Parallel Artificial Membrane Permeability Assay (PAMPA: GIT or BBB mode)

Background: Parallel Artificial Membrane Permeability Assay (PAMPA) is used to determine passive diffusion across an artificial lipid membrane made from lipid-like organic compound or a lipid from natural source and supported by porous filter. Passive diffusion is an important factor in determining absorption of orally administered compounds in the gastrointestinal tract (GIT), penetration of the blood-brain barrier (BBB), as well as general transport across cell membranes. PAMPA provides a simplified approach to permeability by addressing just a single transport mechanism. This avoids the complexities of active transport as well as metabolism and enables ranking of the compounds on a single permeability factor. Depending upon the particular lipid and the buffers used, PAMPA assay could be predictive of gastrointestinal tract absorption (PAMPAGIT), blood-brain barrier permeability (PAMPA-BBB) or transdermal penetration (Skin-PAMPA. Typically, PAMPA experiments are carried out in the early drug discovery phase to select leads with promising oral bioavailability/ brain penetration potential by cost-efficiently ranking candidates within large compound sets. By combining the data generated in PAMPA with more labor-intensive (as well as more predictive) cell-based permeability assays, quick structural modifications of discovery compounds to improve their in vivo characteristics, can be efficiently guided.

Service Details: “PAMPA sandwich” is assembled from two disposable multi-well plates. One plate contains a porous filter disk at the bottom of each well (a donor plate), while the other one is an acceptor plate. The potential permeability contact between the two plates occurs at the filter, which is coated with a solution of lipid material in inert organic solvent to prepare the artificial membrane. Aqueous buffer containing 0.5% DMSO is added to each well of the acceptor plate. 50 µM test compound solutions (containing 0.5% DMSO) are added to the wells of the donor plate. The donor plate is inserted into the acceptor plate and the plates are then incubated at room temperature, in a humid environment, for 1, 2 or 4 hours. Analytical standards are prepared from test compound solution. Test compound permeability is assessed in two independent replicates. Two compounds of known permeability are run as controls on each plate. The donor and acceptor samples for the test and control compounds are quantified by UV or LC-MS/MS using a 3-point calibration curves with the appropriate dilutions of the samples. The experimental analyte recovery is calculated using both donor and acceptor compartment concentrations.

Deliverable: The apparent permeability coefficient for each compound (Papp) is calculated. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 µmol) or 50 µL of 20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient. Brutto formulas are required for all studies involving MS detection.