cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

P-glycoprotein (P-gp) Substrate Identification Assay for Screening and Regulatory Reporting Purposes

Background Information

'P-gp has an important role in limiting entry of various drugs into the central nervous system. In addition, it also plays a part in the intestinal absorption and in the biliary and urinary excretion of drugs.'

[1]The International Transporter Consortium (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9(3)**: 215-236

- P-gp (P-glycoprotein; MDR1, ABCB1) is an important efflux transporter. It is expressed in the gastrointestinal tract, liver, kidney and brain endothelium[1].
- The ITC[1], the EMA guideline[2] and the draft FDA guidance[3] recommend investigating P-gp due to the clinical importance of P-gp in the absorption and disposition of drugs.
- Madin Darby canine kidney (MDCK) cells transfected with the human MDR1 gene overexpress P-gp. The EMA[2] and draft FDA[3] regulatory guidelines recommend polarised MDCK-MDR1 cell monolayers as one of the preferred methods for evaluating the role of P-gp in the efflux of new chemical entities.
- The assay investigates bidirectional transport across the cell monolayer in the presence and absence of a P-gp reference inhibitor, elacridar (screening) or cyclosporin A (regulatory), to determine if active efflux is occurring, and whether this efflux is mediated by P-gp.
- Where MDCK-MDR1 cell assays indicate a compound has inherently low passive permeability, then P-gp membrane vesicles can be used as an alternate *in vitro* test system to identify P-gp substrates (assay available upon request).

Protocol

Test Article Concentration

Screening study - 10 µM plus/minus reference inhibitor (different test compound concentrations available)

Regulatory study - 1, 10, 50 and 100 µM (different concentrations available) plus inhibition at two substrate concentrations (1 and 10 µM)

Assay Conditions

Apical to basolateral and basolateral to apical in presence and absence of elacridar (2 µM; screening) or cyclosporin A (10 µM; regulatory)

Number of Replicates

2 (screening) or 3 (regulatory)

Analysis Method

LC-MS/MS quantification

Integrity Marker

Lucifer Yellow

Data Delivery

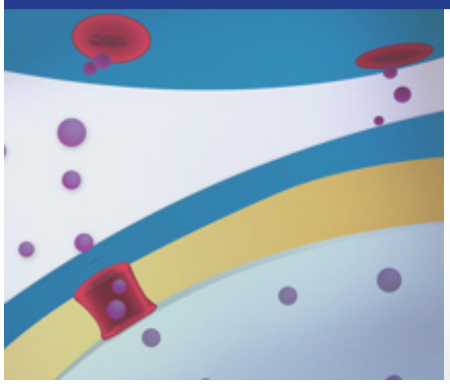
P_{app}

Efflux ratio in presence and absence of reference inhibitor

% Recovery

To find out more contact **enquiries@cyprotex.com**

Bidirectional transport in the presence and absence of a reference P-gp inhibitor can be used to determine if active efflux is occurring and whether the efflux is mediated by P-gp.

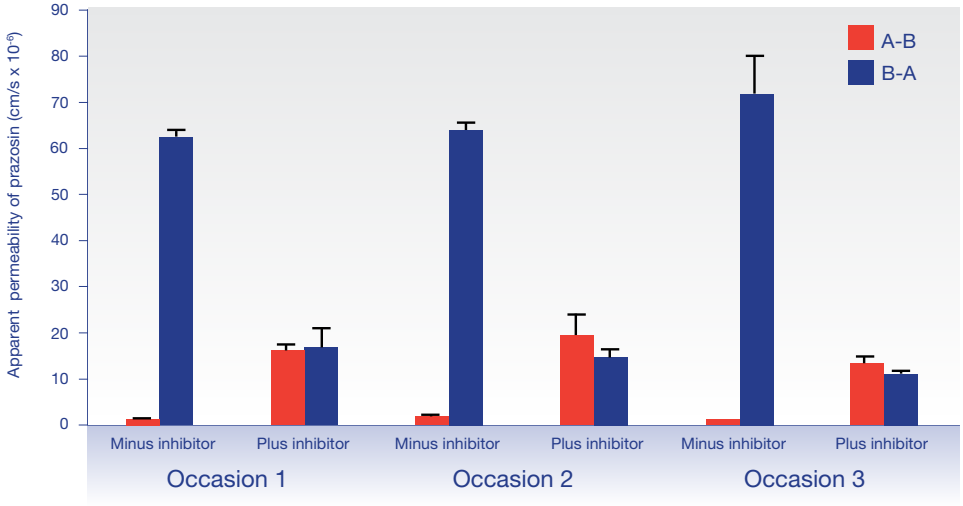


P-gp Substrate Identification Assay

Cyprotex's P-gp substrate identification assay can be used either for screening for potential substrates of P-gp or for regulatory confirmation of a P-gp substrate.

Figure 1

Graph showing effect of the P-gp inhibitor, cyclosporin A (10µM) on the efflux of the P-gp substrate, prazosin. Data show the mean ± standard deviation



Functional activity of P gp in MDCK MDR1 polarised cell monolayers was demonstrated by investigating the inhibition of efflux of the P gp substrate prazosin by the reference inhibitor cyclosporin A.

References

[1] The International Transporter Consortium (2010) Membrane transporters in drug development. *Nat Rev Drug Disc* **9**: 215-236

[2] The European Medicines Agency (EMA) Guideline on the Investigation of Drug Interactions (Adopted 2012)

[3] Draft FDA Guidance for Industry – Drug Interaction Studies – In Vitro Metabolism and Transporter-mediated Drug-Drug Interaction Studies, October 2017