Microsomal Binding Assay (Equilibrium Dialysis)

Background: Nonspecific microsomal binding in the *in vitro* metabolic assays can lead to an underestimation of the microsomal clearance because only the unbound substrate is free to interact with drug metabolizing enzymes in microsomes. Determination of the unbound intrinsic clearance ($Cl_{in,\ u}$) is essential for the accurate comparison of compounds.

Service Details: We determine binding capabilities of drug candidates to mouse, rat or human microsomes by spiking test compounds at the concentration of 2 µM into microsomes in phosphate buffer (0.42 mg of liver microsomal protein per ml) and dialyzing against buffer until equilibrium is achieved. The assay is performed in a multiple-use 96-well dialysis unit HTD96b dialyser (HTDialysis). Each individual well unit consists of 2 chambers separated by a vertically aligned dialysis membrane of predetermined pore size (MWCO 12-14 kDa). Free compound diffuses from the tissue chamber to the buffer chamber until equilibrium is reached. Concentrations of the compounds in the each chamber are determined by LC-MS, and the fraction unbound is calculated. All incubations are performed in duplicates.

Deliverable: The extent of binding is reported as a fraction unbound (fu) value.

Sample Submission: A minimal weighted amount of dry compound (~1 mg) or 100 µL of 10 mM stock DMSO solution is required for this assay. We do not need to know structures of the molecules for ADME testing. However, we ask our customers to provide brutto formulas, if at all possible, for all studies involving MS detection.