In vitro ADME & PK

Cytochrome P450 Time Dependent Inhibition (Single Point)

Background Information

- The inhibition of human cytochrome P450s is one of the most common mechanisms which can lead to drug-drug interactions.
- Time dependent inhibition defines an interaction where there is enhanced inhibition if the test compound is pre-incubated with the metabolising system prior to the addition of substrate.
- If an irreversible interaction occurs, the consequences of time dependent inhibition are considered to be more serious because the inactivated enzyme must be re-synthesised before activity is restored.
- Cyprotex's time dependent inhibition (single point) assay uses industry accepted probe substrates and human liver microsomes.

Protocol

- **CYP Isoforms Available**
  - CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4
- **Substrates**
  - See table 1
- **Number of Replicates**
  - 2
- **Pre-incubation Time**
  - 30 min
- **Test Article Concentration**
  - 25 µM
- **Positive Controls**
  - See Table 1
- **Test Article Requirements**
  - Dependent on number of isoforms assessed
- **Analysis Method**
  - LC-MS/MS (with the exception of ethoxyresorufin for CYP1A)
- **Data Delivery**
  - Mean percentage inhibition following pre-incubation

Related Services

- Cytochrome P450 Time Dependent Inhibition (IC50 Shift)
- Cytochrome P450 Time Dependent Inhibition (kVmax/Km)

To find out more contact enquiries@cyprotex.com
Irreversible and quasi-irreversible inhibition are often viewed as more serious than reversible inhibition, since the inhibitory effect remains after elimination of the parent drug from the body\(^2\).

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Substrate</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Ethoxyresorufin</td>
<td>Furafylline</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion</td>
<td>Ticlopidine</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Pacinaxel</td>
<td>Gemfibrozil glucuronide</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>Ticlopidine</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>5-Methylnicotin</td>
<td>Ticlopidine</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan</td>
<td>Paroxetine</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam</td>
<td>Mifepristone</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Testosterone</td>
<td>Mifepristone</td>
</tr>
</tbody>
</table>

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For the validation, a literature search was performed to identify a selection of compounds which were time dependent inhibitors of the main cytochrome P450 isoforms. These inhibitors were selected and were screened in triplicate on the plate on 3 separate occasions. The results show that there is a high level of consistency over a range of inhibition values.

Mibefradil is both reversible and time dependent CYP3A4 inhibitor as it exhibits inhibitory potential in both the absence and presence of NADPH in the pre-incubation with the inhibition greater in the latter incubation. It is therefore possible to both detect and discriminate between the reversible cytochrome P450 inhibition and the time dependent cytochrome P450 inhibition associated with this test compound.

References

1. FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)