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In-vitro plasma protein binding

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

In vitro plasma protein binding studies in various animal models (Human, rat, mouse, dog, fetal calf serum) are crucial for understanding drug behaviour and predicting human responses. These studies help determine how a drug interacts with plasma proteins, influencing its distribution, metabolism, and elimination.

Protein binding significantly influences drug efficacy and safety profiles. Highly bound compounds (>90%) exhibit greater sensitivity to changes in protein levels due to disease states, age, or co-administered drugs. These data support regulatory submissions and inform clinical trial design by enabling accurate prediction of human pharmacokinetic parameters from preclinical models.
The assay employs equilibrium dialysis, ultrafiltration, or ultracentrifugation methods to separate bound and unbound drug fractions, providing quantitative measurements essential for comprehensive drug development programs.

Safety warnings

Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Summary

1 Plasma protein binding assay is performed in 48-well RED device
This protocol can be used with the following protein plasma:
- Rat
- Mouse
- Fetal calf serum
- Dog
- Human

Sample Preparation

2 The compounds are incubated at 5µM in a shaking incubator at 37ºC

3 The plasma is diluted to 50% with pH 7.4 phosphate buffer and warmed to 37ºC for 10min, test compound is then added to achieve 5µM

Assay

4 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).

5 The samples are incubated for 4 hours to allow equilibrium to be reached

6 Equal volumes from the buffer and the plasma chambers (50µL) are transferred into separate wells of a deep well plate

6.1 50µL of 50% blank plasma are added to the buffer samples, and 50µL of buffer are added to the 50% plasma samples, followed by mixing (this ensures matrix matching of samples from both the free and bound fractions)

6.2 300µL of ice cold acetonitrile containing internal standard are added to precipitate the protein

7 The quenched samples are then centrifuged to precipitate the protein.

Analytical

8 The supernatants are analyzed by LC-MS/MS using generic analytical methods to quantify the concentration of test compound in each chamber.

Data Analysis

9 The fraction unbound is calculated by dividing the concentration in the buffer chamber by the concentration in the plasma chamber.

For each test compound injection,

response ratio = Test peak area / Internal 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,

Fraction Unbound = Concentration buffer chamber / Concentration plasma chamber

% Free = Fraction Unbound X 100

% Bound = 100% - % Free

The % Recovery is calculated via:

% Recovery = (Concentration buffer chamber + Concentration plasma chamber) * 100 / Total quantity incubated

Protocol references

https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05d023a60f6a6c23c3f6d_Concept%20Life%20Sciences_LE_Protein_Binding%20(1).pdf

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