

Cloe Screen 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.'

¹Kariv 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 limits 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.
- Cloe Screen 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 Compound Concentration
5 μ M (different concentrations available)

Number of Replicates
2

Compound Requirements
150 μ L 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.



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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 are scaled to a fraction unbound at 100 %. The application of each method is described in the table below.

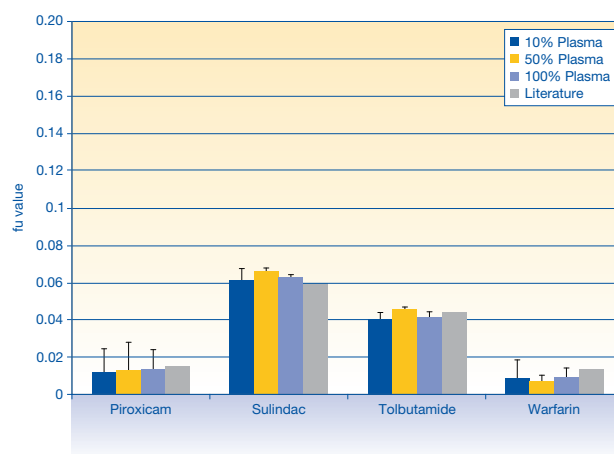
Table 1

Applications for the 3 methods based on differing plasma concentrations.

Option	Applications
10 % plasma	<ul style="list-style-type: none"> Reduced plasma requirement and cost. Highly automated evaluation of large numbers of compounds for early screening. Ideal for differentiating between very highly bound compounds. Not suitable for highly unbound compounds.
50 % plasma	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> '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 Sci* **90** (5): 580-587.

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