cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

PAMPA

Background Information

'The parallel artificial membrane permeability assay (PAMPA), first introduced by Kansy *et al*., has been widely used in the pharmaceutical industry as a high throughput permeability assay to predict oral absorption.'

[1]Di L ,Kerns EH, Fan K, McConnell OJ, and Carter GT. (2003) *Eur J Med Chem* **38**; 223-232.

- The Parallel Artificial Membrane Permeation Assay (PAMPA) is used as an *in vitro* model of passive transcellular permeation.
- PAMPA avoids the complexities of active transport, allowing test compounds to be ranked based on a simple permeability property alone.
- The ability of this assay to evaluate permeability over a large pH range is valuable for an early understanding how new oral compounds might be absorbed across the entire gastrointestinal tract.

Protocol

Test Article Concentration
10 µM

Number of Replicates
4

Membrane Composition
Hexadecane in hexane (5 % v/v)

Incubation Time
5 hours

Temperature
Room temperature

Test Article Requirements
100 µL 10 mM DMSO solution

Integrity Marker
Lucifer Yellow

Analysis Method
LC-MS/MS quantification

Data Delivery
P_{app}
Recovery

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

PAMPA can quickly provide information about passive permeability that is not complicated by other mechanisms such as paracellular transport, active transport and metabolism.

PAMPA

26 Compounds were screened in Cyprotex's PAMPA in quadruplicate on 3 separate occasions. The assay generates consistent, reproducible data over a range of permeability values.

Cyprotex's PAMPA has been successfully trialled by one of our partners and the data compare well with the customer-generated data.

Figure 1

Cyprotex's PAMPA measures passive diffusion of a test compound through an artificial hexadecane membrane.

The protocol was designed in collaboration with our biotechnology partners and follows the method described by researchers at Novartis[2].

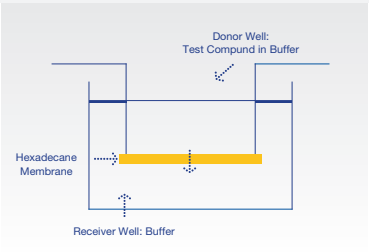


Figure 2

The graph shows the reproducibility of data generated in Cyprotex's PAMPA over 3 separate assays (error bars represent the standard deviation of quadruplicate incubations).

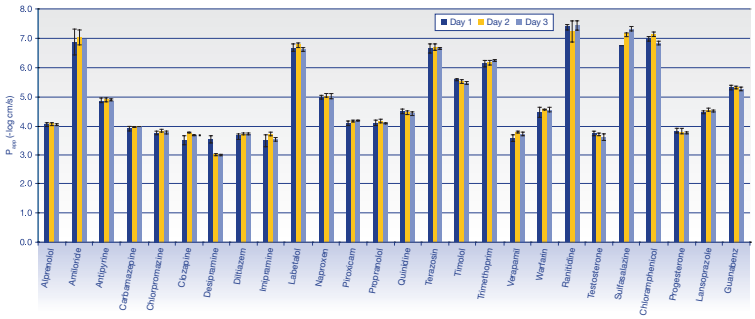


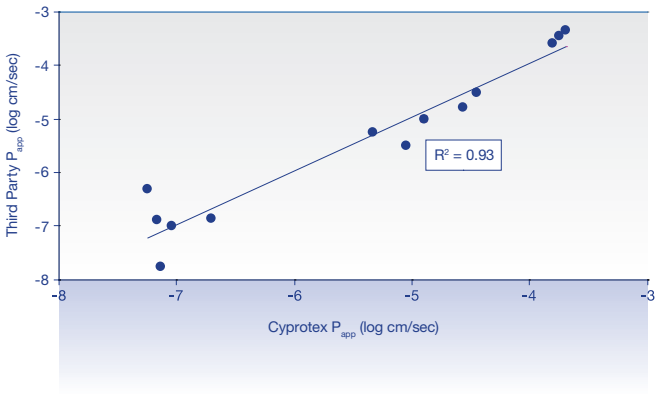
Table 1

Cyprotex log P_{app} data show a high level of similarity to the third party log P_{app} data for the purpose of compound classification into low and high log P_{app}.

Compound name	Mean log P_{app} (Cyprotex)	Mean log P_{app} (Third Party)
Acyclovir	<-5.79	-6.86
Digoxin	<-6.60	-4.85
Ceftriaxone	<-5.89	-6.25
Fluvastatin	-7.24	-6.31
Ranitidine	-7.16	-6.88
Chloramphenicol	-7.13	-7.76
Amiloride	-7.04	-6.99
Suflasalazine	-6.70	-6.85
Guanabenz	-5.32	-5.25
Naproxen	-5.04	-5.50
Antipyrine	-4.90	-5.00
Quinidine	-4.55	-4.80
Lansoprazole	-4.45	-4.53
Verapamil	-3.80	-3.59
Desiparmine	-3.75	-3.47
Testosterone	-3.69	-3.35

Figure 3

Comparison of Cyprotex PAMPA data with third party data.



Cyprotex data correlate well with data generated by one of our partners (R^2 = 0.93). The third party data were produced using the same method as Cyprotex with the exception that a prolonged incubation period and UV absorbance end-point were used. Cyprotex method uses the more sensitive LC-MS/MS end point and a 5 hour incubation.

References

1 Di L *et al*. (2003) *Eur J Med Chem* **38**; 223-232.

2 Wohnsland F and Faller BJ (2001) *Med Chem* **44**; 923-930.