cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Microsomal Stability Assay

Background Information

'The liver microsomal *in vitro* T1/2 approach can be a suitable approach to measure *in vitro* CL_{int} which can be scaled up to the *in vivo* situation and used in the prediction of human clearance.'

[2]Obach RS. (1999) *Drug Metab Dispos* **27 (11)**; 1350-1359

- The liver is the most important site of drug metabolism in the body. Approximately 60% of marketed compounds are cleared by hepatic CYP-mediated metabolism[1].
- Liver microsomes are subcellular fractions which contain membrane bound drug metabolising enzymes.
- Microsomes can be used to determine the *in vitro* intrinsic clearance of a compound.
- The use of species-specific microsomes can be used to enable an understanding of interspecies differences.
- Easy to prepare, use and store enabling cost efficiencies over whole cell models.
- Microsomes are pooled from multiple donors to minimise the effect of interindividual variability.
- Microsomes are fully characterised using probe substrates to ensure activity is maintained between batches.

Protocol

Assay Matrix
Liver microsomes (other tissues and subcellular fractions available on request)

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

Test Compound Concentration
1 µM (different concentrations available on request)

Protein Concentration
0.5 mg/mL (different concentrations available on request)

Time Points
0, 5, 15, 30 and 45 minutes

Cofactor
1 mM NADPH (other cofactors available on request)

Final DMSO Concentration
0.25%

Compound Requirements
50 µL of 10 mM DMSO solution

Controls
0 µM (blank)
Minus cofactor (45 min only)
Positive control compounds with known activity

Analysis Method
LC-MS/MS

Data Delivery
Intrinsic clearance
Standard error of intrinsic clearance
Half life

Subcellular fractions such as liver microsomes are one of the most commonly used *in vitro* models of hepatic clearance in drug discovery.

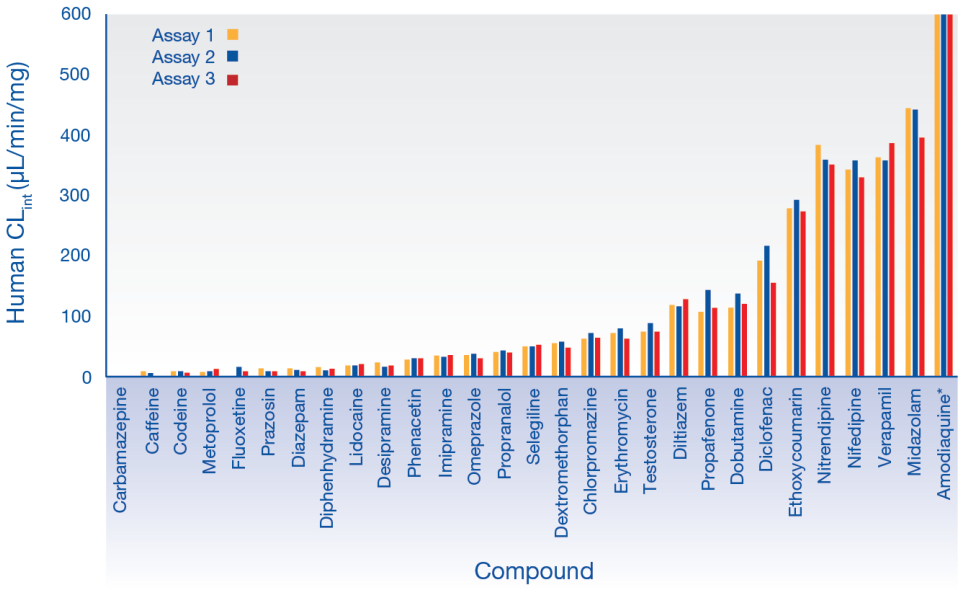

Figure 1

Comparison of CL_{int} values generated in 3 separate assays, based on mean CL_{int} (n=3) per assay. Incubations performed using human liver microsomes 0.5 mg/ml, 0.1 M phosphate buffer pH 7.4, 1 mM NADPH, 1 µM substrate concentration. The graph illustrates the reproducibility of the assay, inter-assay co-efficient of variation was 20.2% (8.5 % excluding compounds with CL_{int} <10.6 µL/min/mg (limit of quantification)).

* CL_{int} values generated for compound outside of axis range (860, 872, 947).

