

## Comparison of Tandem and High Resolution Mass Spectrometry for the Quantification of the Monoclonal Antibody, Trastuzumab in Plasma

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### Abstract

This technology brief demonstrates the sensitive and robust HRMS quantification of trastuzumab from plasma.

The Xevo TQ-XS Tandem Quadrupole is compared with Xevo G2-XS QToF for the bioanalytical quantification of trastuzumab prepared from plasma. For HRMS quantification, best sensitivity and performance was achieved using ToF-MRM mode. In addition, this performance was highly comparable to the Xevo TQ-XS Tandem Quadrupole MS results, achieving LLOQs within 2-fold and 4-orders of linearity. This highly reproducible data demonstrates that the Xevo G2-XS QToF System can be used to provide sensitive, accurate, and robust quantitative results.

### Benefits

- Demand for bioanalytical quantification of proteins via LC-MS has steadily increased as development of protein therapeutics has increased. While tandem quadrupole MS is the most widely adopted

platform for quantification, use of high-resolution mass spectrometers (HRMS), with their ability to provide high selectivity and collect both quantitative and qualitative data, is steadily increasing.

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## Introduction

With the increased focus on developing proteins as new drug candidates, particularly monoclonal antibodies (mAbs), there is great demand for sensitive and robust quantitative bioanalytical methods. With its fast method development times, broad linear dynamic range, sensitivity, and specificity, LC-MS analysis using a tandem quadrupole MS is quickly becoming an attractive alternative to immunoassays. While tandem quadrupole MS systems have traditionally been the ‘go-to’ instrument for bioanalytical quantification, HRMS instruments can now achieve sensitivities and dynamic ranges that are comparable to that of tandem quadrupole instruments.

In addition, HRMS offers better selectivity, providing improved signal:noise (S:N) compared to a unit resolution tandem quadrupole MS and the ability to collect both qualitative and quantitative results in a single analysis. In this work, we demonstrate the sensitive and robust HRMS quantification of trastuzumab from plasma which achieves performance comparable to tandem quadrupole MS.

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## Results and Discussion

The quantitative performance of the high resolution, Xevo G2-XS QToF Quadrupole Time-of-Flight (ToF) Mass Spectrometer was compared to the nominal mass Xevo TQ-XS Tandem Quadrupole Mass Spectrometer for the quantification of trastuzumab in plasma. The unique tryptic peptides of trastuzumab, FTISADTSK and DTYIHWVR, were used for this assessment. Their peptide sequences, precursors, product ions, and corresponding charges states are listed in Table 1. For HRMS analysis,

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several experiments were performed on the Xevo G2-XS QToF, including: ToF-MRM with target enhancement of the product ion (precursor > fragment), ToF-MRM with target enhancement of the precursor ion (precursor > precursor), and a ToF-MS full scan acquisition of precursor ions (full scan > precursor).

Peptide	Precursor charge state	Precursor ion (m/z)	Product ion identification	Precursor ion (m/z)	Cone voltage (V)	Collision energy (eV)
FTISADTSK	[M+2H] <sup>2+</sup>	485.2480	[1H+] <sup>1</sup> /y7	721.3727	40	16
DTYIHWVR	[M+2H] <sup>2+</sup>	545.2774	[1H+] <sup>1</sup> /y4	597.3256	40	23

*Table 1. Sequences, precursors, product ions, and corresponding charge states for trastuzumab tryptic peptides used for quantification.*

A multiple reaction monitoring (MRM) experiment was performed for tandem quadrupole MS analysis on the Xevo TQ-XS System. Chromatographic separation was achieved using an ACQUITY UPLC H-Class System and an ACQUITY UPLC Peptide BEH C<sub>18</sub> Column (p/n: 186003687), using an eight minute gradient (5–50% B) with 0.1% formic acid in water and acetonitrile (flow rate 0.3 mL/min).

Trastuzumab was immunopurified from plasma (50 µL) using a 96-well Protein A agarose-based plate. The postaffinity purified plasma was then digested and peptide-level purification was completed using the ProteinWorks eXpress Digest and µElution SPE Clean-up Kits (p/n: 176003689 and p/n: 186008304). An 8 µL aliquot of the resulting 90 µL SPE eluate was injected for each LC-MS analysis.

