustness and signal stability are key to enabling these high-throughput assays.

- The StepWave XR wide bandpass slotted ion guide within the Xevo TQ Absolute XR Mass Spectrometer maintained instrument performance and signal stability over > 10,000 LC/MS analysis of rat plasma extract.
- The MS source and sampling cone can be quickly removed, cleaned, and reinstalled without the need to break the instrument vacuum.
- waters_connect for Quantitation Software facilitates the rapid simple evaluation of MS performance, via the Dashboard view of internal standards and QCs.

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