Plasma Protein Binding Assay (Equilibrium Dialysis)

Background: The vast majority of small molecule drugs are reversibly bound to blood plasma proteins (albumin, lipoproteins, α1-acid-glycoprotein) soon after the administration. The bound drug molecules fraction is generally considered not available for interaction with their biological targets. Determining the extent of drug-protein binding is among key stages in drug development as it influences compound efficacy, dosing, clearance rate, and potential for drug interactions. Rapid Equilibrium Dialysis (RED) is the “golden standard” method to determine the percentage of the plasma protein binding (%PPB) for a drug candidate.

Service Details: To determine the capabilities of a drug to bind plasma proteins, we spike test compounds at a single concentration (typically 1 μM or 2 μM) into plasma followed by dialysis against the buffer until equilibrium is reached. The assay is performed in a 96-well dialysis unit HTD96b dialyser (HTDialysis). Each individual well consists of 2 chambers separated by a vertically aligned dialysis membrane of certain pore size (MWCO 12-14 kDa). Non-diluted plasma spiked with the compound of interest is added into one chamber, whereas the dialysis buffer is added into the other one. Unbound compound diffuses from the plasma chamber to the buffer chamber until equilibrium is reached. Concentrations of the compound in plasma and buffer are then determined by LC-MS, and the percentage of plasma protein bound compound is calculated. All incubations are performed in duplicates. Whole plasma or an individual plasma protein (albumin, AGP) can be used in this assay. Plasma from different species is available, including human, mouse, dog and rat. Reusable dialysis apparatus is usually employed, however disposable units (RED, Thermo Scientific) are available upon request.

Deliverable: The percentage of plasma protein-bound compound is calculated based on the LC-MS measurements of the compound concentrations in plasma and buffer solutions, full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 μmol) or 50 μL of 10-20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, depending on the particular project. We do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.