

## ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for an Antifolate Agent

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Waters Corporation

用於體外診斷用途。並非在所有國家/地區均可使用。

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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 methotrexate in plasma.



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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 spiking commercially available reference material in plasma. The samples were processed using the following conditions:

### Sample Preparation Conditions

50  $\mu$ L sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

### LC Conditions

Column: ACQUITY UPLC HSS C<sub>18</sub> SB 1.8  $\mu$ m,

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	2.1 mm × 30 mm
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:	0.4 mL/min
Gradient:	77% A isocratic for 5 min

## MS Conditions

Resolution:	MS1 (0.75 FWHM), MS2 (0.75 FWHM)
Acquisition mode:	MRM
Polarity:	ESI (+)

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## Results and Discussion

Performance characteristics of methotrexate on the ACQUITY UPLC I-Class/Xevo TQD IVD System are shown in Table 1. Analytical sensitivity of the chromatographic separation is illustrated in Figure 1.

Compound	Range ( $\mu\text{mol/L}$ )	LLOQ ( $\mu\text{mol/L}$ )	%RSD at LLOQ	Total precision	Repeatability	EQA mean bias
Methotrexate	0.025–10	0.0025	15.7	$\leq 5.5\%$	$\leq 4.0\%$	-5.7%

Table 1. Performance characteristics of methotrexate. Range defined by linear fit where  $r^2 > 0.99$ . LLOQ defined by  $S/N$  (PtP)  $> 10$  and  $\%RSD \leq 20\%$ .  $\%RSD$  at LLOQ determined through analytical sensitivity experiments performed over three occasions ( $n=30$ ). Total precision and repeatability of QCs performed over five occasions in plasma ( $n=25$ ). EQA mean bias determined by comparison of obtained values to the gravimetric reference value.

Note: To convert SI units to conventional mass units divide by 2.2005 ( $\mu\text{mol/L}$  to  $\mu\text{g/mL}$ ).

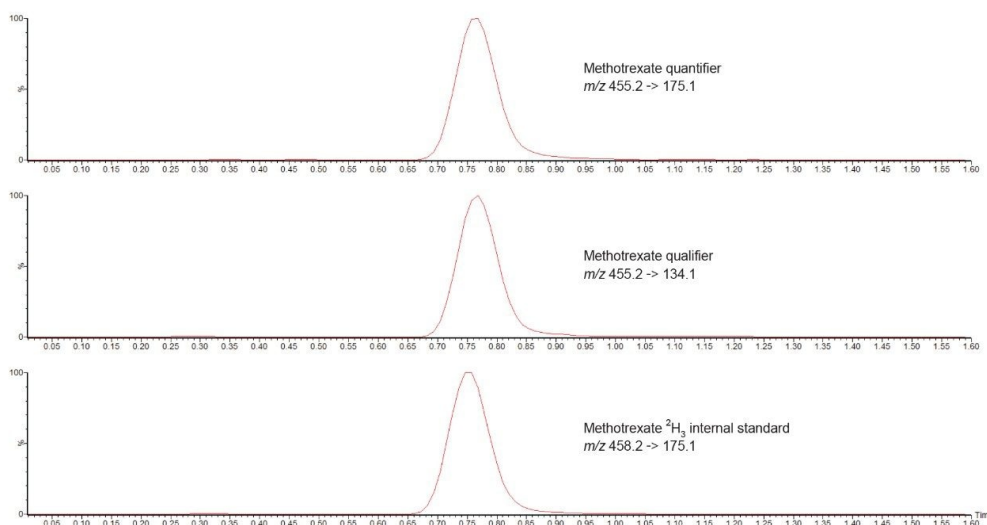


Figure 1. Chromatogram showing the analysis of methotrexate using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

## Conclusion

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver an analytically sensitive and precise method for methotrexate in plasma.

## 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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