A High Throughput LC-MS/MS Method for the Quantitation of Voriconazole in 0.05 mL of Human Plasma

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PURPOSE:
- The purpose of the project was to develop a sensitive and high-throughput LC-MS/MS method suitable for the determination of voriconazole in human plasma samples from bioequivalence or drug interaction studies using a small sample volume.

BACKGROUND:
- Voriconazole is an antifungal medication that is generally used to treat serious, invasive fungal infections.
- Peak plasma concentrations of approximately 2.3 µg/mL are achieved 1 to 2 hours after oral administration of 400 mg in the fasted state.
- The pharmacokinetics are non-linear due to metabolic saturation of the human hepatic cytochrome P450 enzymes, CYP2C19, CYP3A4 and CYP2C9. Voriconazole accounts for a large part of voriconazole metabolism and exhibits genetic polymorphism in humans.
- The range of the assay including dilution integrity is appropriate for analysis of samples from pharmacokinetic and drug interaction studies.
- Using a low sample volume allowed for less blood draw volume and the ability to measure additional analytes from one sample.

METHODS:
- Extraction: Voriconazole human plasma QC E074X samples (0.0500 µg) were diluted with an ammonium acetate buffer, extracted with n-butyl chloride using an automated liquid-liquid extraction procedure that greatly increased sample throughput. The method has successfully been used to support clinical studies.
- Chromatography: Voriconazole was chromatographically separated from other matrix components on an Advanced Chromatography Technologies, AC18 C18 analytical column with an isocratic mobile phase consisting of acetonitrile, water, and formic acid.
- Detection: An API SCIEX 4000, using an ESI interface, detected positive ions in the multiple reaction monitoring mode with a retention time of 1.5 minutes.

RESULTS:
- The calibration standards were linear over the range of 0.02 to 2000 ng/mL. Inter-batch precision (CV) of voriconazole quality control samples was less than 5.2% (Table 1). Inter-batch accuracy (% Bias) of voriconazole quality controls was +12.4 to -2.0% (Table 1).
- Samples having a concentration above the upper limit of the calibration standard range were diluted with blank human plasma, and results show that samples with a concentration up to 15,000 ng/mL could be quantified after the application of an appropriate dilution factor.
- Assay selectivity was demonstrated by the quantitation of ten separate lots of human plasma fortified with known concentrations of voriconazole. No significant matrix effect was observed in any of the ten lots fortified at the LLOQ concentration or at the high quality control sample concentration for voriconazole (Table 2).
- Stability of the analyte in freshly collected human whole blood was established for up to 120 minutes in polypropylene tubes at ambient temperature under white light.
- A post-column infusion showed no areas of significant enhancement or suppression at the retention time of the analyte or internal standard (Figure 2).
- Post-preparative stability in injection solvent (quantitation against freshly extracted standards) was established for 158 hours at 5°C.
- Processed sample stability in injection solvent (quantitation against freshly extracted standards) was established for 163 hours at 5°C.
- Short-term stability in plasma was established for 96 hours at ambient temperature under white light.
- Freeze and thaw stability in plasma was established for six freeze (+2°C) and three cycles (+2°C) and those cycles (ambient temperature).

CONCLUSIONS:
- The bioanalytical assay for the quantitation of voriconazole in human plasma met acceptance criteria for precision, accuracy, linearity, selectivity, and stability. The low sample volume required for analysis enabled the use of an automated liquid-liquid extraction procedure that greatly increased sample throughput.

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<th>5.00 ng/mL</th>
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<td>QC B</td>
<td>QC C</td>
<td>QC A</td>
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<td>306.00</td>
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<td>0.513</td>
<td>6.29</td>
<td>34.30</td>
</tr>
</tbody>
</table>

**Table 1. Inter-batch Precision and Accuracy**

**Figure 1. Voriconazole Structure**

**Figure 2. Post-Column Matrix Infusion**

**Figure 3. Representative Chromatograms of Voriconazole and d3-Voriconazole (IS) from an Extracted Human Plasma (EDTA) Sample For fortified with d3-Voriconazole (IS) Only**

**Figure 4. Representative Chromatograms of Voriconazole and d3-Voriconazole (IS) from an Extracted Blank Human Plasma (EDTA) Sample**

**Figure 5. Representative Chromatograms of Voriconazole and d3-Voriconazole (IS) from an Extracted Human Plasma (EDTA) LLOQ Sample**

**Figure 6. Representative Chromatograms of Voriconazole and d3-Voriconazole (IS) from an Extracted Human Plasma (EDTA) ULOQ Sample**

**Figure 7. Representative Chromatograms of Voriconazole and d3-Voriconazole (IS) from an Extracted Human Plasma (EDTA) QC Sample**

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