Waters™

Application Note

Utilizing the New Xevo™ TQ Absolute XR Mass Spectrometer for the LC-MS/MS Analysis of Serum Estrogens for Clinical Research

David Farrell, Gareth Hammond, Robert Wardle

Waters Corporation, United States

Published on May 20, 2026

Application Brief

Main

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

Abstract

This application note demonstrates the high analytical sensitivity and quantitative performance of the Xevo TQ Absolute XR Mass Spectrometer for the analysis of serum estrogens for clinical research.

Benefits

- Utilizing the Xevo TQ Absolute XR Mass Spectrometer for low level quantification of 17β-estradiol and estrone
- Suitable for simple liquid/liquid sample preparation method

- UHPLC separation of 17β-estradiol and estrone for selective detection

Introduction

The two major biologically active estrogens in non-pregnant humans are 17β-estradiol (E2) and estrone (E1). E2 is produced primarily in the ovaries and tests by the aromatization of testosterone, whereas, most of E1 is derived from androstenedione. E2 can be metabolized to E1 and conversion of E1 to E2 is also possible, making the measurement of both compounds desirable.

The greatest challenge when analyzing E2 and E1 is the requirement to measure down to low concentration levels for certain clinical research applications. Currently, some immunoassay techniques lack analytical sensitivity and more commonly selectivity, while published LC-MS/MS methods use large sample volumes with complex sample extraction, often including derivatization.

Figure 1. ACQUITY UPLC™ I-Class SM-FL IVD System and Xevo TQ Absolute XR Mass Spectrometer.

Enhanced analytical sensitivity, robustness, and reliability over six orders of linear dynamic range is achieved using the Xevo TQ Absolute XR Mass Spectrometer, featuring eXtended Dynamic Range (XDR) and eXtreme Robustness (XR) StepWave™ Ion Guide. When combined with a simple sample preparation method and UHPLC separation of estrogens, the method provides highly selective and analytical sensitivity method for the clinical research analysis of E2 and E1.

Sample Preparation

Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC™-MS/MS) Analysis

E2 and E1 certified reference solutions and their stable labeled internal standards ([13]

C_3) were purchased from Merck (Gillingham, UK). Calibrators were prepared in a surrogate matrix of MSG4000 stripped serum purchased from Golden West Biologicals (Temecula, CA). The calibration range for E2 was 2–1000 pg/mL and 1–1000 pg/mL for E1. The QC samples were prepared in a surrogate matrix of MSG4000 stripped human serum purchased from Golden West Biologicals (Temecula, CA) at 3.0 pg/mL, 10.0 pg/mL and 700 pg/mL. Water, methanol and ethyl acetate were purchased from Fisher Scientific (Loughborough, UK). Ammonium fluoride and hexane were purchased from Merck (Gillingham, UK).

To convert conventional mass units (pg/mL) to SI units (pmol/L), multiply by 3.671 for E2 and 3.699 for E1.

To 250 µL of sample, 20 µL of internal standard was added and mixed. Liquid/liquid extraction was performed by adding 1 mL of 85:15 (v:v) hexane:ethyl acetate, mixing thoroughly for 10 minutes. Samples were centrifuged at 12,000 g for 2 minutes prior to 700 µL of the top organic layer being transferred into a 96-well plate containing 1 mL glass inserts (Waters, p/n: 186000855 < https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186000855-96-well-plate-with-extended-1-ml-glass-inserts-18-pk.html>). Samples were evaporated to dryness and reconstituted in 20 µL of methanol followed by 30 µL of distilled water and mixed thoroughly for 10 minutes.

Samples were subsequently injected onto an ACQUITY UPLC I-Class FL System and Xevo TQ Absolute XR Mass Spectrometer, utilizing a water/methanol/ammonium fluoride gradient and a Waters CORTECS™ Phenyl Column (Waters, p/n: 186008319 < https://www.waters.com/nextgen/global/shop/columns/186008319-cortecs-phenyl-column-90a-27--m-21-mm-x-50-mm-1-pk.html>) heated to 50 °C as shown in Table 1.

