Microsomal Stability

Microsomes (a sub-cellular fraction of cells) are a useful tool for *in vitro* assessments of intrinsic clearance and can be used to predict how rapidly a compound is depleted *in vivo*. Liver microsomes are derived from hepatocytes and contain a variety of enzymes involved in Phase 1 metabolism, such as cytochrome P450s (CYPs).

Compounds are incubated with microsomes in the presence of buffer and enzyme co-factor 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

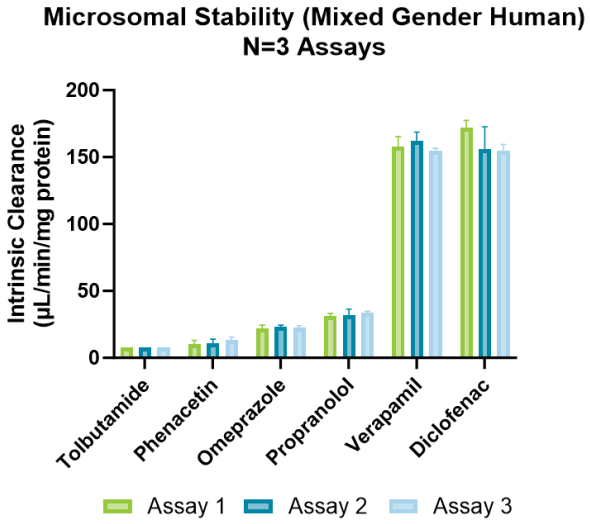
- 0.5 mg/mL liver microsomal protein concentration
- 1 µM compound concentration
- pH 7.4 phosphate buffer
- NADPH co-factor
- 6 time points over 60 minutes
- 1% organic solvent final in the incubation mixture
  - (0.95% acetonitrile, 0.05% DMSO)
- N=2 replicates with NADPH
- N=1 replicate without NADPH
- Analysis by LC-MS
- Low, medium and high CL_{int} marker compounds included
- Metabolite identification possible from study samples
- Various species/strains available

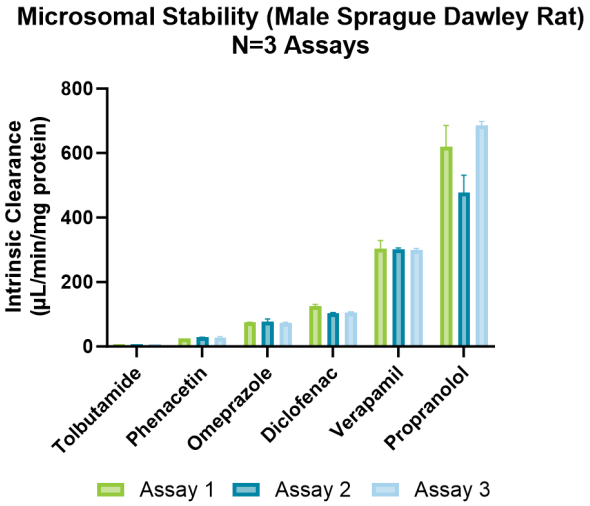
Deliverables

Half-life (min) and CL_{int} (µL/min/mg microsomal protein)

Compound Requirements

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





Liver microsomal stability in Human and Rat for 6 compounds. N=3 experiments each with 3 replicates. Error bars denote standard deviation.

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