In vitro ADME & PK

Cytochrome P450
Time Dependent Inhibition (k_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_inact (maximal inactivation) and K_I (concentration at 50% k_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_inact/K_I assay evaluates the inactivation kinetics of time dependent inhibition at 5 inhibitor concentrations and 7 pre-incubation times.

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_inact/K_I

Related Services
- Cytochrome P450 Time Dependent Inhibition (Single Point)
- Cytochrome P450 Time Dependent Inhibition (IC50 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.’

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

A number of known time dependent inhibitors were characterised in the $k_{\text{inact}}/K_i$ assay and compared with data published in the literature.

**Table 1**

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

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

**References**