Time (min)	Flow Rate (mL/min)	A (%)	B (%)	Curve
Initial	0.3	90	10	Initial
0.50	0.3	60	40	11
3.50	0.3	30	70	6
3.75	0.5	2	98	11
4.25	0.5	90	10	11
4.70	0.3	90	10	11

Table 1. Gradient table for the separation of E2 and E1, having a total run time of 4.75 minutes.

Multiple reaction monitoring (MRM) transitions, quantifier (quan) and qualifier (qual), for the detection of all analytes are shown in Table 2, having a capillary voltage of 2.2kV in electrospray negative ionization mode, with resolution settings at 0.7 FWHM for MS1 and MS2.

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone Voltage (V)	Collision Energy (eV)
E2 (quan)	271.3	145.1	40	38
E2 (qual)	271.3	183.1	40	38
[$^{13}C_3$]-E2	274.3	148.1	40	38
E1 (quan)	269.3	145.1	40	38
E1 (qual)	269.3	183.1	40	38
[$^{13}C_3$]-E1	272.3	148.1	40	38

Table 2. MRM parameters of E2 and E1 quantifier ions, qualifier ions and their internal standards.

Results and Discussion

Chromatographic separation was achieved for E2 and E1, having a total injection cycle time of approximately 5.75

minutes. A typical chromatogram is shown in Figure 2.

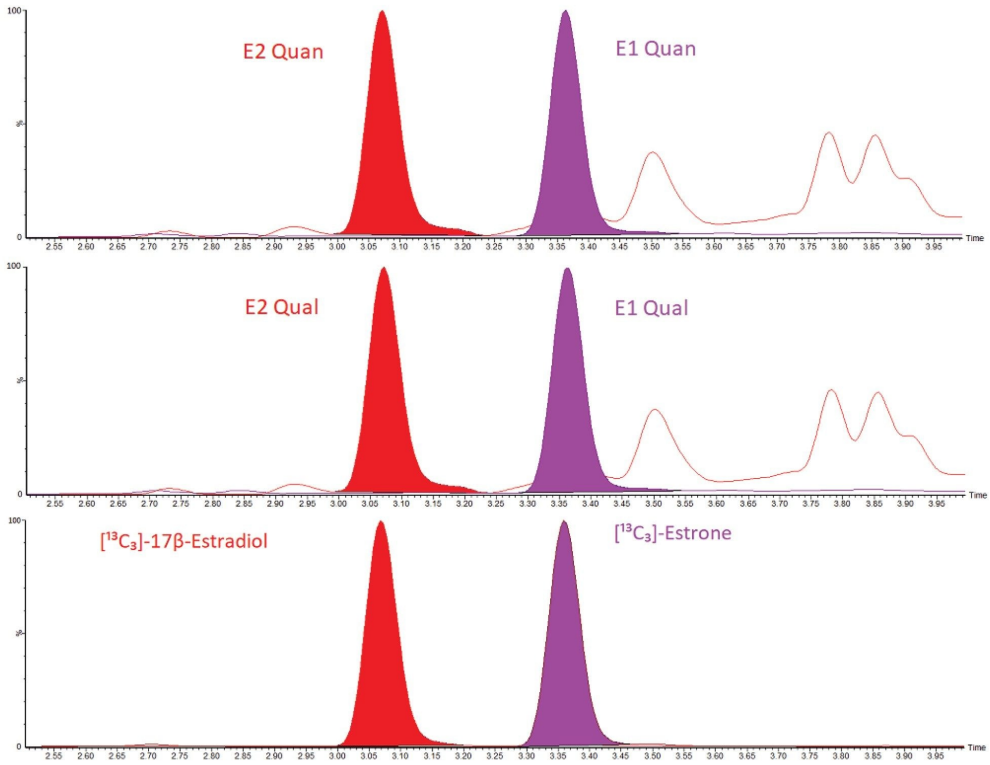

Figure 2. Typical chromatograms of E2 and E1 extracted from a UK NEQAS sample at 22.5pg/mL and 23.6pg/mL respectively.

