

## ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Steroid Prohormones

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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/Xevo TQD 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/Xevo TQD IVD System for the analysis of 25-hydroxyvitamin D2 (25OHD2) and 25-hydroxyvitamin D3 (25OHD3) in serum.



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*ACQUITY UPLC I-Class/Xevo TQD IVD System.*

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## Experimental

The ACQUITY UPLC I-Class/Xevo TQD IVD System was controlled by MassLynx IVD Software (v4.1) and the data processed using the TargetLynx Application Manager. Calibrators and Quality Controls were prepared by

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spiking commercially available reference material in stripped serum and the samples were processed using the following conditions:

## Sample Preparation Conditions

150 µL sample was processed with zinc sulphate, methanol, and centrifuged. Samples were loaded onto Oasis HLB µElution plates, washed, and eluted prior to analysis.

## LC Conditions

Column:	ACQUITY UPLC BEH Phenyl (IVD) 1.7 µm, 2.1 mm × 50 mm
Pre-column:	ACQUITY UPLC Column In-Line Filter
Mobile phase A:	2 mM ammonium acetate + 0.1% formic acid in water
Mobile phase B:	2 mM ammonium acetate + 0.1% formic acid in methanol
Flow rate:	0.45 mL/min
Gradient:	65–80% B over 2.5 minutes, 80% B over 0.2 minutes, 98% B for 0.8 minutes

## MS Conditions

Resolution:	MS1 (0.7 FWHM), MS2 (0.85 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

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## Results and Discussion

Performance characteristics of 25OHD2 and 25OHD3 are shown in Table 1. Chromatographic separation of 25OHD2 and 25OHD3 on the ACQUITY UPLC I-Class/Xevo TQD IVD System is illustrated in Figure 1.

Compound	Range (nmol/L)	LLOQ (nmol)	Total precision	Repeatability	DEQAS mean bias
25OHD2	10–375	3.0	≤7.3%	≤6.5%	N/A
25OHD3	10–375	5.6	≤6.6%	≤6.0%	2.1

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*Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where  $r^2 > 0.99$ . LLOQ defined by allowable precision and bias of 15% and 10% respectively, from samples performed over three days with two runs per day ( $n=90$ ). Total Precision and Repeatability of samples performed over 20 occasions with two runs per day ( $n=80$ ). Vitamin D External Quality Assessment Scheme (DEQAS) Mean Bias determined from NIST assigned DEQAS values.*

*Note: To convert SI units to conventional mass units divide by 2.423 for 25OHD2 (nmol/L to ng/mL) and 2.496 for 25OHD3 (nmol/L to ng/mL).*

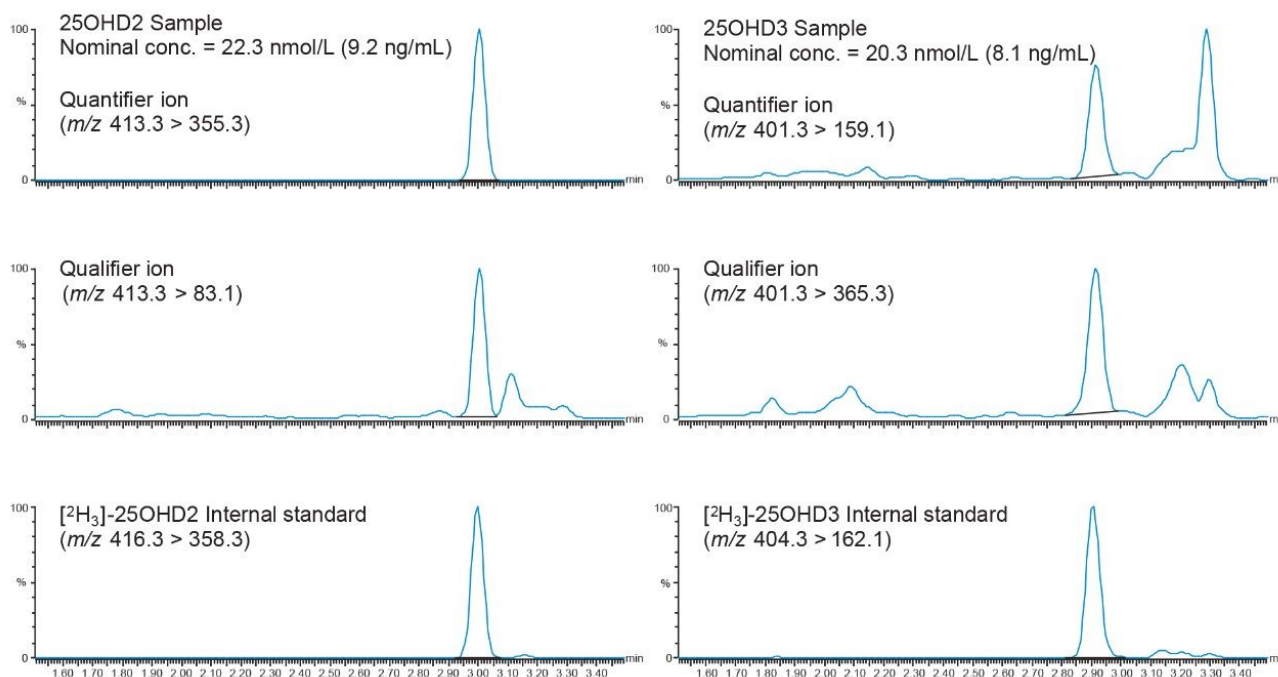


Figure 1. Chromatographic separation of 25OHD2 and 25OHD3 in a low level sample using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

## Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to quantify 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in serum with precision and accuracy.

## 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*

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