CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

PROTEIN BINDING

A drug's efficacy may be affected by the extent of binding to proteins within blood, plasma, tissues including brain tissue. Common proteins in blood that drugs bind to are serum albumin, lipoprotein, glycoprotein, α, β, and γ globulins. Drugs that traverse the BBB may bind brain tissues. The higher the drug free fraction (fu), the more efficiently it can permeate cell membranes or diffuse. If the protein binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:

Protein + drug ⇌ Protein-drug complex

Only the unbound fraction is free to exhibit pharmacologic effects and to undergo metabolism or excretion. Equilibrium dialysis is an accurate and reliable method for determining protein binding affinities (whole blood, plasma or tissues) to chemical substances of low molecular weight. Determining protein binding is a critical phase of drug development as it influences compound dosing, efficacy, clearance rate and potential for drug interactions.

Also, *in vitro* drug binding to microsomes during metabolic stability incubations may lead to underestimations in clearance rates, leading to poor IVIV correlations. If fu inc (free fraction in the incubation) is known it can be accounted for and *in vitro* clearance rates corrected.

Deliverable: % protein binding and fraction unbound, fu using equilibrium dialysis.

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 20µLof 10mM in DMSO or 0.5mg solid.

PROTEIN

Pooled plasma or **whole blood** heparinised, and **rat brain tissue** are sourced from Bioreclamation IVT. Liver or intestinal **Microsomes** from Corning. Human (not brain) & many other species are available.

TEST COMPOUND

Incubation concentration 5µM.

FORMAT

RED device Piercenet. Dialysis membrane (MWCO ~ 8000), 48-well plate, shaking incubator at 37°C.

PROTOCOL

Plasma/blood or tissue homogenate is warmed to 37°C for 10 min. and test compound stock solution is added to acheive a 5µM solution.

500µL of dialysis buffer is added to one side of the chamber of the RED device insert housed within the heated Teflon bock, and the incubation is initiated by the addition of 300µL of the test compound protein solution to the opposing chamber. The final solvent concentrations are 0.95% Methanol and 0.05%DMSO. (99% plasma).

The RED device inserts are covered with sealing tape to minimise evaporation, during orbital shaking (500rpm) at 37°C for 4hr to allow equilibrium to be reached. After 4hr, the chambers are checked to ensure minimal volume change.

Equal volumes from the buffer and the plasma chambers (50µL) are transferred into separate wells of a deep well plate. 50µL of plasma are added to the buffer samples, and 50µL of buffer are added to the plasma samples, followed by mixing (this ensures matrix matching of samples from both the free and bound fractions). 300µL of ice cold acetonitrile containing internal standard are added to precipitate the protein.

Samples are centrifuged (3800rpm = 2700g at 4°C for 20min) to pellet the protein.

CONTROLS

Warfarin & Verapamil for blood & plasma, Clozapine for brain tissue.

QUANTITATION

The supernatants are analysed by LC-MS/MS using Concept Life Sciences generic analytical methods with matrix matched standard curve to quantify the concentration of test compound in each chamber.

DATA ANALYSIS AND RESULTS

For each test compound injection, response ratio $= \frac{\text{Test Peak Area}}{\text{Standard Peak Area}}$

From the standard curve, the response ratio is converted to concentration of test compound.

The binding value of test compound bound is calculated from:

$$\text{Fraction Unbound} = \frac{\text{Concentration Buffer Chamber}}{\text{Concentration Plasma Chamber}}$$

% Free = Fraction Unbound X 100

% Bound = 100% - % Free

The % Recovery is calculated via:

$$\%\ \text{Recovery} = \frac{\text{Quantity Buffer Chamber} + \text{Quantity Plasma Chamber}}{\text{Total Quantity Incubated}} \times 100$$

CONCEPT EQUILIBRIUM PLASMA PROTEIN BINDING