

## ACQUITY UPLC I-Class with Xevo TQ-XS IVD System: Analytical Performance for Estrogens

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Waters Corporation

For *in vitro* diagnostic use. Not available in all countries.

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### Introduction

The Waters ACQUITY UPLC I-Class with Xevo TQ-XS IVD System enables the quantification of organic compounds in human biological liquid matrices.

This document describes a test of the analytical performance of the ACQUITY UPLC I-Class with Xevo TQ-XS IVD System for the analysis of 17 $\beta$ -estradiol (E2) and estrone (E1) in serum.



Figure 1. The Waters ACQUITY UPLC I-Class System and Xevo TQ-XS Mass Spectrometer.

## Experimental

The ACQUITY UPLC I-Class/Xevo TQ-XS IVD System was controlled by MassLynx IVD Software (v4.2) and the data processed using the TargetLynx Application Manager. Calibrators and quality controls were prepared by spiking commercially-available reference material in stripped serum and the samples were processed using the following conditions:

### Sample Preparation Conditions

A 250 µL sample was processed with hexane, ethyl acetate, and centrifuged. Samples were transferred, evaporated to dryness, and reconstituted in methanol and water prior to analysis.

## LC Conditions

Column:	CORTECS Phenyl, 2.7 µm, 2.1 × 50 mm
Mobile phase A:	0.05 mM ammonium fluoride in water
Mobile phase B:	Methanol
Flow rate:	0.3 mL/min
Gradient:	10% B for 0.5 min, 40%–70% B over 3.0 min, 98% B for 0.5 min, 10% B for 0.5 min

## MS Conditions

Resolution:	MS1 (0.7 FWHM), MS2 (0.7 FWHM)
Acquisition mode:	MRM
Polarity:	ESI-

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## Results and Discussion

Chromatographic separation of E2 and E1 on the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System is illustrated in Figure 2, with low level samples of E2 shown in Figure 3. Performance characteristics of E2 and E1 are shown in Table 1.

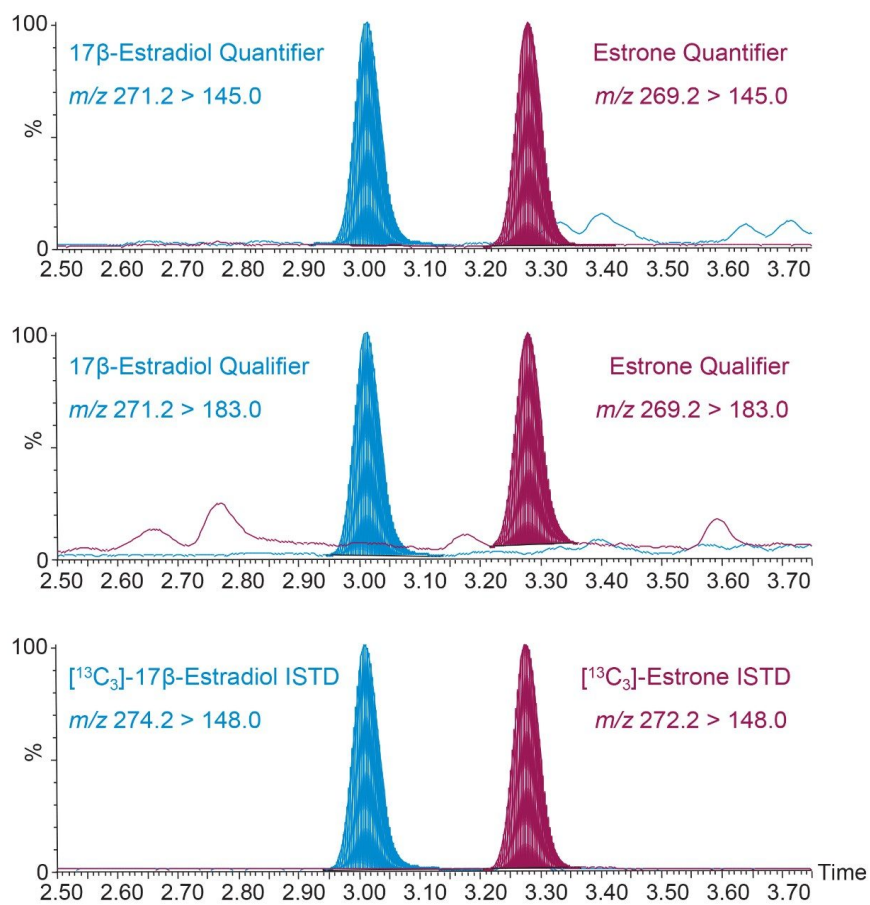


Figure 2. Chromatographic separation of E2 and E1 in a sample using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System.

