



Personalised services in drug discovery

CYP450 inhibition

The cytochrome P450s constitute a superfamily of enzymes that contains almost 50 members.

12 of them are now known to be involved in drug metabolism with 3 families CYP1, CYP2 and CYP3 accounting for almost 90% of drug metabolism. Inhibition of these enzymes by NCE is a major cause of drug-drug interaction and associated side


effects. In vitro CYP450s inhibition assay is a fast and valuable approach in predicting potential in vivo drug-drug interaction early in the drug development process thus saving time and resources.


Other early ADMET assays available

 *In vitro* cytotoxicity assay

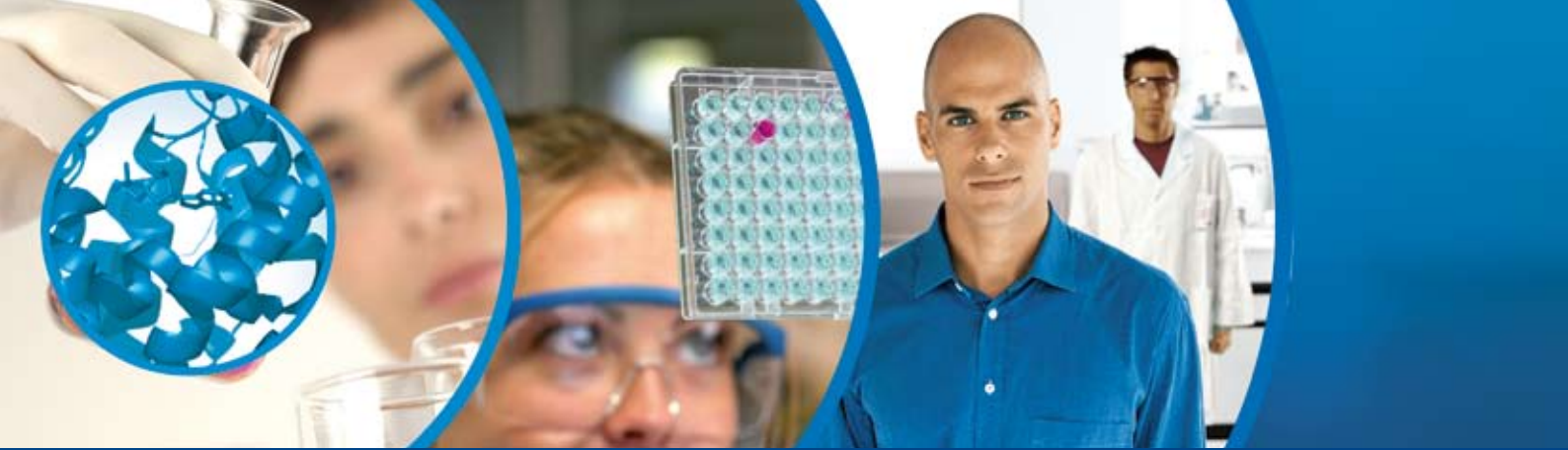
 Kinetic solubility and stability

 Permeability

 Spheroid Model for hepatotoxicity Study

 CYP450 inhibition

 Metabolic assay



CYP450 inhibition

Method

Compound solutions are serially diluted and distributed in triplicate in a 96 well-plate containing appropriate buffer and NADPH regenerating system. Reaction is started by adding the specific isoenzymes and substrates and plates are then incubated at the corresponding CYP450 optimal temperature. After a 30 minutes incubation period detection buffer is added and the formation of the luminescent metabolite is quantified. Known positive inhibitors are used as a positive control and are added in the assay.

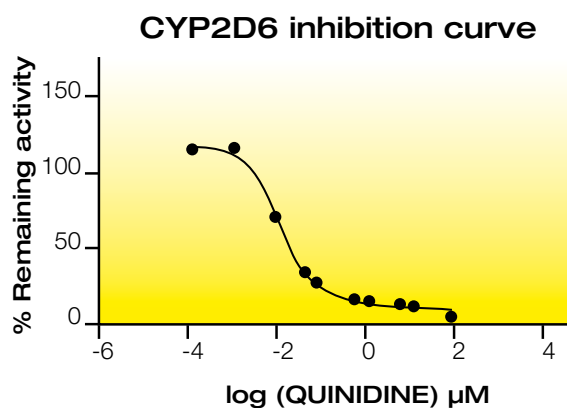
Data analysis:

% remaining of enzyme activity in the presence various concentration of inhibitor is calculated using activity of enzyme in the absence of inhibitor as 100%. IC₅₀ are calculated by fitting the % remaining activity versus compound concentration.

Test concentration and quantity required: reveal assay (1 concentration; 1-10µM) ; focused assay (10 concentrations, 0,1nM-100µM) ; 2 mg of compound required for both tests.

Model validation

IC₅₀ values for 3 known inhibitors were generated from 3 experiments run on different days. Each concentration of compound was run in triplicate in each experiment. As an example, the figure presented shows the inhibition curve of CYP2D6 activity by quinidine.



Inhibitor	Enzyme	Average IC ₅₀ in our model	Published* IC ₅₀ in µM
Ketoconazole	CYP3A4	0,06	0,08
Quinidine	CYP2D6	0,011	0,009
Sulfaphenazole	CYP2C9	0,206	0,23

*Crespi et al., Anal. Biochem. 1997 (248) p188: Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes p450.

Classification

IC ₅₀ < 1µM	Strong inhibitors
1 < IC ₅₀ < 10 µM	Moderate inhibitors
IC ₅₀ > 10 µM	Weak inhibitors