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In vitro **ADME & PK**

Whole Blood Binding

Background Information

'The knowledge of binding properties of drugs is of considerable importance not so much because of possible displacement interactions with other drugs, but because it is assumed that only a small fraction of the circulating drug (the free drug concentration) is able to cross membranes.'

[1]Mazoit JX and Samii K (1999) *Br J Clin Pharmacol* **47 (1)**; 35-42

- The extent of binding to blood influences the way in which a drug distributes into tissues in the body. Extensive blood binding also limits the amount of free compound available to be eliminated from the body which can, in turn, reduce the clearance of the compound.
- Pharmacokinetic parameters are usually determined by analysis of drug concentrations in plasma rather than whole blood. Understanding the extent of whole blood binding in comparison to plasma protein binding is important in identifying if differential binding to a specific component in the blood occurs and in interpreting pharmacokinetic data.
- The method can also be used to investigate non-linear pharmacokinetics via saturation of blood binding.
- Equilibrium dialysis is the most widely accepted method for assessing protein binding as non-specific binding effects are minimised compared with other methods such as ultrafiltration.
- Our whole blood binding assay is performed using an equilibrium dialysis method and delivers a value of fraction of compound unbound to blood (fu).

Protocol

Method
Equilibrium Dialysis

Test Article Concentration
5 µM (different concentrations available)

Number of Replicates
2

Test Article Requirements
120 µL of 10 mM solution in DMSO

Analysis Method
LC-MS/MS quantification
(both blood and buffer standards prepared)

Data Delivery
Fraction unbound in blood
Recovery

To find out more contact **enquiries@cyprotex.com**

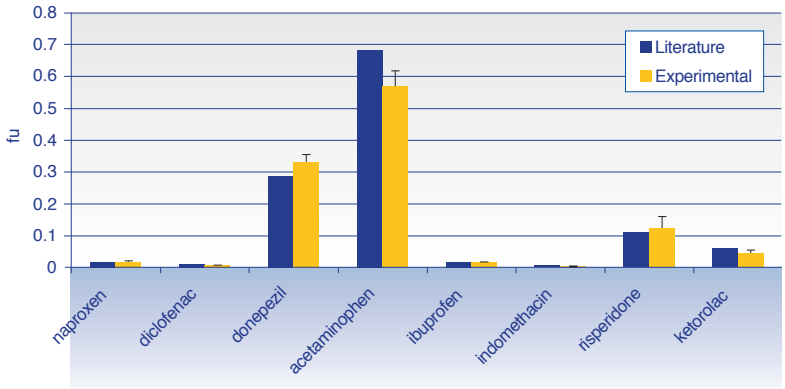
The relative tissue affinities of blood and brain play an important role in determining the extent of CNS penetration observed *in vivo*.[2]

Whole Blood Binding

The whole blood binding method has been developed using a selection of compounds with reported fu values ranging from 0.006 to 0.68% in rat and 0.01 to 0.49% in human blood[2,3]. The experimentally determined fu values were compared to those reported in the literature and inter-assay variability was also assessed.

Figure 1

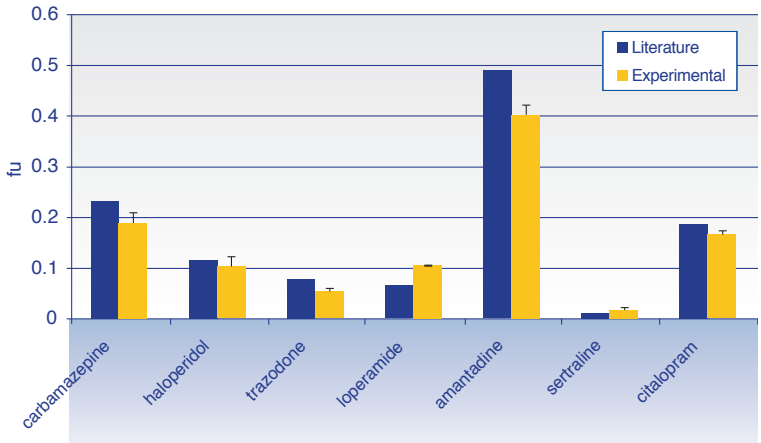
Graph showing the mean fu values for 8 compounds in rat blood and their comparison to literature values[2].



The error bars represent the standard deviation of three separate esperiments. The fu values have good agreement with reported literature values over a wide range of fu (r^2 = 0.99 for rat). The inter-assay variability was low with a coefficient of variation equal to 15% or less.

Figure 2

Graph showing the mean fu values for 7 compounds in human blood and their comparison to literature values[3].



The error bars represent the standard deviation of two separate experiments. The fu values have good agreement with reported literature values over a wide range of fu (r^2 = 0.94 for human).

References

1 Mazoit JX and Samii K (1999) *Br J Clin Pharmacol* **47 (1)**; 35-42

2 Summerfield SG *et al*, (2006) *J Pharmacol Exp Ther* **316 (3)**; 1282-1290

3 Summerfield SG *et al*, (2008) *Xenobiotica* **38 (12)**; 1518-1535