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In vitro **ADME & PK**

Hepatocyte Stability

Background Information

'Human hepatocytes have become the "gold standard" for evaluating hepatic metabolism and toxicity of drugs and other xenobiotics *in vitro*.'

[1]LeCluyse EL and Alexandre E (2010) *Methods Mol Biol* **640**; 57-82

- The liver is the most important site of drug metabolism in the body. Approximately 60% of marketed drugs are cleared by hepatic CYP-mediated metabolism[2].
- Hepatocytes contain the full complement of hepatic drug metabolising enzymes (both phase I and phase II) maintained within the intact cell.
- Hepatocytes can be used to determine the *in vitro* intrinsic clearance of a compound.
- The use of species-specific cryopreserved hepatocytes can be used to enable an understanding of interspecies differences.
- Hepatocytes can be used to profile for metabolites formed by both phase I and phase II enzymes.

Follow on metabolite profiling and structural elucidation

Cyprotex's hepatocyte stability assay can be extended to profile the metabolites that are formed. Cyprotex's biotransformation services are supported by high resolution, accurate mass spectrometry. These services can provide information on an individual species' metabolite profile, or a cross-species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data. Structural elucidation can also be performed on the potential metabolites' MS/MS fragmentation data. All biotransformation studies are performed by a dedicated team of experts.

Please refer to our Metabolite Profiling and Identification section for further details.

Protocol

Cells
Cryopreserved hepatocytes

Species
Human, rat, mouse, dog, primate, minipig, rabbit, guinea pig (other species available)

Test Article Concentration
3 µM (different concentrations available)

DMSO Concentration
0.25%

Incubation Time
0, 5, 10, 20, 40 and 60 min

Test Article Requirements
50 µL of 10 mM DMSO solution

Analysis Method
LC-MS/MS quantification

Assay Controls
Known substrates which undergo either phase I or phase II metabolism

Vehicle control incubation

Data Delivery
Intrinsic clearance
Standard error of intrinsic clearance
Half life

To find out more contact **enquiries@cyprotex.com**

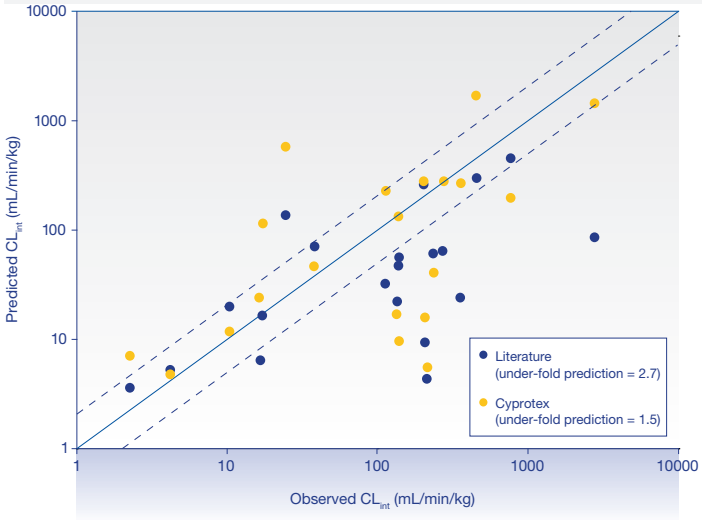
Hepatocytes have the full complement of hepatic drug metabolising enzymes within an intact cell and so are a popular *in vitro* model for determining intrinsic clearance, interspecies difference and metabolite profiling studies.

Hepatocyte Stability Assay

19 compounds were assessed in Cyprotex's human hepatocyte stability assay on three separate occasions. Data were scaled to *in vivo* intrinsic clearance and compared with observed values.

Figure 1

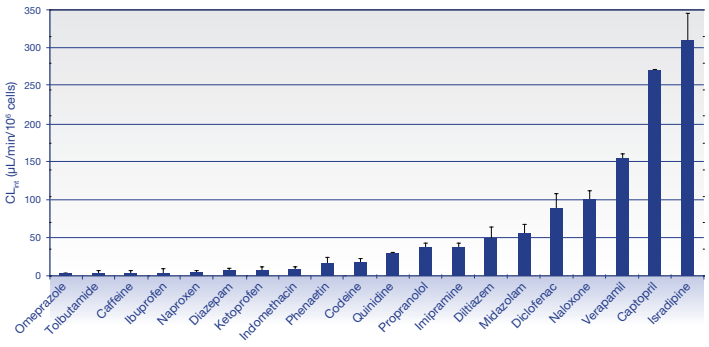
Cyprotex's human hepatocyte stability data and literature values[3,4,5,6] were scaled to *in vivo* intrinsic clearance (predicted CL_{int}) and compared to observed values of intrinsic clearance in 19 compounds.



Human hepatocyte CL_{int} (µL/min10^6 cells) from the Cyprotex assay and from literature[3,4,5,6] were scaled to *in vivo* CL_{int} (mL/min/kg) using a hepatocellularity of 99 x 10^6 cells/g liver and human liver weight of 21.4 g liver/kg. CL_{int} predictions were assessed for the predicted error (difference between the predicted and observed *in vivo* value). The bias of CL_{int} prediction was assessed from the geometric mean of the ratio of predicted and observed value and the fold under-prediction calculated. The data from the Cyprotex assay showed greater predictive capability when compared with data from the literature. Using literature values, the fold under-prediction was 2.7. Using Cyprotex values, the fold under-prediction was 1.5.

Figure 2

Graph illustrating intrinsic clearance data for 19 compounds generated in Cyprotex's hepatocyte stability assay. The data show the mean ± standard deviation of 3 separate incubations.



The graph shows consistency of data between separate runs of the assay. Pooled hepatocytes typically from 5 different donors are used for the human hepatocyte stability assay to reduce the problems associated with inter-individual variability.

References

1 LeCluyse EL and Alexandre E (2010) *Methods Mol Biol* **640**; 57-82.
2 McGinnity DF *et al*, (2004) *Drug Metab Dispos* **32**; 1247-1253.
3 Soars MG *et al*, (2002) *J Pharmacol Exp Ther* **301(1)**; 382-390.
4 Shibata Y *et al*, (2002) *Drug Metab Dispos* **30(8)**; 892-896.
5 Lau YY *et al*, (2002) *Drug Metab Dispos* **30(12)**; 1446-1454.
6 McGinnity DF and Riley RJ (2004) *Drug Metab Rev* **36** (S1); 211.