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Early drug discovery ADME assays, such as fast Caco-2 screens, can help in rejecting test compounds that lack good pharmaceutical profiles. A cost-effective, high-throughput method – parallel artificial membrane permeability analysis – that uses a phospholipid artificial membrane that models passive transport of epithelial cells, is becoming increasingly popular. The pION PAMPA Evolution 96 System (with Double-Sink and Gut-Box) is a new surrogate assay that predicts the gastrointestinal tract absorption of candidate drug molecules at different pH conditions. This paper describes Beckman Coulter's Biomek FX Single Bridge Laboratory Automation Workstation PAMPA Assay System that features a 30-minute incubation time using an on-deck integrated Gut-Box and a SpectraMax microplate spectrophotometer. The permeability coefficients of drug standards with diverse physicochemical properties were compared from both PAMPA and Caco-2 assays automated using the Biomek FX Workstation. These automated assays can be used for high-throughput ADME screening in early drug discovery.

Introduction

Incorporating predictive ADME assays in earlier stages of drug discovery can help in rejecting molecules that lack necessary pharmacological properties. Drug bioavailability is influenced by factors including absorption and metabolism. The U.S. FDA issued the Biopharmaceutics Classification System, which is the guidance for using *in vitro* models to assess drug bioavailability. The para-

llel artificial membrane permeability assay (PAMPA) using membranes coated with a concentrated mixture of phospholipids in dodecaine has been widely accepted as a surrogate model for high-throughput permeability assays measuring passive transport.

The Biomek FX Laboratory Automation Workstation was used to automate the PAMPA Evolution 96 permeability assay. The PAMPA assay with Gut-Box and SpectraMax Plate Reader (Molecular Devices, Sunnyvale, CA, USA) is integrated on the Biomek FX Laboratory Automation Workstation (Figure 1). Drug permeability and membrane retention for seven reference drugs of different physicochemical properties were determined at different pH conditions and are reported here. The rank order of these reference drugs match well with the conventional Caco-2 permeation assay. The automated PAMPA Evolution 96 assay can be used for high-

Automation of a Double-Sink PAMPA Permeability Assay on the Biomek[®] FX Laboratory Automation Workstation

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Figure 1. The Biomek FX-ADMETox Workstation with integrated pION Gut-Box and Molecular Devices' SPECTRAMax 384 Plus.

throughput primary screening of drug candidates for ADME properties.

Experimental Conditions

All liquid-handling steps for the PAMPA assay are performed on the Single Bridge Multichannel Biomek FX Laboratory Automation Worksta-

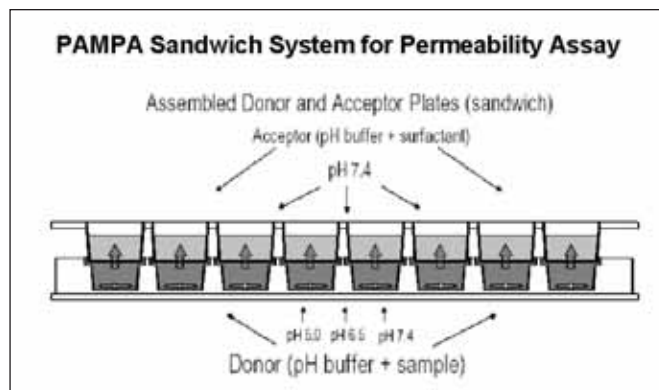


Figure 2. A PAMPA Sandwich Plate System for the determination of drug permeability through a phospholipid mixture under different pH conditions.

