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# ACQUITY UPLC I-Class/Xevo TQD IVD System: Analytical Performance for Azole Antifungals

## 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 fluconazole, hydroxyitraconazole, itraconazole, posaconazole, voriconazole, and voriconazole-N-oxide in serum.

## EXPERIMENTAL DETAILS

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 serum and the samples were processed using the following conditions:

### Sample preparation conditions

50 µL sample was processed with methanol and centrifuged, then subsequently diluted with water prior to analysis.

### LC conditions

| | |
|---|---|
| Column: | ACQUITY UPLC BEH $C_{18}$<br>1.7 µm, 2.1 mm × 30 mm |
| Mobile phase A: | 2 mM Ammonium acetate<br>+ 0.1% formic acid in water |
| Mobile phase B: | 2 mM Ammonium acetate<br>+ 0.1% formic acid in methanol |
| Flow rate: | 0.8 mL/min |
| Gradient: | 75% A initial, gradient 7 until 97% B at 2.1 minutes, then hold 75% A until 2.5 minutes |

### MS conditions

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


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

## RESULTS

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

| Compound | Range (µg/mL) | LLOQ (µg/mL) | %RSD at LLOQ | Total precision | Repeatability |
|---|---|---|---|---|---|
| Fluconazole | 0.5–100 | 0.3750 | 13.1 | ≤2.7% | ≤2.6% |
| Hydroxyitraconazole | 0.05–10 | 0.0500 | 18.8 | ≤11.5% | ≤10.0% |
| Itraconazole | 0.05–10 | 0.0375 | 17.2 | ≤8.9% | ≤8.6% |
| Posaconazole | 0.05–10 | 0.0500 | 15.2 | ≤7.7% | ≤5.2% |
| Voriconazole | 0.05–10 | 0.0250 | 15.8 | ≤2.6% | ≤1.5% |
| Voriconazole N-Oxide | 0.05–10 | 0.0375 | 16.1 | ≤5.4% | ≤3.5% |

*Table 1. Performance characteristics of the analytes evaluated. Range defined by linear fit where $r^2$ >0.99. LLOQ defined by S/N (PtP) >10 and %RSD ≤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 stripped serum (n=25).*

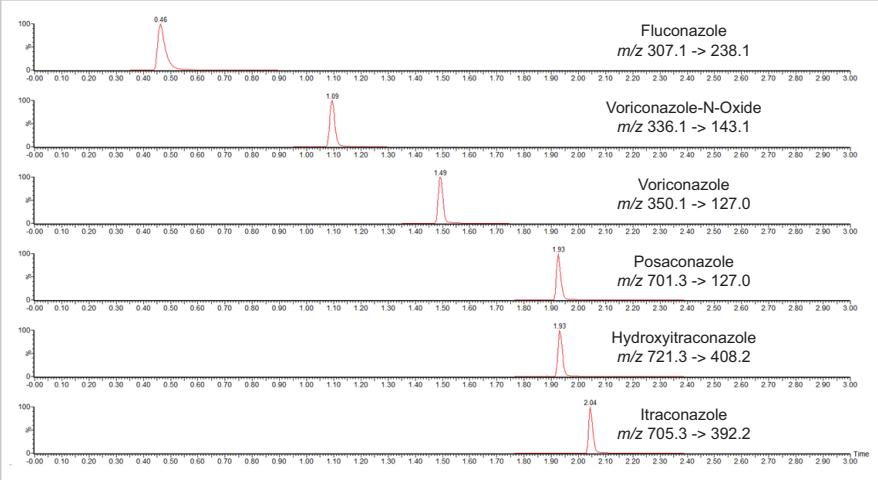


*Figure 1. Chromatographic separation of a range of azole antifungals using the ACQUITY UPLC I-Class/Xevo TQD IVD System.*

## CONCLUSIONS

The Waters ACQUITY UPLC I-Class/Xevo TQD IVD System has demonstrated the capability to deliver an analytically sensitive and precise method for fluconazole, hydroxyitraconazole, itraconazole, posaconazole, voriconazole, and voriconazole-N-oxide in serum.

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

**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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