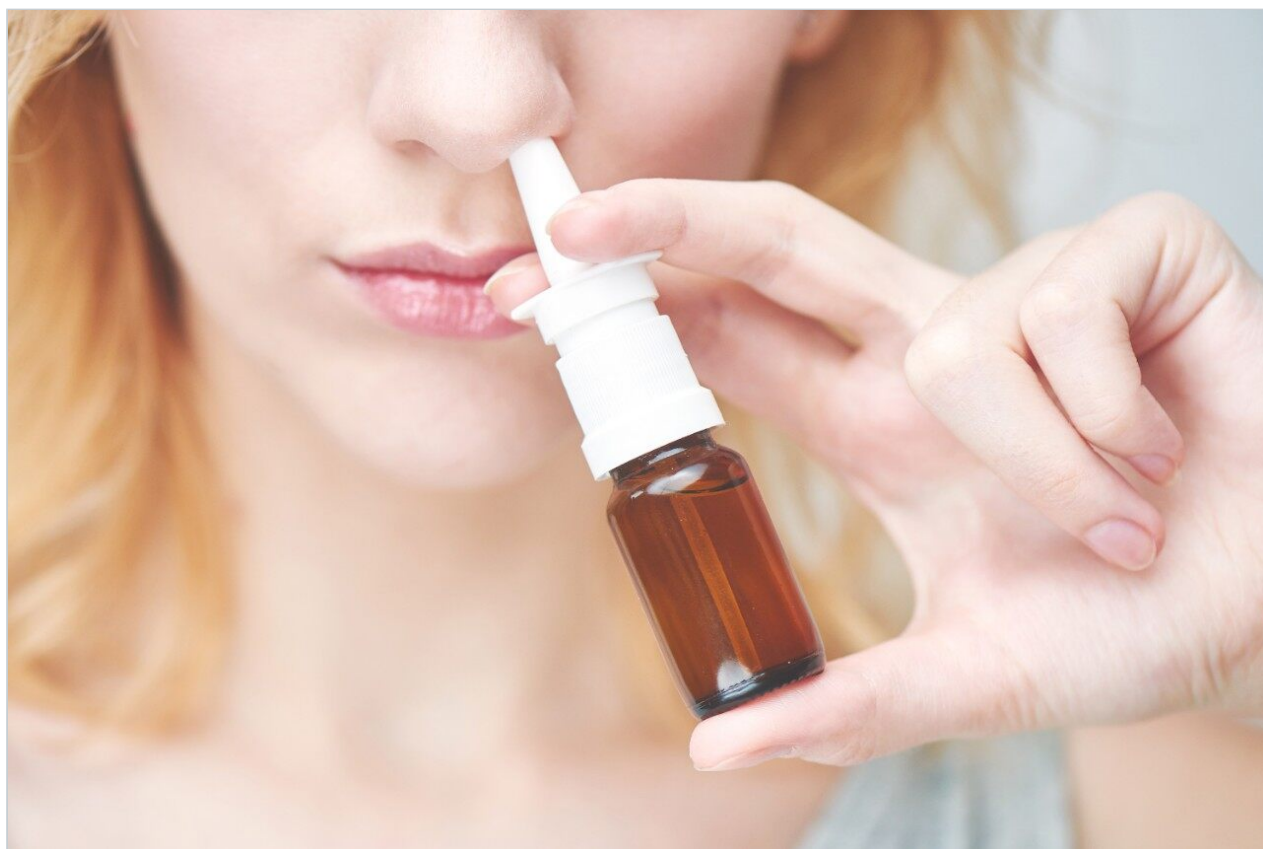


## Accurate Bioanalytical Peptide Quantification of Salmon Calcitonin Using High Resolution Mass Spectrometry (HRMS)

---

Mary E. Lame, Nikunj Tanna

Waters Corporation



*For research use only. Not for use in diagnostic procedures.*

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

---

## Abstract

This Application brief demonstrates sensitive and robust quantitative performance of the Xevo G2-XS QToF Mass Spectrometer for peptide bioanalysis.

### Benefits

The Xevo G2-XS QToF Mass Spectrometer used for quantification of salmon calcitonin (extracted from human serum) achieved excellent sensitivity and dynamic range, demonstrating its suitability for peptide bioanalysis.

