In Vitro Plasma and Microsomal Protein Binding

Determine the free concentration of drug candidates

Drugs can bind to plasma proteins, a process that can significantly impact their overall distribution and clearance and potentially alter their pharmaceutical efficacy and potential toxicity. For example, high plasma protein binding can extend the half-life of a drug by decreasing its clearance.

Determining the plasma protein binding of a drug candidate is critical for understanding its metabolic and pharmacokinetic profile and for evaluating its potential risk for drug-drug interactions (DDIs). The results can support clinical study design and human dosage considerations. Plasma protein binding data are also used when designing IND-enabling studies outlined in regulatory guidelines that suggest evaluating physiologically relevant concentrations of a drug candidate and considering the fraction unbound (f_u) in circulation.

BioIVT uses rapid equilibrium dialysis (RED) to determine drug f_u in plasma. The drug f_u represents the percentage of the drug candidate that is not bound to proteins and is, therefore, free to exert intended therapeutic effects and is available to be metabolized by enzymes or for active transport. Factors determining f_u include affinity of a drug candidate to different plasma proteins, concentration of the plasma protein, and relative concentration of the drug candidate to the protein.

Study Design

Element	Feature		Standard
Design	Preliminary evaluation	Determination of equilibrium time	✔
		Evaluation of stability	✔
		Non-specific binding assessment	✔
		Replicates	2
		Time points	3
		Drug candidate concentrations	2
	Main experiment	Protein binding measurement in plasma or microsomes using RED device	✔
		Replicates	3
		Time points – determined in preliminary evaluation	1
		Drug candidate concentrations	3
	Analysis by LC-MS/MS (or other appropriate method)		✔
Deliverables	Fraction unbound (f_u)		✔
	Standard report including methods, data, results and conclusions		✔

Methodological Considerations and Test Systems

BioIVT's standard equilibrium dialysis study design uses the RED device. The drug candidate is added to one chamber containing plasma and allowed to reach equilibrium across the dialysis membrane with a chamber filled with buffer. Following incubation of the RED device at 37 °C, aliquots are removed from both chambers at the appropriate time points, and the amount of drug candidate in each chamber is measured by LC-MS/MS or other appropriate method.

The standard design starts with a preliminary evaluation to optimize the following assay conditions for the drug candidate being tested:

- **Equilibrium time:** Determining the time taken for the free drug candidate to reach equilibrium across the dialysis membrane, which is evaluated in the buffer.
- **Stability:** As plasma and microsomes contain drug metabolizing enzymes, the drug candidate needs to be sufficiently stable over the incubation period to be measured.
- **Non-specific binding:** Hydrophobic drug candidates might bind non-specifically to the membrane and/or the chamber of the device.

Once the appropriate experimental conditions have been found, the main experiment determines the f_u in plasma using the RED device.

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Study Deliverables

BioIVT's standard protein binding reports include the methods, preliminary evaluation data (stability, time to equilibrium, and non-specific binding) as well as the results from the main experiment determining f_u, as illustrated in Table 1.

Data from a plasma protein binding study can help to explain the following observed pharmacokinetic effects of a drug:

- Distribution of the drug candidate into body tissues
- Therapeutic effects of the drug candidate
- Reduction in the amount of drug candidate available to be metabolized or cleared from the body and impact its DDI potential.

Microsomal Protein Binding

BioIVT also offers microsomal protein binding using the same method and study design. These data are needed for pharmacokinetics-related modeling and definitive *in vitro* study design. For example, the microsomal unbound fraction ($f_{u,mcs}$) can be used to calculate unbound K_i values in enzyme inhibition studies according to regulatory guidelines and assist with modeling per the equation below:

$$K_{i,u} = f_{u,mcs} \times \frac{IC_{50}}{2}$$

with $K_{i,u}$ being the unbound inhibition constant for direct inhibition.

Additional Considerations

Neglecting to accurately quantify the unbound fraction of a drug candidate with a definitive study can lead to misinformed dose determination in human clinical trials. Even a small inaccuracy can mean a big difference. This is particularly important for drug candidates with high protein binding, which is categorized by a binding affinity of greater than 99%. Knowing whether a drug is 98.9% or 99.8% bound can de-risk changes in free drug exposure and can impact the design of *in vivo* studies.

In collaboration with research partners, BioIVT also offers definitive plasma protein binding studies using two additional techniques:

- Ultrafiltration
- Ultracentrifugation

These techniques are helpful when equilibrium dialysis is not applicable, such as for the evaluation of large molecules or drug candidates that are non-specifically binding to the membrane or the device.

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Table 1. Protein binding and f_u determination of a drug candidate in rat and human plasma.

Drug candidate (µM)	Matrix	Species	Mean reported conc. (nM) ± SD	Mean f_u	% Free	% Binding	% Recovery
0.1	Buffer	Rat	0.497 ± 0.009	0.232	23.2	76.8	96.5
	Plasma		2.16 ± 0.19				
1	Buffer		146 ± 1.34	0.232	23.2	76.8	88.6
	Plasma		632 ± 10.7				
10	Buffer		1600 ± 55.9	0.229	22.9	77.1	93.1
	Plasma		6960 ± 297				
0.1	Buffer	Human	0.403 ± 0.012	0.172	17.2	82.8	93.8
	Plasma		2.34 ± 0.09				
1	Buffer		113 ± 7.22	0.154	15.4	84.6	95.4
	Plasma		732 ± 4.05				
10	Buffer		1220 ± 13.7	0.153	15.3	84.7	93.7
	Plasma		8020 ± 184				