**High-throughput equilibrium dialysis to determine protein binding characteristics of voriconazole**

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**BACKGROUND & OBJECTIVES**

Plasma protein binding (PPB) is an important aspect of a drug’s pharmacokinetics. Equilibrium dialysis (ED) is the analytical method of choice¹. Voriconazole, a triazole with unpredictable pharmacokinetics, binds to plasma proteins for 58%. This was determined with ED in premarketing studies, using a Dianorm dialyzer for 2 hours, with human plasma spiked with 1 mg/L voriconazole against a 0.1 M phosphate buffer pH 7.4². Nowadays, a newer high-throughput ED assay (HT-ED) is available³ (HTDialysis LLC, USA). We have tested its applicability for the PPB of voriconazole.

**METHODS**

- HT-ED: 96-well plate (HTDialysis, model HTD96b)
  - Buffer compartment: PBS
  - Investigational compartment: plasma spiked with voriconazole
  - Diffusion of unbound drug
  - Semi-permeable membrane
  - PPB% = \frac{[\text{voriconazole}]_{\text{PBS}}}{[\text{voriconazole}]_{\text{plasma}}} \times 100
  - Shaker incubation
  - Voriconazole analysis: LC-MSMS⁴

**RESULTS**

- Median voriconazole PPB:
  - 47.6% (IQR 45.3% - 50.0%)
- PPB Independent of voriconazole plasma concentration
  - (p=0.653)
  - (tested concentration: 0.7-11.2 mg/L)
- Mass balance:
  - Negligible drug loss through adsorption to the device
- Temperature:
  - No difference in PPB at 25°C versus 37°C (p=0.713)
- Freeze-thaw cycles:
  - No difference in PPB during 1-3-5 freeze-thaw cycles (p=0.108)
- Frozen Storage at -20°C:
  - No difference in PPB (up to 12 months)

**PRACTICAL ASPECTS**

<table>
<thead>
<tr>
<th></th>
<th>Premarking ED²</th>
<th>HT-ED</th>
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<tbody>
<tr>
<td>PPB (median)</td>
<td>58%</td>
<td>47.6%</td>
</tr>
<tr>
<td>Material</td>
<td>Dianorm dialyzer</td>
<td>HTD dialysis: 96-well plate</td>
</tr>
<tr>
<td>Volume of buffer/plasma</td>
<td>1 ml</td>
<td>150 µl</td>
</tr>
<tr>
<td>Equilibrium time</td>
<td>2 h</td>
<td>4 h</td>
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<tr>
<td>Voriconazole concentration</td>
<td>1 mg/L</td>
<td>0.7-11.2 mg/L</td>
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<tr>
<td>Detection method</td>
<td>Liquid scintillograph</td>
<td>LC-MSMS⁴</td>
</tr>
<tr>
<td>Buffer</td>
<td>Phosphate buffer (0.1 M)</td>
<td>PBS (0.012 M)</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>252</td>
</tr>
</tbody>
</table>

- Clinical relevance of the difference in PPB, as determined with the two methods, is unknown

**CONCLUSION**

HT-ED is a robust assay for determination of voriconazole PPB and can easily be used to determine unbound concentrations in daily practice. It can be performed without influence of temperature, batching, freezing and thawing.

**REFERENCES**

1. Wan & Rehngren, 2006, J Chromatograph
2. Roffey et al., 2003, Drug Metab & Dispo
3. Banker et al., 2003, J Pharm Sci
4. Pauwels et al., 2012, Clin Chim Acta
5. Lagrange et al., 2000, J Pharmaceut & Biomed

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