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In vitro **ADME & PK**

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.'

[1]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.

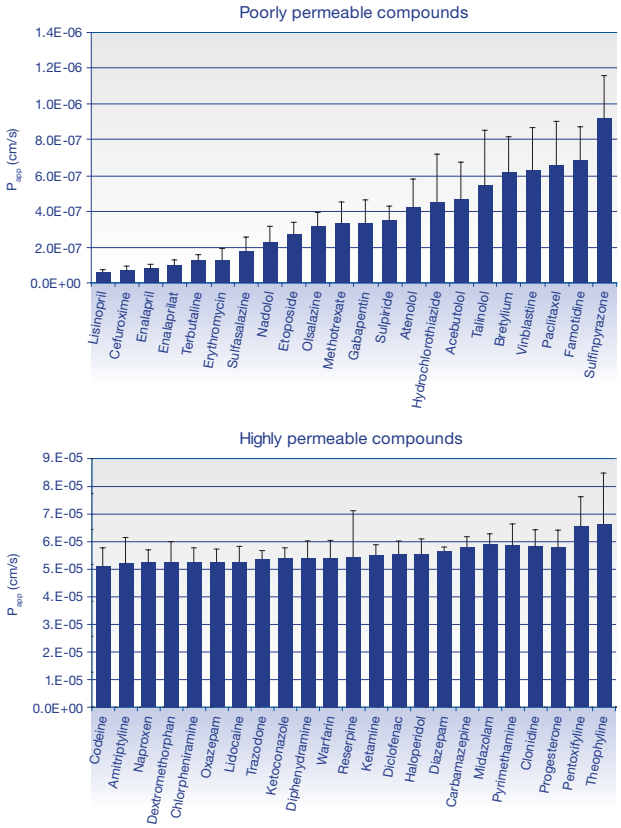
Cyprotex's Caco-2 Permeability

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

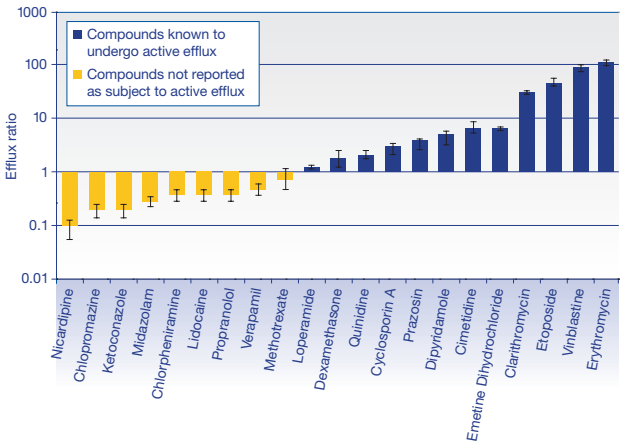
Graph illustrates the consistency of Cyprotex's Caco-2 Permeability data over 3 separate experiments for the apical to basolateral assay.



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

[1] Wang Z *et al.* (2000) *J Mass Spectrom* **35 (1)**; 71-6