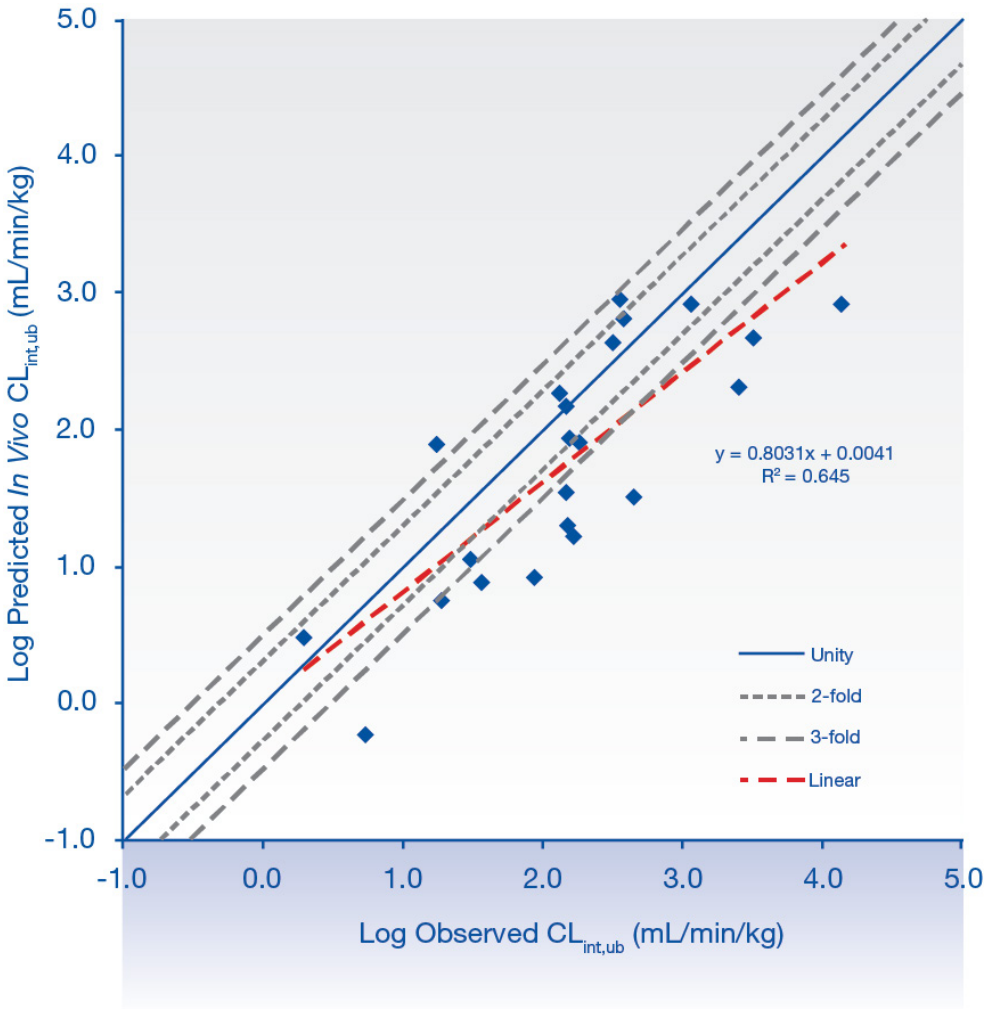

Figure 2

In vitro/in vivo clearance correlation in Cyprotex's human microsomal stability assay. *In vitro* CL_{int} data, for 22 literature compounds including acid, base and neutral compounds, was scaled (predicted $CL_{int,ub}$) and compared to values of *in vivo* intrinsic clearance back-calculated from observed *in vivo* clearance using the well-stirred model.

Dashed line shows line of regression. Dotted lines show 2-fold and 3-fold range from unity line (solid).

A range of literature compounds were assessed in the Cyprotex microsomal stability assay (1µM, 45 minute incubation, n=3 assays) and intrinsic clearance (CL_{int} µL/min/10^6 cells) determined. Predicted *in vivo* CL_{int} (mL/min/kg) values were calculated using a microsomal protein value of 40 mg/g liver, a human liver weight of 25.7 g liver/kg[3] and taking into account fu_{inc} (fraction unbound *in vitro* incubation)[4]. Observed *in vivo* $CL_{int,ub}$ were back-calculated from observed microsomal clearance using the well-stirred model, published intravenous human blood clearance values, human liver blood flow of 20.7 ml/min/kg and f_{ub} (fraction unbound in blood)[4]. A 2-3 fold under prediction of *in vivo* clearance was observed in human liver microsomes consistent with other reports in the literature[5].

References

[1] Cluyse EL and Alexandre E (2010) Isolation and culture of primary hepatocytes from resected human liver tissue *Methods Mol Biol* **640**; 57-82

[2] Obach RS. (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of *in vitro* half-life approach and nonspecific binding to microsomes *Drug Metab Dispos* **27(11)**; 1350-1359.

[3] Davies B. and Morris T. (1993) Physiological parameters in laboratory animals and humans *Pharma Res* **10(7)**; 1093-1095

[4] Riley RJ *et al.* (2005) A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes *Drug Metab Dispos* **33**; 1304-1311

[5] Wood FL *et al.* (2005) Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology *Drug Metab Dispos* **45(11)**; 1178-1188

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