

Determination of - Plasma Protein Binding (PPB) by Ultrafiltration - Blood partitioning

Purpose

The binding of small molecules and peptides to serum proteins is a very important parameter for drug metabolism and pharmacokinetic studies. If a molecule is highly bound to plasma proteins, the amount of drug available to diffuse into the target tissue may be significantly reduced and the efficacy of the drug may consequently be poor. Determining the level of binding, therefore, is critical and will directly correlate with *in vivo* efficacy of the molecule. A rapid technique to determine plasma protein binding is ultrafiltration centrifugation. Usually the concentration of a test compound in the central compartment is determined by the measuring the plasma concentrations. As there can be a significant difference between the concentration within blood cells and plasma, the information on blood partitioning thus can be important for a comprehensive understanding of pharmacokinetics.

Model validation

Plasma protein binding of four drugs with different affinities to plasma protein were chosen for assay validation. The data presented in **Table 1** demonstrate that the PPB values obtained by this method correlate well with the published values. The mass balance results are consistent with low non-specific binding of the reference compounds.

Table 1: Comparison of published PPB values to data measured at Pharmacelsus

Drug	Published PPB Value	Pharmacelsus Experimental PPB Value	mass balance
Methotrexate	21%	22.4 %	98.7
Caffeine	10-37.5%	28.4%	90.6%
Propranolol	81-93%	99.1%	87.6%
Warfarin	98-100%	98.6%	92.1%

Assay protocols

Plasma protein binding: Test solutions are prepared by adding an aliquot of concentrated test compound to rat or human plasma. These solutions are pre-incubated at 37°C for 1 hour. The spiked plasma is then transferred into pre-conditioned ultrafiltration devices and centrifuged for 30 minutes

at 1400 g. Ultrafiltrates are analyzed for drug concentrations by an appropriate method of analysis (HPLC or LC-MS/MS) and the degree of binding to the plasma proteins (PPB) is calculated by the following equation:

$$\% \text{ PPB} = 100 - \frac{\text{Compound}_{\text{ultrafiltrate}}}{\text{Compound}_{\text{total}}} \times 100$$

To estimate the recovery of the test compound from the matrix a portion of phosphate buffered saline (10 mM, pH 7.4) or ultrafiltrated plasma is treated in the same manner. This value indicates the potential for non-specific binding during the test.

Blood partitioning: Whole blood of the corresponding species is incubated with the test compound. After separation of blood cells and plasma by centrifugation, the remaining concentration of test compound in plasma is determined by LC-MS/MS (sample preparation by protein precipitation using acetonitrile). A positive control is used with spiked plasma to prove plasma stability.

Please don't hesitate to contact us for a customized quotation

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