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In vitro **ADME**

Plasma Protein Binding

Background Information

'Equilibrium dialysis is the preferred method to determine the free drug fraction, because it is less susceptible to experimental artifacts.'

[1]Kariv I, Cao H and Oldenburg KR. (2001) *J Pharm Sci* **90 (5)**; 580-587.

- The extent of binding to plasma influences the way in which a drug distributes into tissues in the body.
- Extensive plasma protein binding also limites the amount of free compound available to access sites of action in the cell, and metabolism and elimination may be slower.
- Equilibrium dialysis is the most widely accepted method for assessing plasma protein binding as non specific binding effects are minimised compared with other methods such as ultrafiltration.
- Cyprotex's Plasma Protein Binding assay is performed using an equilibrium dialysis method and delivers a value of fraction of compound unbound to proteins (fu).
- There is a choice of three methods for assessing plasma protein binding using three different percentages of plasma to provide flexibility depending on budget and compound characteristics.

Protocol

Method
Equilibrium Dialysis
(at 10%, 50%, or 100% plasma)

Test Article Concentration
5 µM0 (different concentrations available)

Number of Replicates
2

Compound Requirements
100 µL of 10 mM solution

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

Data Delivery
Fraction unbound in 100% plasma
Recovery

Equilibrium dialysis is the preferred method for evaluating plasma protein binding.

Plasma Protein Binding

3 different methods have been validated based on performing the equilibrium dialysis at different plasma concentrations (10% plasma, 50% plasma and 100% plasma). For the 10% and 50% plasma methods the fraction unbound values scaled to a fraction unbound at 100%. The application of each method is described in the table below.

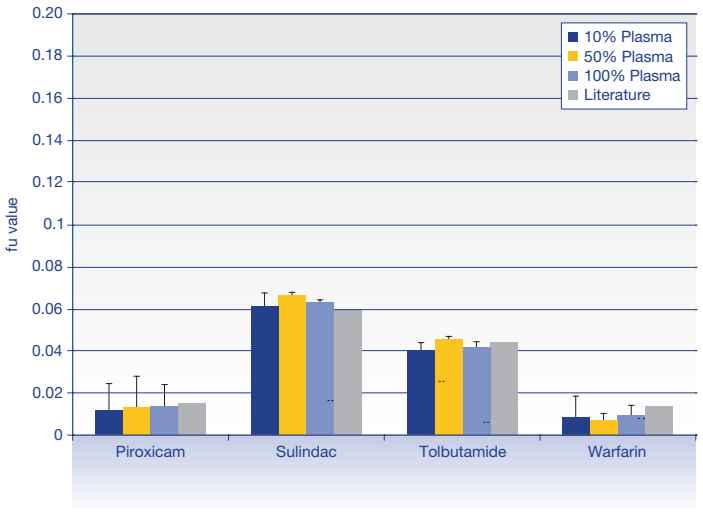
Table 1

Applications for the 3 methods based on differing plasma concetrations

Option	Applications
10% plasma	• Reduced plasma requirement cost. • Highly automated evaluation of large number of compounds for early screening. • Ideal for differentiating between very highly bound compounds. • Not suitable for highly unbound compounds.
50% plasma	• Reduced plasma requirement and cost. • Highly automated evaluation of plasma protein binding using a higher concentration of plasma. • Recommended for differentiating between highly unbound compounds.
100% plasma	• 'Gold standard' assay. • Evaluation of protein binding using 100% plasma. • Applicable to all stages of preclinical ADME.

Figure 1

Graph showing the fraction unbound of 4 compounds using 10%, 50% and 100% plasma, and their comparison to literature values (Goodman and Gilman, 1996).



The fraction unbound has been scaled to 100% for compounds that were screened using 10% and 50% plasma. The error bars represent the standard deviation of 3 separate experiments.

References

1 Kariv I *et al*. (2001) *J Pharm Scie* **90 (5)**; 580-587.

2 Goodman and Gilman's: The Pharmacological Basis of Therapeutics. 1996.