Calibration curves were shown to be linear over the calibration ranges described, having correlation coefficients of >0.999 and %bias of within ±15% (±20% for Calibrator 1) for E2 and E1 across five occasions.

Precision performance was assessed by extracting and analyzing five replicates of the low, mid and high QC samples on each of five occasions. Within-run and total precision were ≤3.2% CV for all steroid hormones for all concentration levels and is summarized in Table 3.

	E2		E1	
	Repeatability (%CV)	Total (%CV)	Repeatability (%CV)	Total (%CV)
QC L – 3.0 pg/mL	3.0	3.2	2.9	2.9
QC M – 10.0 pg/mL	1.4	2.2	1.5	2.7
QC H – 700 pg/mL	1.6	2.3	1.7	3.0

Table 3. Precision performance summary of E2 and E1 extracted and analyzed on the ACQUITY UPLC I-Class FL System and Xevo TQ Absolute XR Mass Spectrometer.

Analytical sensitivity was assessed by spiking Golden West Biologicals MSG4000 stripped serum at low concentration levels. Ten replicates of each sample were then extracted and analyzed on each of four occasions. %CVs of ≤20% and biases of ≤15% were obtained at 1.0 pg/mL for E2 and E1, however, a signal to noise ratio (peak to peak) of ≥10:1 was not achieved at this level for E2, therefore a lower limit of quantification (LLOQ) of 3 pg/mL was selected for this analyte. Figure 3 shows typical chromatograms of E2 and E1 at these concentrations. While an LLOQ of 1 pg/mL was not achieved, a blank stripped serum sample can be differentiated from the same serum spiked at 1 pg/mL of E2.

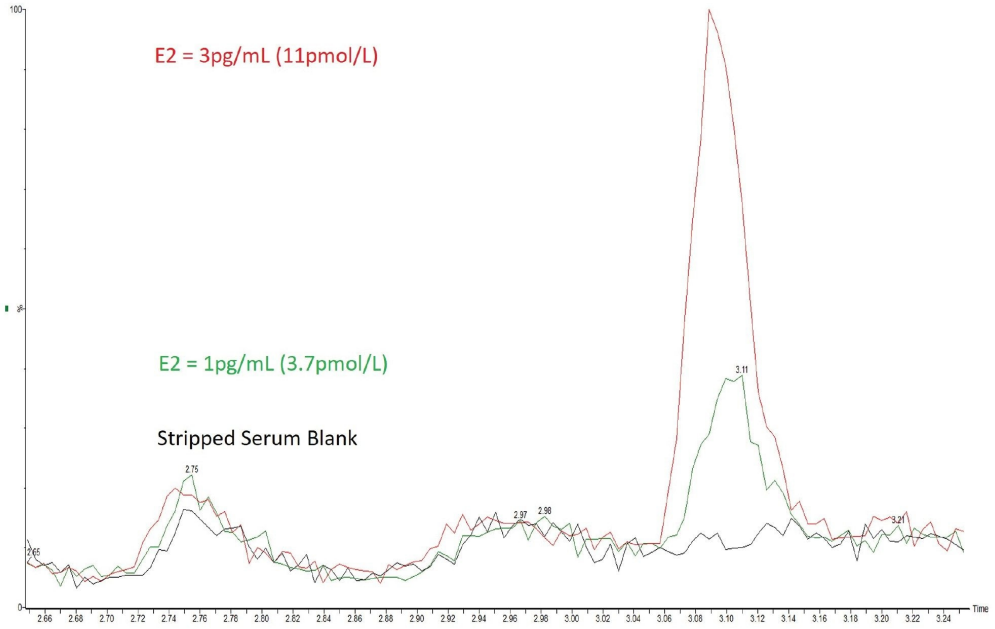

Figure 3. Example of chromatograms of a blank and low level samples in stripped serum for E2.