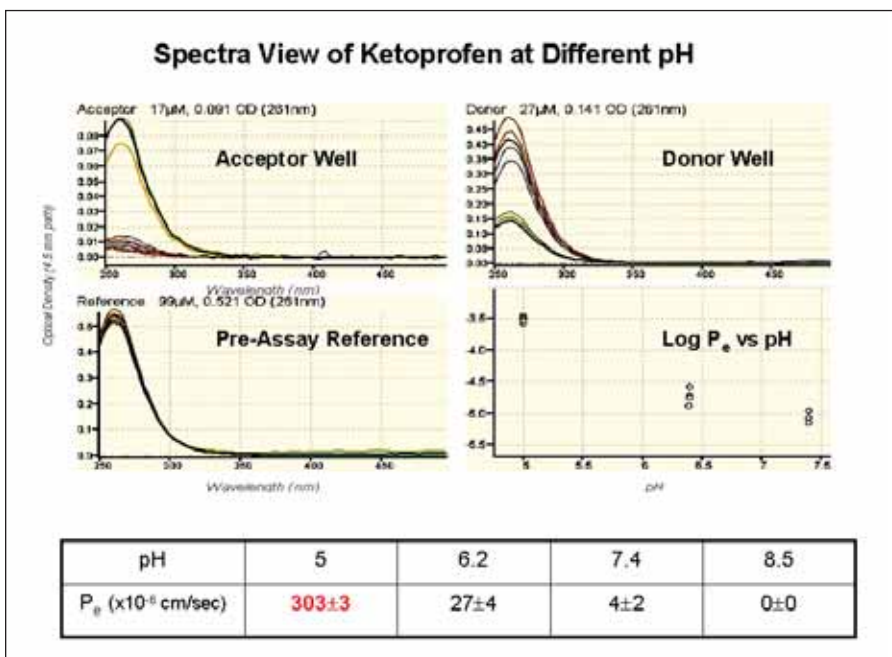


Figure 3. UV spectral scans of ketoprofen at different pH levels and a plot of the calculated $\text{Log } P_e$ vs. pH.

Table I. A PAMPA assay, that differentiates drugs with optimal permeability coefficient at different pH conditions

P_e ($\times 10^{-6}$ cm/sec)	pH = 5	pH = 6.2	pH = 7.4	pH = 8.5
Verapamil	161±12	734±118	1073±172	1423±407
Propranolol	96±40	675±83	1000±128	1945±199
Ketoprofen	304±3	27±4	4±2	0±0
Metoprolol	29±19	22±3	218±37	748±237
Carbamazepine	130±12	107±9	137±45	89±11
Atenolol	0±0	0±0	0±0	0±0
Ranitidine	0±0	0±0	0±0	0±0

tion, driven from pION's (Woburn, MA) PAMPA Evolution 96 Command Software. The PAMPA Evolution 96 Permeability Assay Kit includes System Solution, Acceptor Sink Buffer (ASB), Double-Sink Lipid Solution and a PAMPA Sandwich plate, preloaded with magnetic disks (Figure 2). Test compounds used in this analysis include 10 mM stock solutions of ketoprofen, verapamil, metoprolol, carbamazepine, ranitidine, propranolol and atenolol. The PAMPA assay is initiated by transferring 3 μL of lipid to the support membrane in the acceptor well, followed by addition of 200 μL of ASB (pH 7.4). Then, 180 μL of diluted test compound (50 μM in system buffer at pH 5.0, 6.2, 7.4 and 8.5) is added to the donor wells.

The PAMPA sandwich plate is assembled and placed on the Gut-Box for the course of the assay. The Gut-Box generates a rotating magnetic field that cre-

ates an aqueous boundary layer in the donor wells that mimics the condition in the human gut and shortens the required incubation time for the assay from 4 h to 30 min. The distribution of the compounds in the donor and acceptor buffers (100 μL aliquot) is determined by the measure of the UV spectra from 200 to 500 nm using the SpectraMax reader. The permeability coefficient is determined using the absorbance from 200 to 500 nm using the following formula:

$$P_e = -[2.303V_D/A(t-\tau_{SS})](1/1+r_a) \cdot \log_{10} [-r_a + (1+r_a/1-R) \cdot C_D(t)/C_D(0)]$$

Where P_e is the effective permeability; V_D is the donor well volume (cm^3); V_A is the acceptor well volume; A is the filter area (cm^2); t is the

interval of time (sec); τ_{SS} is the steady-state time needed to fill up the membrane; $r_a = (V_D/V_A)P_e(A \text{ to } D)/P_e(D \text{ to } A)$; R is the membrane retention (related to the membrane/water partition coefficient); $C_D(0)$ is the sample concentration in the donor well at time 0 (mole/ cm^3); $C_D(t)$ is the sample concentration in the donor well at time t .

Results

We show the ultraviolet spectral scans of a test drug compound from replicate wells and multiple pH conditions in Figure 3. The scans are overlaid to demonstrate the reproducibility of the data and to enable the identification of outliers. As shown in the summary table (Table 1), the PAMPA assay differentiates the permeability coefficient of the test compound drug standards under different pH conditions. The rank order of the drug standards correlates with that determined using a Caco-2 system on both the Biomek FX and the Biomek 3000 (data not shown).

Conclusions

The Double-Sink PAMPA permeability assay mimics *in vivo* conditions by the use of a chemical sink in the acceptor wells and pH gradient in the donor wells. The use of the pION Gut-Box integrated on the deck has shortened the PAMPA assay incubation time to 30 min.

The permeability coefficient and rank order of the seven test drug compounds correlates with data obtained using the *in vitro* Caco-2 assay and *in vivo* permeability properties measured in rat intestinal perfusions. The automated Double-Sink PAMPA permeability assay, using the Biomek FX Workstation, can be used for high-throughput ADME screening in early drug discovery.

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