

# Caco-2 Permeability

## Background Information



‘Studying the permeability of compounds across a Caco-2 cell monolayer is an established *in vitro* model to screen for oral absorption and to evaluate the mechanism of transport. Using LC-MS/MS for the analysis of samples derived from Caco-2 cells studies allows the rapid and accurate determination of drug transport across the Caco-2 cell monolayer.’

<sup>1</sup>Wang Z, Hop C.E., Leung K.H. and Pang J. (2000) *J Mass Spectrom* **35 (1)**; 71-6

- Cyprotex’s Caco-2 permeability assay uses an established method that measures the rate of flux of a compound across polarised Caco-2 cell monolayers and from which the data generated can be used to predict *in vivo* absorption of drugs.
- The Caco-2 cell line is derived from a human colon carcinoma. The cells have characteristics that resemble intestinal epithelial cells such as the formation of polarised monolayer, well-defined brush border on the apical surface and intercellular junctions.
- Assessing transport in both directions (apical to basolateral (A-B) and basolateral to apical (B-A)) across the cell monolayer enables an efflux ratio to be determined which provides an indicator as to whether a compound undergoes active efflux.
- The P-glycoprotein (P-gp) inhibitor, verapamil, can be included to identify whether active efflux is mediated by P-gp (alternatively for definitive P-gp substrate identification, we have a P-gp substrate identification assay using the MDCK-MDR1 cell test system in which human P-gp is expressed in isolation and unlike Caco 2, is not subject to potential efflux interference by BCRP).
- The BCRP inhibitor, fumitremorgin C, can be included to identify whether active efflux is mediated by BCRP (see Cyprotex’s BCRP substrate identification assay).

### Protocol

#### Test Article Concentration

10 µM

#### Passage Number

40-60

#### Period of Cell Culture

20 days

#### Number of Replicates

2

#### Incubation Time

120 min

#### Temperature

37°C

#### Test Article Requirements

100 µL of 10 mM DMSO solution

#### Integrity Marker

Lucifer Yellow

#### Control Compounds

Atenolol, propranolol and talinolol

#### Analysis Method

LC-MS/MS quantification

#### Data Delivery

$P_{app}$   
Efflux ratio  
% Recovery

**Cyprotex's Caco-2 assay is performed** in a 96-well format providing a cost-effective and highly reproducible method of assessing the permeation potential of test compounds.



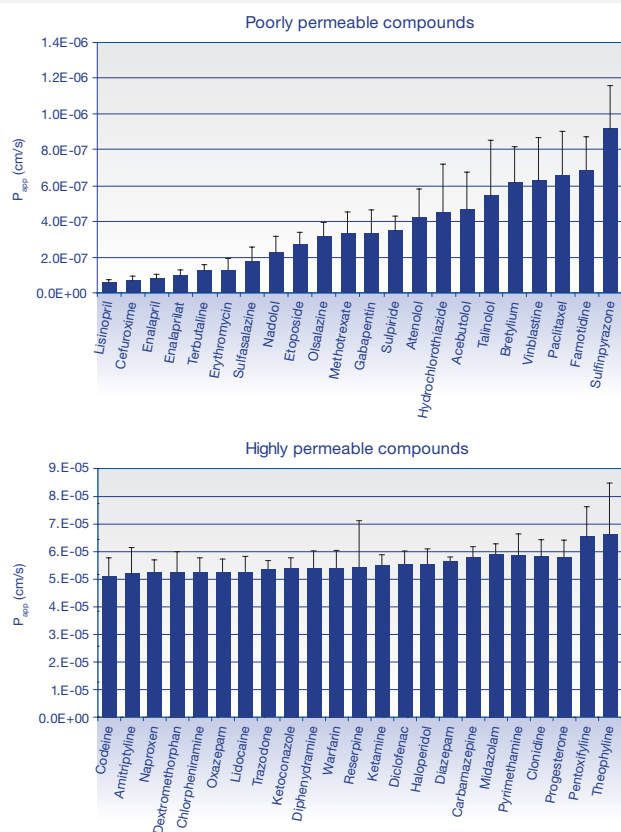
### Cyprotex's Caco-2 Permeability

For the validation, a set of compounds were screened through Cyprotex's Caco-2 Permeability assay over 3 separate experiments. Data generated were reproducible over a range of permeabilities.

The bidirectional assay is able to correctly distinguish between those compounds which are reported to undergo active efflux and those which are not.

**Figure 1**

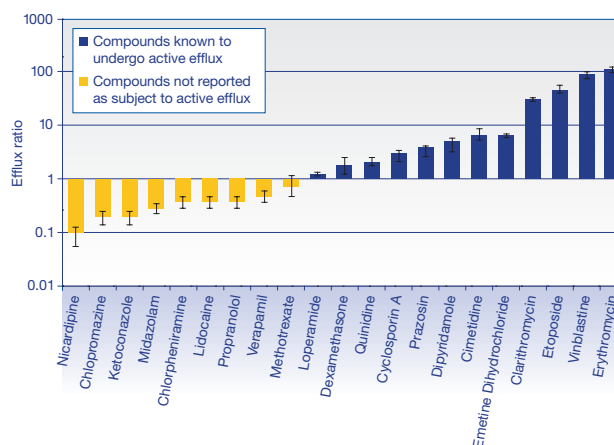
Graph illustrates the consistency of Cyprotex's Caco-2 Permeability data over 3 separate experiments for the apical to basolateral assay.



These data illustrate the high level of reproducibility provided by this assay for a set of compounds with a range of permeabilities.

**Figure 2**

Graph displays the efflux ratio of a set of 21 compounds generated by Cyprotex's Caco-2 permeability assay.



Cyprotex's bi-directional Caco-2 permeability assay can identify and quantify level of active efflux. Screening compounds in both the A to B and B to A direction provides a ratio of B-A/A-B (efflux ratio). When a compound has an efflux ratio of greater than 2, it suggests that the compound may be subject to active efflux.

#### References

<sup>1</sup> Wang Z et al. (2000) *J Mass Spectrom* **35** (1): 71-6