---

## Introduction

As the pharmaceutical industry shifts from small to large molecule therapeutics, the demand for sensitive and robust bioanalytical LC-MS quantification continues to increase. With fast method development times, sensitivity and large dynamic range, tandem quadrupole MS is well-established for bioanalysis. However, as the size and complexity of these biomolecules has increased, interest in HRMS for quantification is also increasing due to its many benefits, including increased mass range, selectivity, and ability to collect both quantitative and qualitative data. In addition, HRMS instruments can now achieve sensitivities and dynamic ranges that are comparable to that of tandem (triple) quadrupole instruments with the benefit of improved selectivity. In this work, we demonstrate the sensitive and robust HRMS quantification of salmon calcitonin, a 3.4 kDa polypeptide.

Using simple SPE sample preparation, analytical scale chromatographic separation with an ACQUITY UPLC, and HRMS quantification with the Xevo G2-XS QToF, we demonstrate sensitive, accurate, and robust quantification of salmon calcitonin from serum with quantitative performance comparable to the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. This method achieves 50 pg/mL limits of detection with excellent accuracy (90–110%) and reproducibility (CVs <10%), using only 100  $\mu$ L of serum.

---

## Results and Discussion

The quantitative performance of the Xevo G2-XS QToF MS was evaluated with focus on the 4+ (859.146  $m/z$ ) and 5+ (687.911  $m/z$ ) precursors for salmon calcitonin. The different acquisition modes available on the HRMS instrument, full scan, ToF MRM (Precursor > Precursor) with Target Enhancement (TE), and ToF MRM (Precursor > Product) with Target Enhancement (TE) were evaluated. For tandem MS analysis, a multiple reaction monitoring (MRM) experiment was performed on the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. Chromatographic separation was achieved using an ACQUITY UPLC and CORTECS UPLC C<sub>18</sub> +, 90Å, 1.6  $\mu$ m, 2.1  $\times$  50 mm Column (p/n:176003167), using a 4.5 minute gradient (5–75% B) with 0.1% formic acid in water and acetonitrile (flow rate 0.4 mL/min). The mixed-mode solid phase extraction (SPE) of salmon calcitonin from serum is described in full detail in a recent Waters Application Note (720006342EN).

Figure 1 demonstrates the power of HRMS for bioanalysis for a 500 pg/mL serum extraction of salmon calcitonin using ToF MRM (Precursor > Precursor) for the 4+ precursor. With its high mass accuracy and ability to extract very narrow mass windows, selectivity of the assay is greatly improved. As seen in this figure, using a mass extraction window of 20 mDa provided the best S/N, eliminated endogenous interferences, and yielded the best overall sensitivity.

