CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

TIME-DEPENDENT INHIBITION

Time-dependent inhibition (TDI) of CYP metabolism can generally be classified into three types: reversible, quasi-irreversible and irreversible. In general, TDI results from irreversible covalent binding or quasi-irreversible non-covalent tight binding of a chemically reactive intermediate to the enzyme that catalyses its formation. This results in loss of enzyme function, and can cause clinically relevant drug-drug interactions (DDI). In some cases reversible inhibition of a metabolite(s) generated in situ could give rise to TDI.

TDI inhibitory effect lasts longer than reversible inhibition and persists after elimination of the parent drug from the body because CYP activity can only recover with new enzyme synthesis.

Deliverable: Time-dependent inhibition (%) of test compound against CYP1A2, CYP2C9, CYP2C19, CYP2D6 & CYP3A4.

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 50µLof 10mM in DMSO or 0.5mg solid.

ENZYMES

Pooled Human Liver Microsomes.

Table 1. Substrates & standard inhibitors

CYP	SUBSTRATE AT KM	METABOLITE PRODUCED	STANDARD INHIBITOR
1A2	Phenacetin	Acetaminophen	Furafylline
2C9	Piclofenac	4'OH Diclofenac	Tienilic acid
2C19	S-mephenytoin	4'OH Mephenytoin	Ticlopidine
2D6	Bufuralol	1'OH Bufuralol	MDMA
3A4	Midazolam	1'OH Midazolam	Troleandomycin & Mifepristone

TEST COMPOUND

Single concentration 25µM, in duplicate.

FORMAT

96-well plate, 200µL incubation volume.

PROTOCOL

HLMs are added to wells of 96-well microplate. Parallel pre-incubations of test compounds are set up in the absence and also presence of NADPH for 30 min. After this pre-incubation period, aliquots are transferred to a secondary incubation plate containing the substrate (at 4 x Km) cocktail and NADPH in all wells, and this is further incubated for 15 min at 37°C.

The quenched samples are stored at -20°C for a minimum of 4hr, then centrifuged (3800rpm at 4°C for 20min) to precipitate the protein.

QUANTITATION

The supernatants analysed by LC-MS/MS using Concept Life Sciences generic analytical methods to measure metabolite formation.

DATA ANALYSIS AND RESULTS

Metabolite formation in presence of test compound is compared with the control and expressed as % control activity. The % control activities of the parallel incubations of test compound in the absence and in the presence of NADPH are compared to generate a % TDI.

$$\%\ TDI = \left(1 - \frac{\%\ Control\ activity\ WITH\ NADPH}{\%\ Control\ activity\ WITHOUT\ NADPH}\right) X100\%$$

+VE CONTROLS

One control compound for each CYP but two for CYP3A4, are used in each assay run (see bar chart below for identities).