Matrix effect investigations were previously performed on a Xevo TQ Absolute Mass Spectrometer using serum from six individuals. The endogenous peak areas were separately quantified and post-spiked samples at low and high concentration levels were adjusted using the mean peak areas to enable comparison to solvent spiked samples. While some suppression and enhancement in the matrix factor results were observed when looking at the peak areas, these were compensated for by the internal standard (Table 4).

Compound	Spiked Concentration (pg/mL)	Matrix Factor based on Peak Area (Range)	Matrix Factor based on Concentration (Range)
E2	20	0.917 (0.873 – 1.063)	0.962 (0.911 – 1.023)
	450	0.897 (0.874 – 0.938)	0.940 (0.912 – 0.973)
E1	20	1.313 (1.201 – 1.429)	0.976 (0.915 – 1.031)
	450	1.253 (1.188 – 1.321)	0.938 (0.908 – 0.957)

Table 4. Matrix factor summary for E2 and E1.

Accuracy was assessed by analyzing 39 UK NEQAS E2 samples with calculated concentrations compared to the mass spectrometry laboratory trimmed means. The correlation for E2 can be seen in Table 5 and Figure 4, showing excellent agreement with the EQA scheme.

Scheme	Deming Fit Equation	Linear Fit (r^2)	Bland Altman Mean Bias
UK NEQAS E2	y = 1.17 + 1.012x	0.998	-2.50%

Table 5. Accuracy summary for E2.

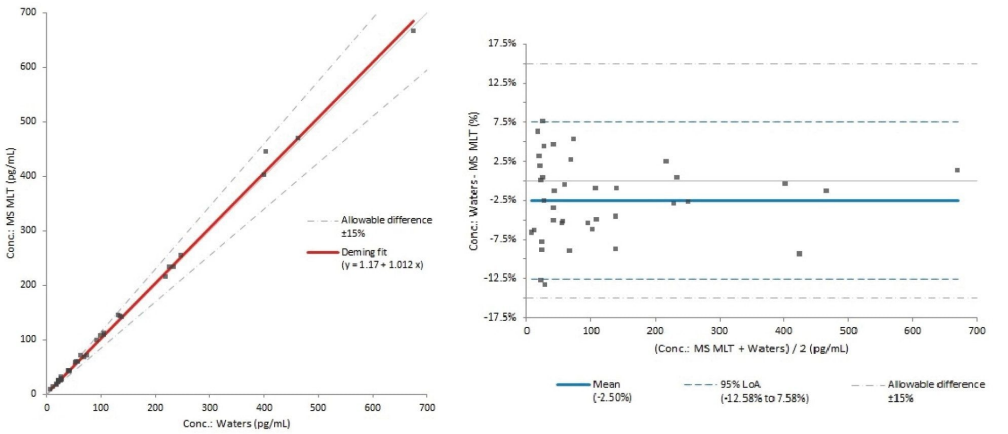

Figure 4. Deming Fit and Bland-Altman plots for comparison with the UK NEQAS E2 scheme.

Conclusion

The Xevo TQ Absolute XR Mass Spectrometer has demonstrated excellent analytical sensitivity and quantitative performance for the analysis of E2 and E1 for clinical research, having the following method performance characteristics:

- Calibration curves had correlation coefficients (r^2) of >0.999 for E2 and E1 for all runs.
- Within-run and total precision results of ≤3.2% CV.
- Analytical sensitivity concentrations of 3.0pg/mL (11.1 pmol/L) for E2 and 1.0 pg/mL (3.7 pmol/L) for E1 were achieved, having a %CV of ≤20%, bias of ≤15% and S:N (ptp) of >10:1. Furthermore, a blank stripped serum sample was able to be distinguished from the same matrix sample spiked with 1 pg/mL of E2 from only 250 µL of sample, without the need for derivatization.
- Little to no ion suppression was observed from six individuals when comparing calculated concentrations to control samples.
- The method was shown to be accurate when compared to the UK NEQAS E2 scheme, having a Deming Fit of y = 1.17 +1.012x and a Bland-Alman Mean Bias of -2.50%.

Featured Products

Xevo TQ Absolute XR <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html>

720009343, May 2026