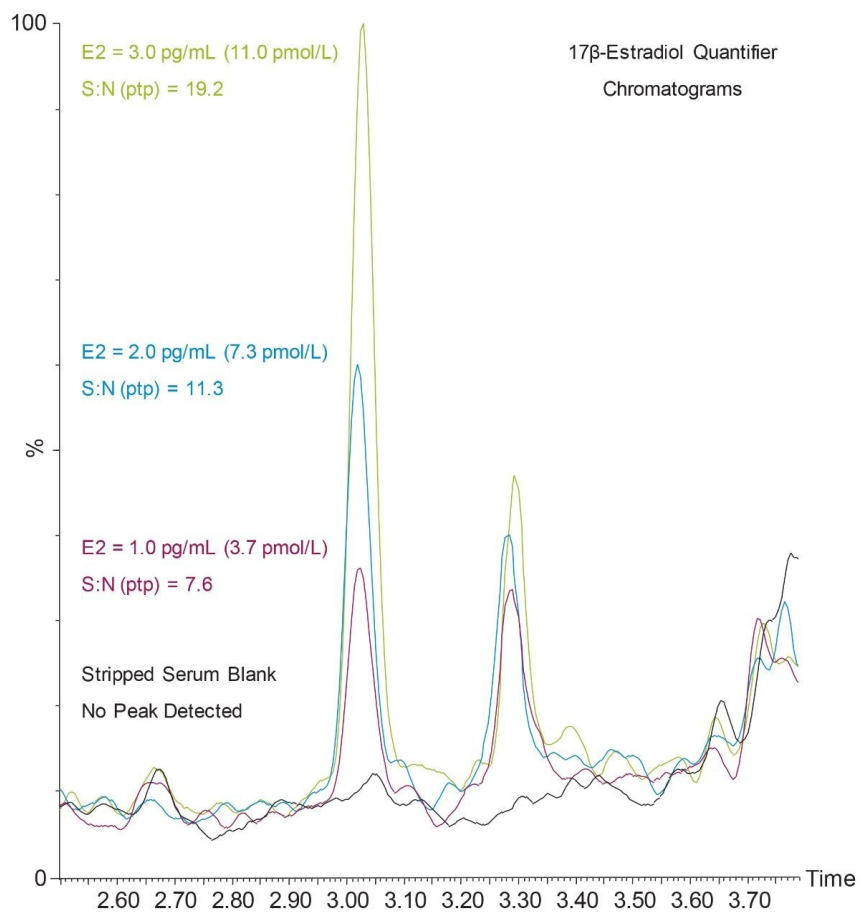


Figure 3. Stripped serum spiked with low levels of E2 using the ACQUITY UPLC I-Class/Xevo TQ-XS IVD System.

Compound	Range (pmol/L)	LLOQ (pmol/L)	Total precision	Repeatability	CDC HoSt mean bias	EQA LC-MS mean bias
17β-Estradiol (E2)	11.1-3700	11.1	≤4.5%	≤4.5%	7.0%	1.7%
Estone (E1)	7.4-3700	7.4	≤3.5%	≤4.8%	N/A	N/A

Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where  $r^2 > 0.99$ . LLOQ defined by allowable precision of 20% and signal-to-noise ratio of >10:1, from samples performed over five days with one run per day ( $n = 25$ ). Total precision and repeatability of samples performed over five occasions with one run per day ( $n = 25$ ). Central for Drugs Control Hormone Standardization Program (CDC HoSt) mean bias determined from assigned values. EQA LC-MS mean bias determined from LC-MS method means of UK NEQAS Estradiol Programme.

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

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## Conclusion

The Waters ACQUITY UPLC I-Class with Xevo TQ-XS IVD System has demonstrated the capability to deliver analytical sensitivity, precision, and accuracy for the analysis of 17β-estradiol and estrone in serum.

## Disclaimer

The analytical performance data presented here is for illustrative purposes only. Waters does not recommend or suggest analysis of the analytes described herein. These data are intended solely to demonstrate the performance capabilities of the system for analytes representative of those commonly analyzed using liquid chromatography and tandem mass spectrometry. Performance in an individual laboratory may differ due to a number of factors, including laboratory methods, materials used, intra-operator technique, and system conditions. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the analytes in this analysis.

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