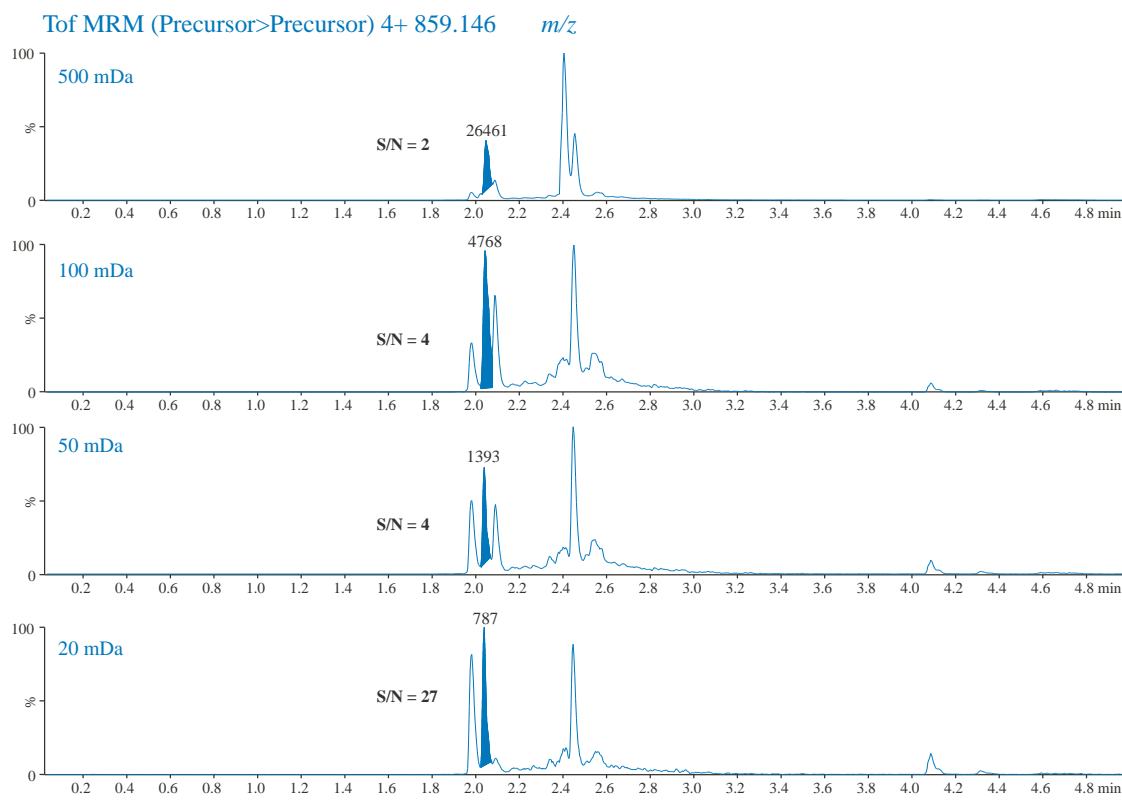


Figure 1. Representative chromatogram highlighting the increase in sensitivity and specificity for the Xevo G2-XS QTof MS using ToF MRM (Precursor>Precursor) for the 4+ precursor of salmon calcitonin extracted from plasma (500 pg/mL) using various mass extraction windows from 500–20 mDa.

Instrument	Precursor	MRM	Calibration curve (pg/mL)	Weighting	Linear fit (R <sup>2</sup> )	% Accuracy range
Xevo TQ-XS	4+	687.3>830.3	25–1,500	1/x	>0.99	85–115
Xevo G2-XS QTof	4+	687.911>687.911	50–1,500	1/x	>0.99	85–115
Xevo TQ-XS	5+	859.2>1106.7	25–1,500	1/x	>0.99	86–112
Xevo G2-XS QTof	5+	859.146>859.146	50–1,500	1/x	>0.99	85–110

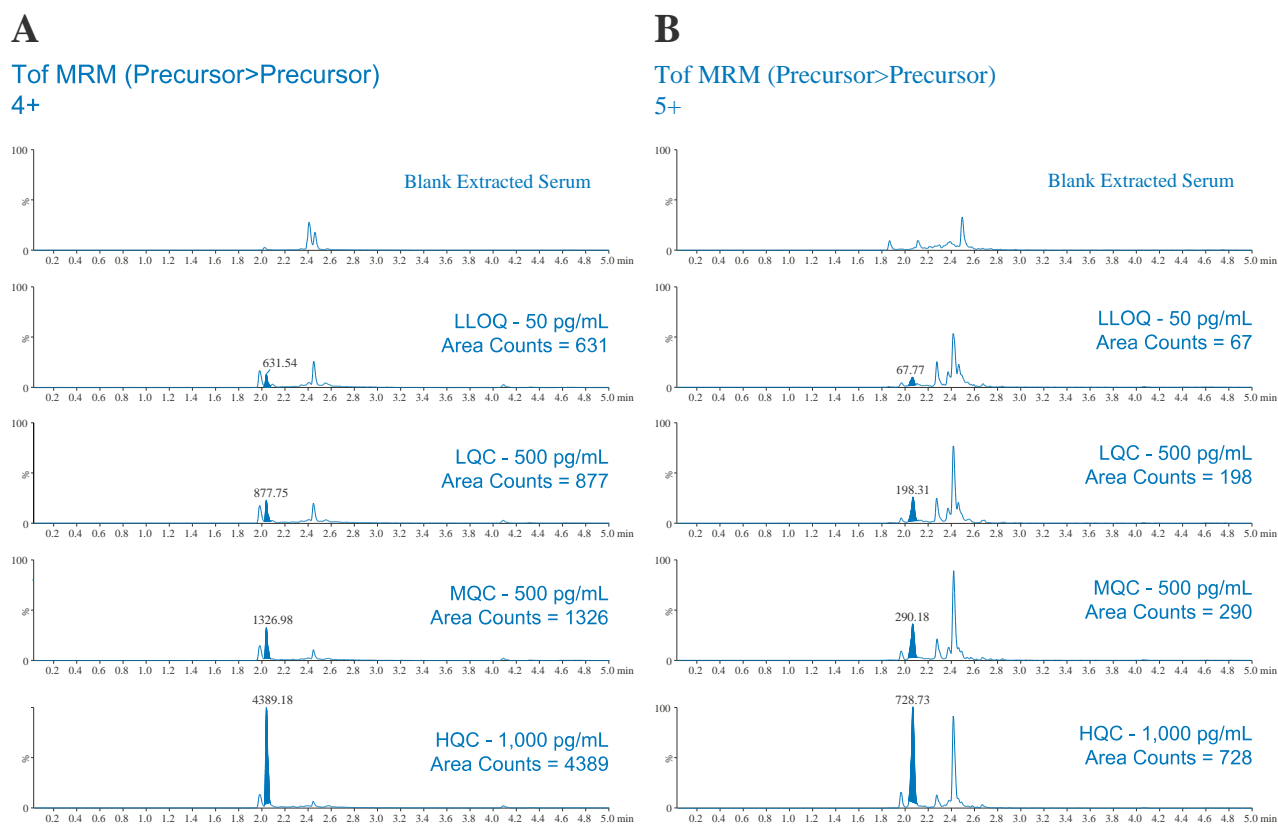
Table 1. Linear dynamic range and standard curve statistics for the 4+ and 5+ precursor of salmon calcitonin extracted from plasma, analyzed on the Xevo G2-XS QTof and Xevo TQ-XS tandem MS.

