Hepatocyte Stability

Hepatocytes are the primary epithelial cell type present in the liver and comprise approximately 80% of the liver's mass[1], they are an important *in vitro* ADME tool widely utilised in drug discovery and development for hepatic drug metabolism, uptake and toxicity studies.

Hepatocytes contain both phase I and phase II metabolising enzymes, as well as the necessary co-factors, enabling them to be used to study drug metabolism[2]. The phase I enzymes, principally cytochrome P450s, catalyse both oxidative and reductive reactions, while phase II enzymes catalyse conjugation reactions.

In this assay, compounds are incubated with hepatocytes at 37°C. Aliquots of the incubation mixture are removed over the course of the assay and the proteins precipitated to prevent further enzymatic activity. The samples are then analysed, and the intrinsic clearance calculated.

Standard Assay Conditions

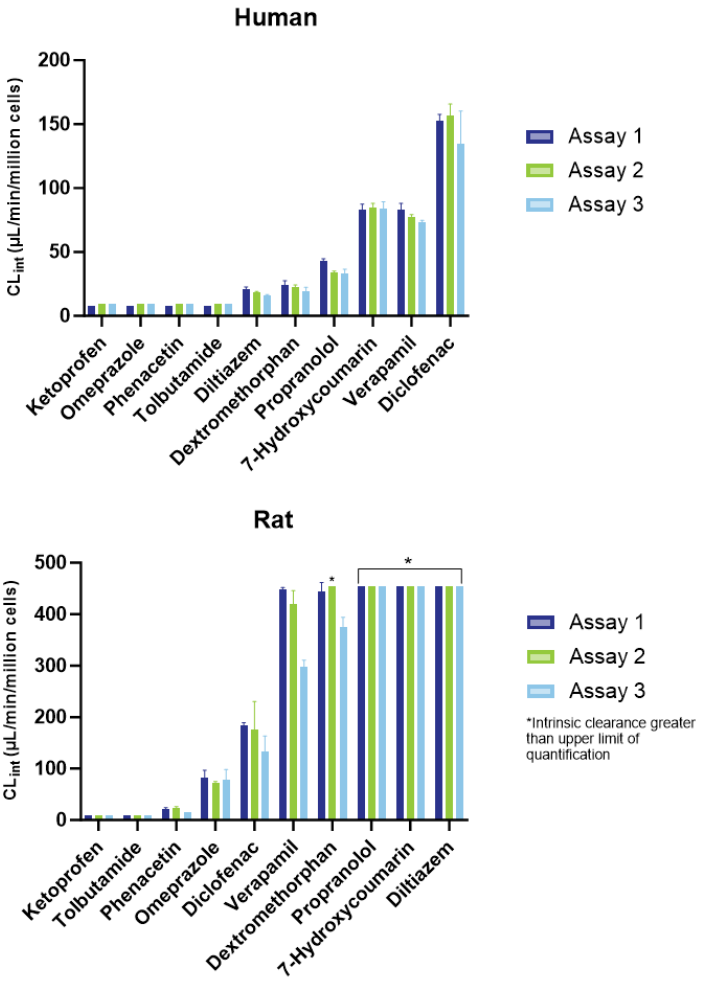
- 0.5 million cells/mL
- 1 µM compound concentration
- 6 time points over 60 minutes
- 1% organic solvent final in the incubation mixture
  - 0.95% acetonitrile, 0.05% DMSO
- N=2 replicates
- Analysis by LC-MS
- Marker compounds included
- Metabolite identification possible from study samples
- Various species/strains available

Deliverables

Half-life (min) and CL_{int} (µL/min/million cells)

Compound Requirements

100 µL of a 10 mM stock or 1 mg of solid (accurately weighed to 2 decimal places)



Hepatocyte stability in Human and Rat for 10 compounds. N=3 experiments each with 3 replicates. Error bars denote standard deviation.

References

[1] Hansel *et al* (2014) The History and Use of Human Hepatocytes for the Study and Treatment of Liver Metabolic Diseases, Curr Protoc Toxicol., 62, 14.12.1-14.12.23.

[2] Li *et al* (1999) Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug–drug interaction potential, Chemico-Biological Interactions, 121, pp. 17-35.

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