

Cloe Screen S9 Stability

cyprotexexperts in **ADME**

Background Information



'Human liver S9 fraction is a preparation containing both the microsomal and cytosolic fractions of the cell. This system offers the most complete representation of DMEs, as it incorporates both the majority of phase I (mainly microsomal) and phase II (often cytosolic) enzymes, allowing a relatively complete metabolite profile to be achieved.'

¹Plant N. (2004) *Drug Discovery Today* 9 (7); 328-336

- The liver is the main site of drug metabolism and therefore *in vitro* studies are predominantly focused on using hepatocytes or subcellular hepatic fractions such as microsomes or S9.
- Subcellular fractions are easy to prepare, use and store enabling cost efficiencies over whole cell models.
- The S9 fraction (post-mitochondrial supernatant fraction) consists of microsomes and cytosol.
- The advantage of using S9 fraction for *in vitro* screening is that it contains a wide variety of both phase I and phase II enzymes.
- S9 can be supplemented with cofactors such as UDPGA and PAPS to investigate Phase II metabolic pathways.

Follow on metabolite profiling studies

The Cloe Screen S9 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)

S9 Concentration
1 mg/mL (different concentrations available)

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

Cofactors
NADPH, UDPGA (others available on request)

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

S9 has the advantage of containing a relatively complete complement of hepatic enzymes as it contains both Phase I and Phase II enzymes.

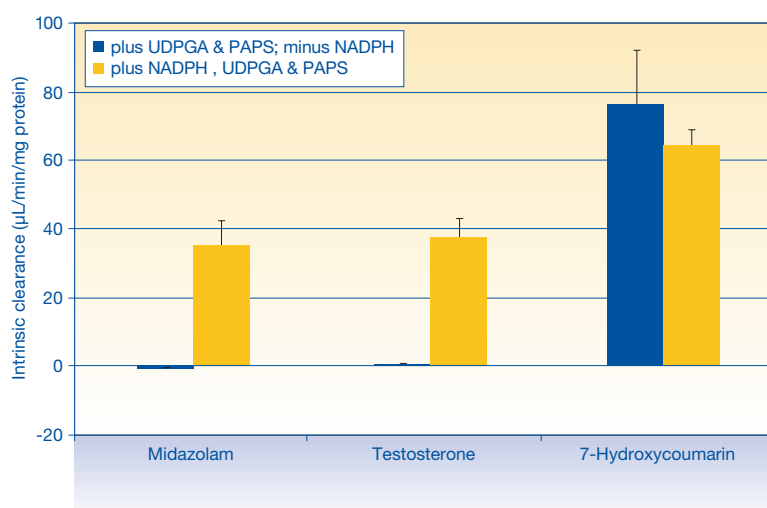


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3 Compounds were incubated with mouse liver S9, UDPGA and PAPS in the presence and absence of NADPH.

Figure 1

Cloe Screen S9 Stability data for midazolam, testosterone and 7-hydroxycoumarin.



7-Hydroxycoumarin which is predominantly metabolised by glucuronidation and sulphation (and not phase I metabolism) is metabolised in the presence of UDPGA and PAPS regardless of the presence of NADPH. However, midazolam and testosterone require the presence of NADPH for phase I metabolism prior to metabolism by phase II enzymes.

References

¹ Plant N. (2004) *Drug Discovery Today* 9 (7); 328-336.