Caco-2 Permeability Assay

Background: Solubility and intestinal permeability are the most important factors determining oral absorption of drugs. Caco-2 (human colon adenocarcinoma cells) assay is a widely used model in drug discovery for evaluation of compound permeability properties. When grown to confluence and allowed to differentiate the cells form a monolayer resembling luminal epithelium of human intestine by structure and properties. Caco-2 cells have a variety of active transporters, which are relevant to the absorption process in the gastro-intestinal tract. Therefore, in contrast to the PAMPA (parallel artificial membrane permeability) assay, Caco-2 method is more suitable for the prediction of in vivo drug efflux. Importantly, this assay has a good correlation with in vivo studies of absorption. For Caco-2 permeability assay, cells are grown on semipermeable supports inside inserts in multi-well plates. The system is composed in the way that a semi-permeable support separates apical and basolateral compartments, as differentiated Caco-2 monolayer is asymmetrical. Therefore, this system enables measurements of drug transport in both directions (apical to basolateral or basolateral to apical/A-B and B-A), across the cell monolayer. After certain incubation time, the solutions of tested compounds and samples from appropriate compartments are taken and analyzed by LC-MS/MS. Based on compound concentrations measured, apparent permeability coefficient (Papp), reflecting the ability of a compound to penetrate cell monolayer, is calculated. Assessing transport across the monolayer in both directions (A-B and B-A) enables determination of an efflux ratio, which is an indicator as to whether a compound undergoes active efflux. A P-glycoprotein (P-GP) inhibitor, typically verapamil, can also be included to identify whether active transport is mediated by this efflux pump.

Service Details: Caco-2 (human colorectal adenocarcinoma line) was purchased from ATCC (cat.#HTB-37) and cultured according to the supplier's recommendations. The Caco-2 assay is carried out in 24-well insert plates (Millipore). Prior to use, the integrity of Caco-2 monolayers is verified by transepithelial electrical resistance (TEER) measurements. The assay is performed by spiking a compound (in duplicates, at 10 μM) into the apical and/or basolateral compartments of the trans-well insert, and monitoring the appearance of this compound on the basolateral and/or apical side at a predetermined time point. Incubation time for tested compounds is 2 h; buffer pH is 7.4 (or 6.5) in the donor and 7.4 in the acceptor compartments. High and low permeability controls are run with every experimental batch to verify assay validity. Due to the long set-up time for the assay, the lead time could be up to 3-4 weeks. Similar permeability assay using MDCK (Madin- Darby canine kidney) cell line is also available upon request.

Deliverable: Based on compound concentrations measured by LC-MS/MS, apparent permeability coefficient (Papp), reflecting the ability of a compound to penetrate cell monolayer, is calculated. Efflux ratios and P-gp substrate liability are determined if applicable. 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.