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In vitro **ADME & PK**

Cytochrome P450 Time Dependent Inhibition (IC_{50} Shift)

Background Information

'The IC_{50} shift method has the capability for simultaneous detection of both reversible inhibitors and time-dependent inhibitors, plus the added benefit for potentially predicting drug-drug interactions'

[1]Grimm SW, Einolf HJ, Hall SD, He K, Lim H-K, John Ling K-H, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Horn RV, Wang RW, Wong YN, Yang TJ and Obach RS. (2009) *Drug Metab Dispos* **37**; 1355-1370

- Inhibition of cytochrome P450 enzymes is one of the most common mechanisms resulting in clinically relevant drug-drug interactions. This inhibitory effect can either be a reversible or irreversible (time dependent) interaction.
- Time dependent inhibition (TDI) of cytochrome P450 is of particular concern as typically *de novo* synthesis of the enzyme is required in order to restore activity. The consequences of TDI can be termination of drug development, drug withdrawal or serious restrictions of use.
- Cyprotex's IC_{50} shift assay determines the IC_{50} (inhibitor concentration which results in 50% inhibition of activity) following a pre-incubation in the absence and presence of NADPH. This assay enables discrimination between compounds which cause reversible, irreversible, or both reversible and irreversible inhibition.

Related Services

- Cytochrome P450 Time Dependent Inhibition (Single Point)
- Cytochrome P450 Time Dependent Inhibition (k_{inact}/K_I)

Protocol

CYP Isoforms Available
CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4

Substrates
See table 1

Test System
Human liver microsomes

Pre-Incubation Time
30 min (+/- NADPH) and 0 min

Test Article Concentration
0.1, 0.25, 1, 2.5, 10, 25 µM
(different concentrations available)

Positive Controls
See Table 1

Test Article Requirements
150 µL of 10 mM solution

Analysis Method
LC-MS/MS

Data Delivery
IC_{50}
Standard error of IC_{50}
Shifted IC_{50}

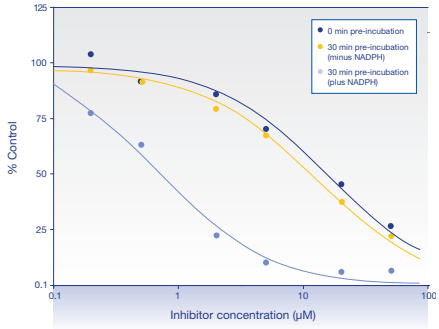
Failure of several late stage clinical candidates has been attributed to TDI, and this mechanism is also suspected to play a role in liver toxicities often observed in preclinical species[2].

Cytochrome P450 Time Dependent Inhibition (IC_{50} Shift)

A number of known time dependent inhibitors were screened in the IC_{50} shift assay in triplicate on the plate on three separate occasions. Inhibitors which were known to be solely reversible inhibitors were screened as negative controls alongside the time dependent inhibitors. The results show a high level of consistency over a range of inhibition values.

Figure 1

IC_{50} shift data for phenacetin O-deethylation inhibition by furafylline, a time dependent inhibitor.



Furafylline was pre-incubated with human liver microsomes in the presence and absence of NADPH prior to the addition of the CYP1A2 substrate, phenacetin. The 17 fold shift in IC_{50} value ($IC_{50\ (30\ Minus)}$ = 10.1 μM; reversible inhibition component, $IC_{50\ (30\ Plus)}$ = 0.586 μM; time-dependent effect) when NADPH is included in the pre-incubation indicates that furafylline is a time dependent inhibitor.

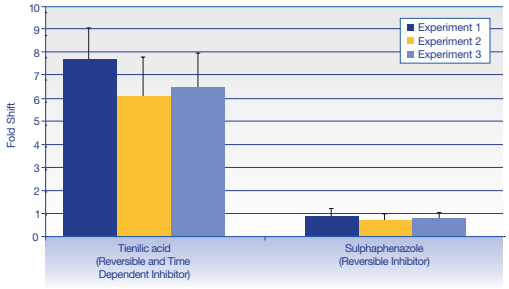
Table 1

Cytochrome P450 isoform specific substrates and positive control compounds used in the Cyprotex IC_{50} Shift assay.

Isoform	Substrate	Positive Control
CYP1A2	Phenacetin	Furafylline
CYP2B6	Bupropion	Thiotepa
CYP2C8	Paclitaxel	Gemfibrozil glucuronide
CYP2C9	Diclofenac	Tienilic acid
CYP2C19	*S*-Mephenytoin	Fluoxetine
CYP2D6	Dextromethorphan	Paroxetine
CYP3A4	Midazolam	Mifepristone
CYP3A4	Testosterone	Mifepristone

Figure 2

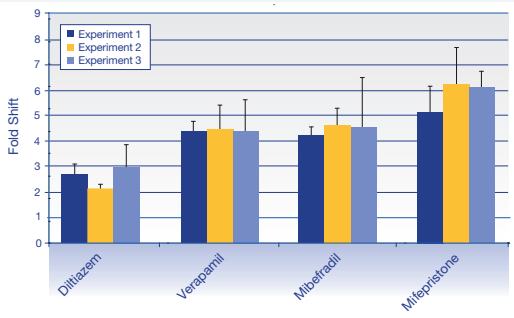
Graph illustrates the fold shift in IC_{50} for tienilic acid (a reversible and time dependent inhibitor) and sulphaphenazole (a reversible inhibitor). Data are mean ± standard deviation of triplicate incubations for three separate experiments.



It is recommended that compounds which exhibit an IC_{50} shift of ≥ 1.5 with a 30 min pre-incubation are classified as time dependent inhibitors[1]. Tienilic acid, a known reversible and time dependent inhibitor, gives a mean fold shift of 6.8, whereas sulphaphenazole, which is solely a reversible inhibitor, gives a mean fold shift of 0.84.

Figure 3

Graph illustrating mean fold shift in IC_{50} of midazolam 1-hydroxylation by four known CYP3A4 time dependent inhibitors.



The data illustrate the mean fold shift in IC_{50} over three experiments with the error bars representing the triplicate incubations on each run of the assay.

References

[1] Grimm SW et al, (2009) *Drug Metab Dispos* **37**; 1355-1370.
[2] Zimmerlin A *et al*, (2011) *Drug Metab Dispos* **39(6)**; 1039-1046.