A summary of standard curve performance for the FTISADTSK and DTYIHWVR peptides using both the Xevo TQ-XS and Xevo G2-XS QToF with the various acquisition modes is highlighted in Table 2. Best overall quantification performance was achieved using the tandem quadrupole Xevo TQ-XS MS, with lower limits of quantification (LLOQs) between 10–25 ng/mL and linear dynamic range  $\geq 4.3$  orders of magnitude. While all three HRMS modes on the Xevo G2-XS QToF MS showed excellent linearity (R<sup>2</sup> values  $\geq 0.99$ ), best sensitivity and performance, which was comparable to the Xevo TQ-XS, was achieved using ToF-MRM with linear dynamic range  $\geq 4.0$  orders of magnitude and LLOQs between 25–50 ng/mL. QC performance, highlighted in Table 3, was excellent for both tandem quadrupole and HRMS MRM analysis with mean accuracies and % CV' s  $\pm 15\%$ . Chromatographic performance for the FTISADTSK tryptic peptide of trastuzumab is highlighted in Figure 1.

(A) FTISADTSK						
MS/Acquisition mode	Curve (µg/mL)	Log <sub>10</sub> range	Weighting	Linear fit (R <sup>2</sup> )	% Accuracy range	LOD (µg/mL)
TQ-XS/MRM	0.010–250	4.4	1/X <sup>2</sup>	0.988	85.0–111.6	0.005
G2-XS/ToF-MRM	0.025–500	4.3		0.991	89.2–114.4	0.010
G2-XS/Precursor > Precursor	0.250–100	2.6		0.991	91.9–109.5	0.250
G2-XS/Full scan > Precursor	0.250–100	2.6		0.997	97.3–102.7	0.250

  

(B) DTYIHWVR						
MS/Acquisition mode	Curve (µg/mL)	Log <sub>10</sub> range	Weighting	Linear fit (R <sup>2</sup> )	% Accuracy range	LOD (µg/mL)
TQ-XS/MRM	0.025–500	4.3	1/X <sup>2</sup>	0.992	89.0–108.3	0.025
G2-XS/ToF-MRM	0.050–500	4.0		0.995	95.5–105.6	0.050
G2-XS/Precursor > Precursor	2.500–500	2.3		0.994	94.5–106.4	2.500
G2-XS/Full scan > Precursor	1.000–500	2.7		0.991	89.6–107.8	1.000

Table 2. Linear dynamic range and standard curve statistics for the FTISADTSK (A) and DTYIHWVR (B) trastuzumab tryptic peptides using the Xevo TQ-XS Tandem Quadrupole MS and Xevo G2-XS QToF MS.

(A) Xevo TQ-XS/MRM				
Peptide	Trastuzumab QC spike concentration (µg/mL)	Mean (N=3) calculated trastuzumab QC concentration (µg/mL)	Mean (N=3) % accuracy	% RSD
FTISADTSK	0.050	0.055	109.9	4.2
	0.500	0.532	106.4*	7.5
	5.000	5.047	100.9*	3.1
	50.000	48.677	97.4	4.7
DTYIHWVR	0.050	0.045	89.7	4.7
	0.500	0.553	110.5	4.0
	5.000	5.243	104.8	2.3
	50.000	53.569	107.133	5.0

  

(B) Xevo G2-XS QToF/Tof-MRM				
Peptide	Trastuzumab QC spike concentration (µg/mL)	Mean (N=3) calculated trastuzumab QC concentration (µg/mL)	Mean (N=3) % accuracy	% RSD
FTISADTSK	0.050	0.055	110.1*	1.0
	0.500	0.483	96.5	10.9
	5.000	5.235	104.7	7.4
	50.000	57.404	114.9*	6.1
DTYIHWVR	0.500	0.437	87.5	4.4
	5.000	4.646	92.9	6.3
	50.000	56.109	112.2	1.4

\*Indicates that 1 of 3 data points was excluded from the standard curve.

Table 3. QC sample statistics for the FTISADTSK and DTYIHWVR trastuzumab tryptic peptides, using the Xevo TQ-XS Tandem Quadrupole MS, MRM analysis (A) and Xevo G2-XS QToF MS, Tof- MRM analysis (B)

## Conclusion

In this work we compared the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer to the Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometer for the bioanalytical quantification of trastuzumab prepared from plasma. For HRMS quantification, best sensitivity and performance was achieved using Tof-MRM mode. In addition, this performance was highly comparable to the Xevo TQ-XS Tandem Quadrupole MS results, achieving LLOQs within 2-fold and 4-orders of linearity. This highly reproducible data demonstrates that the Xevo G2-XS QToF System can be used to provide sensitive, accurate, and robust quantitative results.

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Xevo TQ-XS Triple Quadrupole Mass Spectrometry <<https://www.waters.com/134889751>>

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ACQUITY UPLC Peptide BEH C18, 300Å, 1.7 µm, 2.1 mm X 150 mm Column

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<https://www.waters.com/waters/partDetail.htm?partNumber=176003689>>

ProteinWorks µElution SPE Clean-up Kit <

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