ADME: CYP450 metabolism-dependent inhibition in human liver microsomes

Background:

Inhibition of cytochrome P450 enzymes is a well-recognized cause of drug-drug interactions and occurs by two general mechanisms: direct inhibition and metabolism-dependent inhibition (MDI).[1] In typical drug discovery and development, and in line with FDA and EMEA requirements, MDI is evaluated *in vitro* in human liver microsomes by determining if the inhibitory potency of a test compound increases following an incubation period.

MDI requires biotransformation of an inhibitor in the presence of cofactor (NADPH) and results in an increased inhibitory potency. The IC_{50} is determined following pre-incubation with test compound in the presence and absence of NADPH. A positive MDI result is inferred from a decrease in IC_{50} following pre-incubation with NADPH.

Assay description

CYP450 isoform
CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4

Compound concentration
0-100µM (IC_{50})

Compound requirements
1-2 mg of dry matter

Incubation details
isoform specific substrate (Table 1)
isoform specific time of incubation

Assay controls
isoform specific controls (Table 1)

Detection method
LC-MS/MS with internal standard

[1] Parkinson et al 2011, Drug Metab Dispos 39, 1370

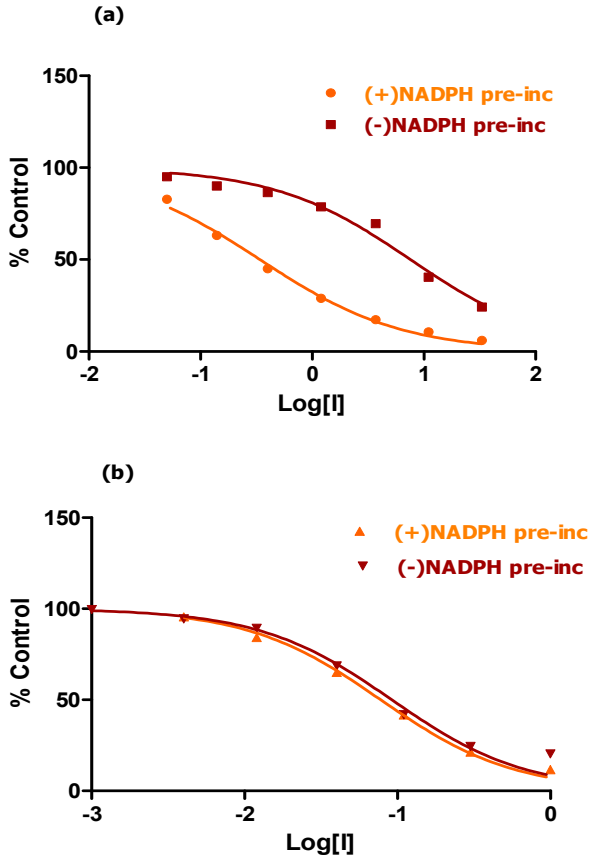

Figure 1.
IC_{50} fold shift for midazolam 4-hydroxylation by: (a) troleandomycin (metabolism-dependent inhibitor) and (b) ketoconazole (reversible inhibitor)

Assay details adjustable to client's and/or project specific requests

Contact us:
Adrijana Vinter, Business Development
adrijana.vinter@glpg.com
+385 91 265 5527

Mila Vrančić, Business Development
mila.vrancic@glpg.com
+385 91 265 5528

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Assay controls

Table 1. CYP450 isoform specific substrates and controls

Isoform	Substrate	Positive control	Negative control
CYP1A2	Phenacetin	Furafylline	α-naphtoflavone
CYP2C9	Diclofenac	Tienilic acid	Sulfaphenazole
CYP2C19	S-mephenytoin	Ticlopidine	Tranylcypromine
CYP2D6	Bufuralol	Paroxetine	Quinidine
CYP3A4	Midazolam/Testosterone	Troleandomycin	Ketoconazole

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Adrijana Vinter, Business Development
adrijana.vinter@glpg.com
+385 91 265 5527
Mila Vrančić, Business Development
mila.vrancic@glpg.com
+385 91 265 5528