Permeability & Transporter Services

Part I: Permeability

Highlights

- Either unidirectional or bidirectional permeability assay
- Adjustable timepoints, concentrations, number of replicates and pH values
- Lucifer Yellow assay or TEER measurement to monitor cell monolayer integrity
- Customized approaches to improve solubility and reduce non-specific binding
- Differentiated protocols for discovery and IND filing

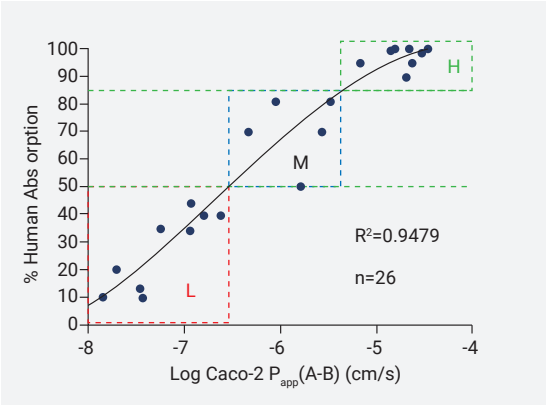
Introduction

Most discovery projects focus on developing orally administered drugs that are primarily absorbed across the intestinal mucosa. *In vitro* permeability assays are commonly used to evaluate the suitability of potential oral and/or CNS therapeutics to determine intestinal or brain penetration. Therefore, it is essential to select an appropriate *in vitro* permeability model in drug development that provides reliable data to predict intestinal absorption and brain penetration in humans accurately.

Available Models

Models		Strengths	Applications
Non-cell-based Models	BBB-PAMPA	▪ High-throughput ▪ To predict transcellular transport ▪ To evaluate permeability over a large pH range to better mimic gastrointestinal tract (Egg-PAMPA & Pion GIT PAMPA) ▪ Cost-efficiency	BBB Permeability
	Egg-PAMPA		Permeability Screening & GIT Permeability
	Pion BBB PAMPA		Brain Penetration
	Pion GIT PAMPA		Permeability Screening & GIT Permeability
Cell-based Models	Wild-type MDCK II/ MDR1-MDCK II (NKI)	▪ High-throughput ▪ Passive and active transport ▪ Better mimic *in vivo* system ▪ More instructive for optimizing lead compounds	Permeability Screening
	Wild-type MDCK I/ MDR1-MDCK I (NIH)		Brain Penetration
	Caco-2		GIT Permeability

Validation Data



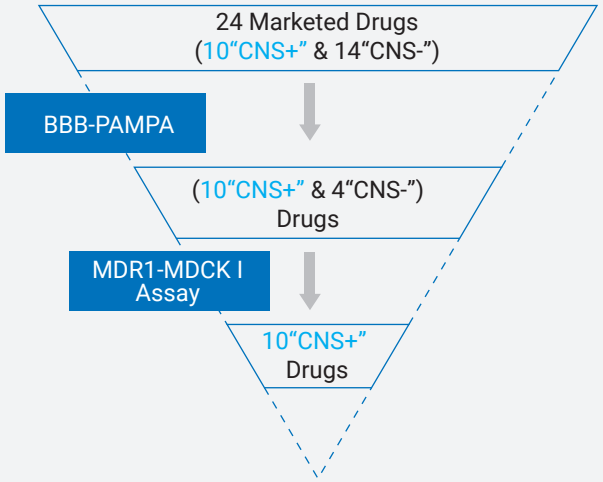
Intestinal Permeability Assessment

The validation of a Caco-2 permeability test was carried out with 26 marketed drugs with different human fraction absorbed data (%Fa). The results were reproducible, and the P_{app} (A-B) values of these model drugs correlated well with %Fa values (R^2 = 0.9479) (Fig. 1).

Fig. 1. A correlation between human absorption versus estimates from Caco-2 logP_{app}(A-B) derived from a collection of 26 marketed drugs (data from 7 repeated runs, triplicates per compound per run)

“Screening Funnel” for Brain Penetration Assessment

- Ten “CNS+” drugs (high brain penetration, triangles) and 14 “CNS-” drugs (low brain penetration, dots) were included.
- All these drugs were tested in BBB-PAMPA and ten “CNS-” drugs (red dots) were excluded due to limited BBB penetration.
- The others were tested in MDR1-MDCK I (NIH) assay and four “CNS-” drugs (blue dots) were further excluded due to high P-gp efflux.
- The remaining 10 compounds were potential “CNS+” (high brain penetration) compounds and were classified correctly.



