

Microsomal Binding

Background Information



'It has been recognized that nonspecific microsomal binding in the *in vitro* metabolic assays can significantly affect the observed kinetics of metabolism and hamper the accurate prediction of clearance, and there are now several examples where knowledge of the extent of microsomal binding can lead to a better understanding of the relationship between *in vitro* metabolism and *in vivo* pharmacokinetics.'

¹Austin RP, Barton P, Cockroft SL, Wenlock MC and Riley RJ. (2002) *Drug Metab Dispos* **30 (12)**; 1497-1503.

- Drug that is sequestered in microsomes *in vitro* is presumed to be unavailable for direct interaction with metabolising enzymes, just as drug that is bound to plasma proteins and tissue macromolecules *in vivo* is presumed to be unable to be directly acted on by drug metabolising enzymes.
- Microsomal binding is an important factor in the prediction of *in vivo* pharmacokinetics from *in vitro* drug metabolism data.
- It has been recognised that correcting for nonspecific binding in the *in vitro* microsomal stability assays can improve the accuracy of *in vivo* metabolic clearance prediction^{2,3,4}.
- Knowledge of $f_{u,inc}$ has also been shown to be important for the prediction of *in vivo* drug-drug interactions.
- Cyprotex's Microsomal Binding assay uses equilibrium dialysis to determine the extent at which a compound binds to microsomes (fraction unbound data).

Protocol

Method

Equilibrium Dialysis

Test Article Concentration

3 μ M (different concentrations available)

Protein Concentration

0.5 mg/mL (different concentrations available)

Number of Replicates

2

Temperature

37°C

Test Compound Requirements

150 μ L of 10 mM solution

Analysis Method

LC-MS/MS quantification (both microsome and buffer standards prepared)

Data Delivery

Fraction unbound in incubation at 0.5 mg/mL
Recovery

Microsomal binding can be used to improve the prediction of *in vivo* pharmacokinetics from *in vitro* metabolism data.



Microsomal Binding

14 compounds were screened in Cyprotex's Microsomal Binding assay using human liver microsomes (n = 6 per run) on 3 separate occasions. The data are highly reproducible for a range of fraction unbound values.

Figure 1

The graph shows the reproducibility of 14 compounds (n = 6 replicates) within 1 run of Cyprotex's Microsomal Binding assay (error bars represent the standard deviation).

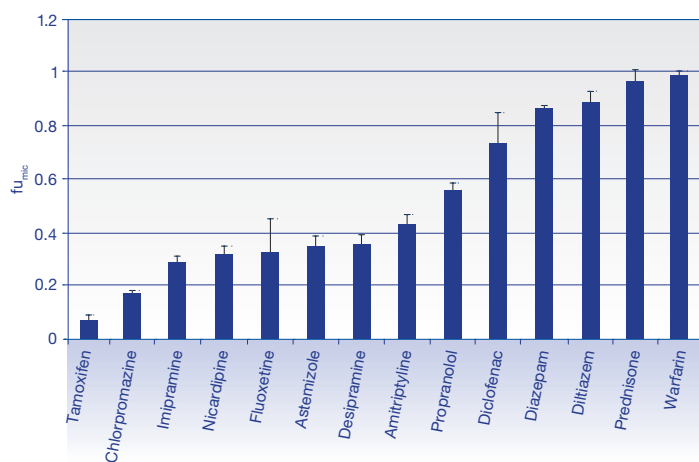
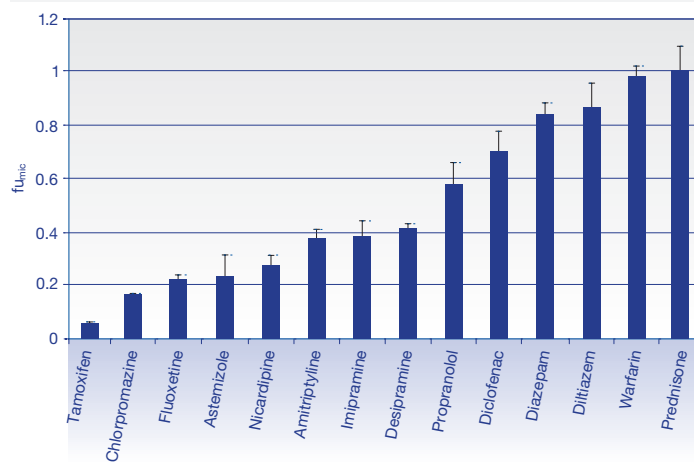


Figure 2

The graph shows the reproducibility of 14 compounds between 3 separate runs of Cyprotex's Microsomal Binding assay (error bars represent the standard deviation).



References

- ¹ Austin RP et al. (2002) *Drug Metab Dispos* **30** (12); 1497-1503.
- ² Carlile DJ et al. (1999) *Br J Clin Pharmacol* **47** (6); 625-635.
- ³ Obach RS. (1997) *Drug Metab Dispos* **25** (12); 1359-1369.
- ⁴ Obach RS. (1999) *Drug Metab Dispos* **27** (11); 1350-1359.