Best overall quantification performance was achieved using the targeted ToF MRM (Precursor > Precursor) mode with TE, using very low collision energy for the 4+ and 5+ precursors of salmon calcitonin. This performance was comparable to the quantification data observed on the Xevo TQ-XS Tandem Quadrupole

using MRM (Precursor > Product) mode. A summary of standard curve performance for the 4+ and 5+ precursors of salmon calcitonin for both the Xevo G2-XS QTof and Xevo TQ-XS MS is highlighted in Table 1. Using the Xevo G2-XS QTof and the targeted ToF MRM (Precursor > Precursor) method, the lower limit of quantification (LLOQ) achieved was 50 pg/mL with a dynamic range from 50–1,500 pg/mL. The calibration curve was linear with  $r^2$  values >0.99 (1/x weighting) with mean accuracy of all calibration points between 85–105% and 85–110% for the 4+ and 5+ precursors, respectively. Quality Control (QC) performance of salmon calcitonin was excellent, with mean accuracies between 93–108% and CV's <11% for both the 4+ and 5+ precursors, respectively (Table 2). QC chromatographic performance is highlighted in Figure 2 for 4+ (panel A) and 5+ (panel B) precursors.

Precursor	QC level	Expected concentration (pg/mL)	Mean (N=3) observed concentration (pg/mL)	Mean (N=3) % accuracy	%CV
4+	LQC	100	104.10	104.1	2.99
	MQC	500	444.70	88.9	0.35
	HQC	1000	968.37	96.8	9.53
5+	LQC	100	101.50	101.5	2.23
	MQC	500	436.07	87.2	2.80
	HQC	1000	866.77	86.7	4.97

*Table 2. QC sample statistics for the 4+ (panel A) and 5+ precursor (panel B) of salmon calcitonin extracted from plasma using the Xevo G2-XS QTof MS, ToF- MRM (Precursor > Precursor) analysis.*



*Figure 2. Representative QC chromatograms for 4+ (panel A) and 5+ precursors (panel B) of salmon calcitonin extracted from plasma using Tof-MRM (Precursor > Precursor) analysis on the Xevo G2-XS QToF MS.*

## Conclusion

This application brief demonstrates the ability to perform highly sensitive HRMS quantitative analysis of the peptide, salmon calcitonin, extracted from human serum using the Xevo G2-XS QToF MS, achieving an LLOQ of 50 pg/mL. In addition, this performance was comparable to the Xevo TQ-XS Tandem Quadrupole MS results, achieving LLOQs within 2-fold. This highly reproducible data (CVs <10%) demonstrates that the HRMS Xevo G2-XS QToF can be used to provide sensitive, robust, and accurate quantification of peptides from biological matrices.

---

## Featured Products

Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry <<https://www.waters.com/134798222>>

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

ACQUITY UPLC System <<https://www.waters.com/514207>>

720006382, September 2018

©2019 Waters Corporation. All Rights Reserved.