An LC-MS/MS Method for Quantifying Azole Antifungal Medications in Plasma

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Introduction

• Azole antifungal medications (voriconazole, fluconazole, itraconazole, hydroxy-itraconazole, and posaconazole):
  - inhibit ergosterol synthesis → cell membrane permeability in fungi
  - are used for treatment or prophylaxis of invasive fungal infections, especially in immunocompromised patients

• Therapeutic drug monitoring is recommended for azole antifungals due to:
  - variable pharmacokinetic properties
  - certain trough plasma concentration thresholds that are required for efficacy
  - correlation of toxicity with elevated trough plasma concentrations of itraconazole

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Range (μg/mL) of Trough Plasma Concentrations</th>
<th>Toxic Range (μg/mL) of Trough Plasma Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voriconazole</td>
<td>1.0 – 5.5</td>
<td>&gt; 6.0</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>5.0 – 20.0</td>
<td></td>
</tr>
<tr>
<td>Itraconazole (localized infection)</td>
<td>&gt; 0.5</td>
<td></td>
</tr>
<tr>
<td>Itraconazole (systemic infection)</td>
<td>&gt; 1.0</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>&gt; 0.7</td>
<td></td>
</tr>
</tbody>
</table>

from ARUP

Methods

• Samples for method development and validation:
  - 500 μL of plasma spiked with voriconazole-d₃ internal standard at 10 μg/mL and alkalinized with sodium hydroxide at 0.9%

• Sample clean-up:
  - liquid/liquid extractions with 3 mL of 30% ethyl acetate/70% hexane
  - extracts dried and reconstituted in 65 μL of 50% methanol/0.05% acetic acid

• LC-MS/MS system:
  - Varian 1200L triple quadrupole

  - 27 μL of sample injected
  - mobile phase gradient of 35-100% methanol (containing 0.1% formic acid throughout) over 6 min at a flow rate of 0.2 mL/min
  - C₁₈ guard column and C₁₈ column (2.1 x 150 mm, 3.5 μm) maintained at 35°C
  - positive electrospray ionization (ionization voltage 5000 V) with nitrogen drying gas at 400°C

Results

• Post-column infusion studies showed separation of azole peaks from zones of ion suppression

• Baseline separation of various azoles

• Preliminary lower limits of quantitation are <0.1 μg/mL

• Linearity study based on CLSI Guideline EP6-A showed linear relationship between 0.05-33 μg/mL for itraconazole and between 0.05-50 μg/mL for each of the other azoles

Objective

• To develop and validate an LC-MS/MS method to be used for therapeutic drug monitoring of azole antifungal medications

Disclosure

• This project has received financial support from Pfizer

Future Directions

• Full validation of the method will be performed, including precision, accuracy, and lower limit of quantitation studies