

Cloe Screen Microsomal Stability

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.'

²Obach 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 metabolism¹.
- 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.

Follow on metabolite profiling studies



The Cloe Screen Microsomal Stability assay can be extended to profile the main breakdown product that is formed. Options include a low resolution analysis to identify whether a metabolite is formed, or a cross species comparison to identify potential differences in metabolism which could in turn help to interpret pharmacology and toxicity data. We can also perform ion-transition analysis in order to understand the derivation of metabolites. Please refer to our Cloe Select Metabolite Profiling and Identification section for further details.

Protocol

Test Compound Concentration
3 μ M (different concentrations available)

Microsome Concentration
0.5 mg/ml (different concentration available)

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

Cofactor
NADPH

Final DMSO Concentration
0.25 %

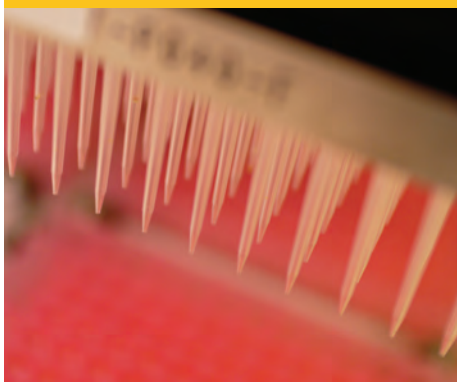
Compound Requirements
50 μ L of 10 mM solution

Controls
0 μ M (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.



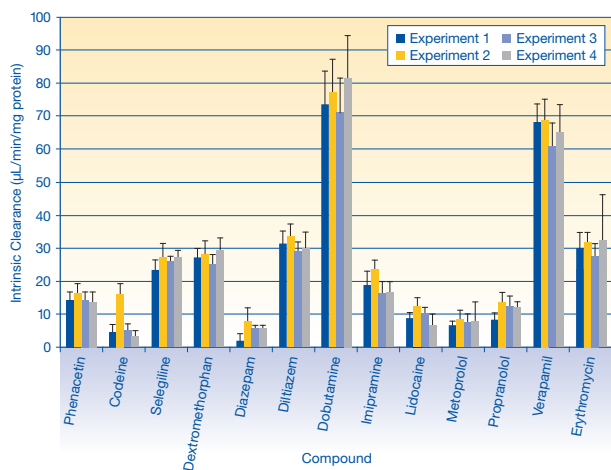
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A set of known drugs were screened in the Cloe Screen Microsomal Stability assay in quadruplicate on 4 separate occasions. The data show reproducibility over a range of intrinsic clearance values.

Data generated in Cloe Screen Microsomal Stability compare well with literature data.

Figure 1

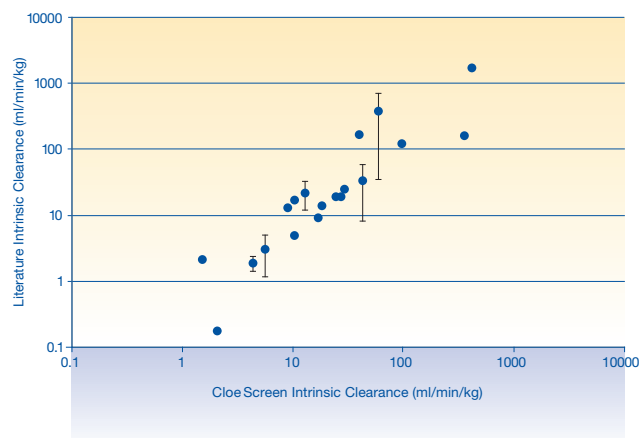
Mean intrinsic clearance data for 13 compounds obtained using the Cloe Screen Microsomal Stability assay (error bars represent the standard deviation from quadruplicate incubations within each run of the assay).



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

Figure 2

Comparison of Cloe Screen 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 \pm standard deviation) on the graph. Literature data taken from Riley *et al.* (2005)³

References

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