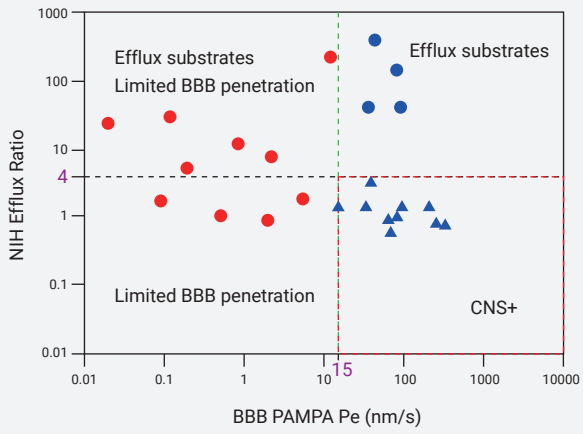
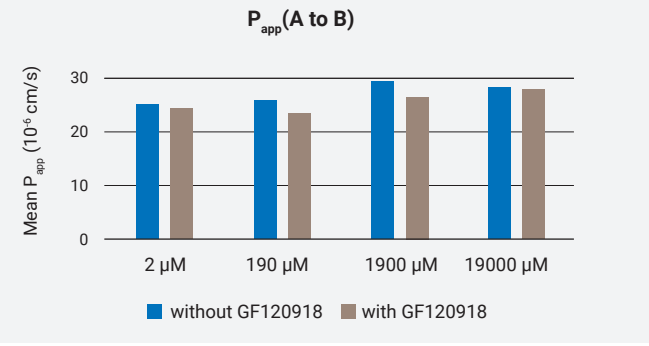


Fig. 2. BBB penetration assessment for 24 marketed drugs

Case Sharing: Permeability Assessment for BCS Classification or BE Waiver

Metronidazole is an antibiotic. When administered orally in tablet form, it is absorbed entirely, showing a bioavailability of greater than 90%. Based on the clinical dosage and the solubility, four dosing concentrations were designed to be 2, 190, 1900, and 19000 μM. Metronidazole was tested in Caco-2 cells at four concentrations in the absence and presence of the efflux inhibitor, Elacridar. The results showed that metronidazole was a high permeability compound and was a poor or non-efflux substrate (Fig. 3).



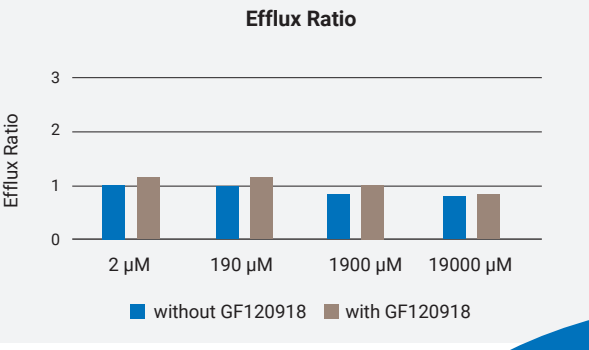


Fig. 3. Permeability assessment for metronidazole in Caco-2 cells

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Permeability & Transporter Services

Part II: ABC Transporters

Highlights

- Caco-2 cell model, MDR1 transfected MDCK I and MDCK II cell models and inside-out membrane vesicle models
- Substrate and inhibition evaluation as well as K_m and V_{max} determination
- Customized approaches to improve solubility and reduce non-specific binding
- Differentiated protocols for discovery and IND filing

Introduction

ABC transporters actively transport endogenous and exogenous substrates through biological membranes in body tissues by coupling ATP binding, hydrolysis, and phosphate release, so they play a critical role in drug disposition by affecting absorption, distribution, and excretion. As reported in numerous publications, ABC transporters influenced the pharmacokinetics characteristics of many drugs as well as the related transporter-mediated drug interactions.

