Pion

Application Note

AN. 213-1 Rev. 1

A Low-cost Permeability Screen: BBB PAMPA

Dana C. Lipp
Pion Inc.
Billerica, MA, USA
www.pion-inc.com

Summary

A high throughput, 96 well permeability method (PAMPA) has been developed using an artificial membrane constructed from porcine brain lipid (PBL) extract that shows good correlation (r^2=0.79) with rat brain perfusion data using thirty test compounds. The *in vitro* results were more predictive of *in situ* brain penetration than published cell-based MDCK data. BBB PAMPA costs 1,000 times less than *in vivo* testing. Complete PAMPA Systems including a UV plate reader, all reagents, plates, control test samples, data collection/processing software ensure excellent system performance right out of the box.

Introduction

Delivering drugs across the blood-brain barrier to their therapeutic targets constitutes a considerable challenge common to pharmaceutical developers. Costly *in vivo* brain penetration tests limit the screening of potential drugs. Researchers could evaluate more compounds earlier in the discovery process with a more cost-effective and API-sparing screen.

Method Description

A PAMPA sandwich (parallel artificial membrane permeability assay) is constructed of a lower (donor) plate containing the drug solution and an upper drug-free compartment (acceptor). A filter membrane separating the donor and acceptor is freshly coated with PBL (Pion BBB-1 lipid) just before use. A Pion Gut-Box™ provides stirring to reduce the thickness of the unstirred water layer at the donor solution/membrane interface. After incubation at room temperature, the relative drug concentration in each compartment is determined with a UV plate reader and the intrinsic permeability is calculated. Membrane retention is calculated using mass balance. PAMPA Command Software (Figure 1.) provides control of the plate reader, spectral data processing, calculations and automatically reports results in both graphical and tabular form.

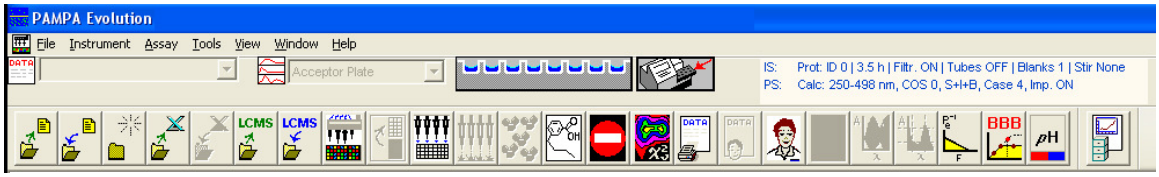

Figure 1. PAMPA Evolution Command Software

Results

A correlation between rat brain perfusion data and BBB PAMPA using 39 diverse compounds supplied by Astra Zeneca was developed using a variety of models as shown in Figure 2. The best correlation coefficient obtained is shown in Figure 3, which was produced after further refinement of the lipid model.

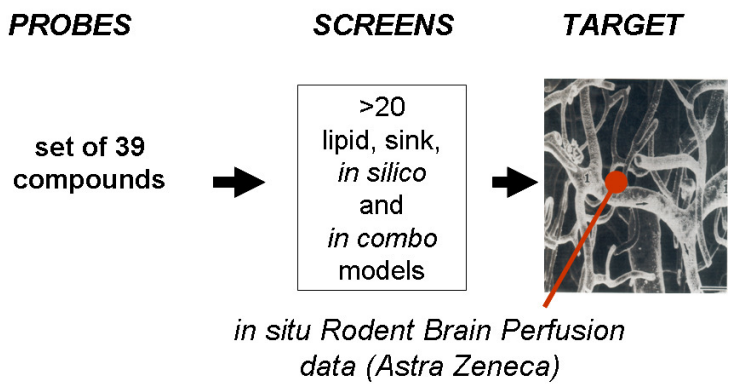

Figure 2. Development of BBB PAMPA screens required evaluation of over 20 different models.

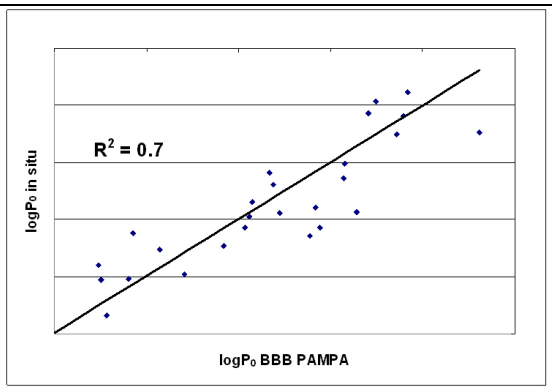

Figure 3. Intrinsic BBB PAMPA and rat brain penetration data corrected for ionization

Conclusions

The correlation between the BBB PAMPA model confirms a good linear correlation over at least fivel orders of magnitude for passively transported CNS drugs establishes additional validity to this new model.

While *in situ* brain perfusion measurements cost about $5-8,000 per molecule and the *in vitro* cellular methods cost about $50-100 per molecule, BBB PAMPA can be done for less than $10 per compound. This cost is low enough to justify screening of libraries of over 100,000 molecules. And when applied to screening large libraries, PAMPA can make a significant contribution to NIH efforts.

The PAMPA Explorer Complete contains everything required to run the assay in-house. Test compound measurements in our worldwide permeability database, combined with Pion technical support will ensure a quick and confident startup experience.