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In vitro **ADME & PK**

Microsomal Stability

Background Information

'The liver microsomal *in vitro* T1/2 approach can be a suitable approach to measure *in vitro* CLint 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

Test Article Concentration
3 µM (different concentrations available)

Microsome Concentration
0.5 mg/mL (different concentrations available)

Time Points
0, 5, 15, 30, 45 min

Cofactor
NADPH

Final DMSO Concentration
0.25%

Test Article Requirements
50 µL of 10 mM 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

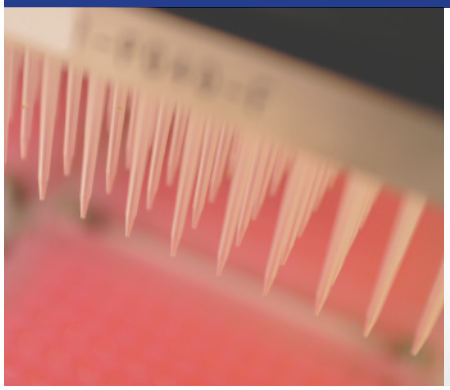
Follow on metabolite profiling studies

Cyprotex's microsomal 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.

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

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



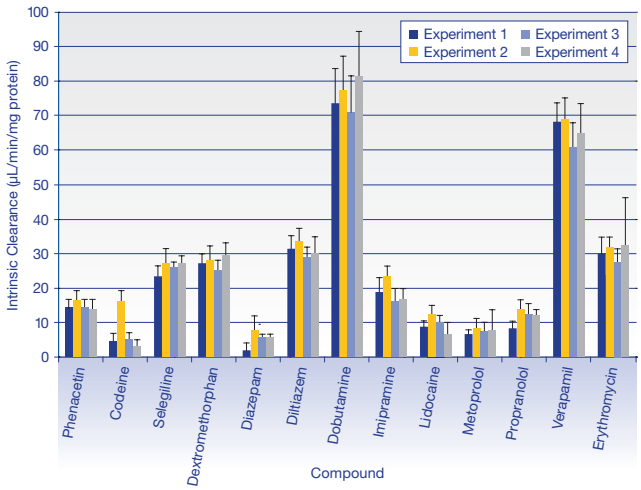
Microsomal Stability

A set of known drugs were screened in Cyprotex's Microsomal Stability assay in quadruplicate on 4 separate occasions. The data show reproducibilty over a range of intrinsic clearance values.

Data generated in Cyprotex's Microsomal Stability compare well with literature data.

Figure 1

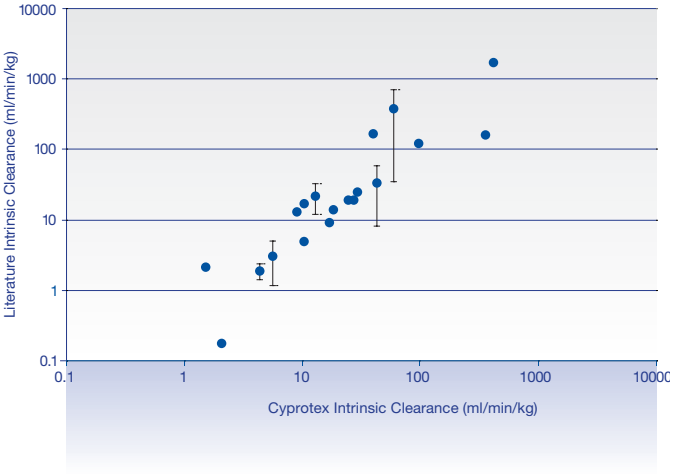
Mean intrinsic clearance data for 13 compounds obtained using Cyprotex's Microsomal Stability assay (error bars represent the standard deviation from quadruplicate incubations within each run of the assay).



The graph shows consistency of data both within the assay and between separate runs of the assay.

Figure 2

Comparison of Cyprotex's Human Microsomal Stability intrinsic clearance data with literature data.



The inter-laboratory variability in literature data can be considerable as shown by the error bars (mean ± standard deviation) on the graph. Literature data taken from Riley *et al*. (2005)[3]

References

1 McGinnity DF *et al*. (2004) *Drug Metab Dispos* **32**; 1247-1253.
2 Obach RS. (1999) *Drug Metab Dispos* **27 (11)**; 1350-1359.
3 Riley RJ *et al*. (2005) *Drug Metab Dispos* **33**; 1304-1311.