In vitro ADME & PK

Cytochrome P450 Time Dependent Inhibition ($k_{\text{inact}}/K_I$)

Background Information

- Time dependent inhibition of cytochrome P450, often caused by an irreversible or quasi-reversible interaction, can lead to clinically relevant drug-drug interactions or non-linear pharmacokinetics of a drug. In addition, these interactions are typically a consequence of reactive metabolite formation which is also associated with toxicity via covalent binding to cellular macromolecules.

- Characterisation of the $k_{\text{inact}}$ (maximal inactivation) and $K_I$ (concentration at 50% $k_{\text{inact}}$) parameters is frequently performed during drug development to evaluate risk of time dependent inhibition and decide if a clinical interaction study is required.

- Cyprotex's $k_{\text{inact}}/K_I$ assay evaluates the inactivation kinetics of time dependent inhibition at 5 inhibitor concentrations and 7 pre-incubation times.

Protocol

- Substrates and CYP Isoforms
  Phenacetin (CYP1A2), bupropion (CYP2B6), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4) (others available on request)

- Test System
  Human liver microsomes

- Pre-incubation Times
  7 Pre-incubation times (including 0 min)

- Test Article Concentrations
  5 Concentrations plus vehicle control

- Number of Replicates
  2

- Analysis Method
  LC-MS/MS

- Data Delivery
  $k_{\text{inact}}/K_I$

Related Services

- Cytochrome P450 Time Dependent Inhibition (Single Point)
- Cytochrome P450 Time Dependent Inhibition (IC$_{50}$ Shift)

To find out more contact enquiries@cyprotex.com
When TDI is the mode of inhibition, the inhibitory interaction will generally be greater over time following multiple dosing and be longer lasting after discontinuation of the inhibitor than in a situation when the inhibitory interaction is reversible\textsuperscript{3}.  

Cytochrome P450 Time Dependent Inhibition (k\textsubscript{inact}/K\textsubscript{I})

A number of known time dependent inhibitors were characterised in the k\textsubscript{inact}/K\textsubscript{I} assay and compared with data published in the literature.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exponentially Determined Values at Cyprotec</th>
<th>Literature Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution factor</td>
<td>Concentration range (µM)</td>
</tr>
<tr>
<td>Diliazem</td>
<td>1:10</td>
<td>0.5-50</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>0.2-20</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>1:10</td>
<td>0.2-20</td>
</tr>
<tr>
<td>Mifepristone</td>
<td>1:10</td>
<td>0.3-30</td>
</tr>
<tr>
<td>Verapamil</td>
<td>1:10</td>
<td>0.2-20</td>
</tr>
</tbody>
</table>

The table illustrates that data generated at Cyprotec compares well with literature data.

### References