Available Models

Transporter	Models	Agency Requirement
P-gp	MDR1-MDCK II, MDR1-MDCK I, Caco-2 Vesicle-MDR1	FDA, EMA, NMPA, PMDA, and ICH
BCRP	Caco-2 Vesicle-BCRP	FDA, EMA, NMPA, PMDA, and ICH
BSEP	Vesicle-BSEP	EMA, PMDA, and
MRP2	Vesicle-MRP2	PMDA and ICH
MRP4	Vesicle-MRP4	PMDA
MRP1	Vesicle-MRP1	Not mentioned
MRP3	Vesicle-MRP3	

Model Selection Strategies

The table below summarizes the major advantages and limitations of the three types of models commonly used in DMPK studies.

Models	Strengths	Limitations	Applications
Caco-2 cells	▪ Transporter expression similar to small intestine	▪ Multiple transporters expressed ▪ Test compounds should have transmembrane capability	Regular small molecules
MDR1 transfected MDCK cells	▪ High signal/noise ratio ▪ High specificity	▪ Test compound should have transmembrane capability	Regular small molecules
P-gp, BCRP, BSEP, MRP1/2/3/4 vesicles*	▪ More suitable for compounds with poorly passive permeability	▪ May fail to identify potential substrates that are highly permeable compounds or highly non-specific binding	PROTACs, oligonucleotides, polypeptides, liposomes, and poorly permeable small molecules

*Sf9 insect cell-derived or HEK293 cell-derived inside-out membrane vesicles overexpressing a single transporter

Validation Data: Assessing P-gp & BCRP Substrates in Caco-2 Cells

In the Caco-2 cell model, WuXi AppTec DMPK has screened the relatively specific inhibitors of P-gp and BCRP by using digoxin and estrone 3-sulfate as probe substrates, respectively. Verapamil (Ver, 30 μM) and zosuquidar (Zos, 1 μM) were selected as P-gp inhibitors, and novobiocin (Nov, 30 μM) and sulfasalazine (Sul, 100 μM) were selected as BCRP inhibitors. At the concentrations tested, inhibitors of both transporters did not interfere with each other, demonstrating a good specificity (Fig. 4).

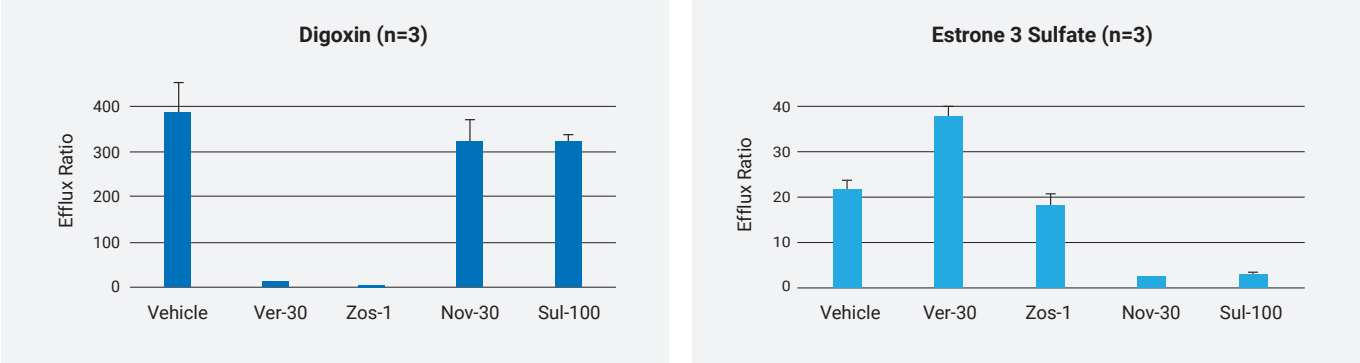

Fig. 4. Inhibitor selection for P-gp & BCRP substrate assessment in Caco-2 cells

Case Sharing: Advantages of Using Vesicles for Poorly Permeable Compounds

While whole-cell models possess a limitation in assessing transporter inhibition by poorly permeable compounds due to the inhibitor's inability to access the inhibition site, this challenge can be effectively addressed using inside-out membrane vesicles. As shown in Fig. 5a, Compound A, an extremely low permeable compound, didn't show the potential to inhibit P-gp in MDR1-MDCK II cells (IC_{50} greater than the highest dosing concentration of 15.0 μM), while inhibited P-gp with the IC_{50} of 1.79 μM in P-gp vesicles (Fig. 5b).

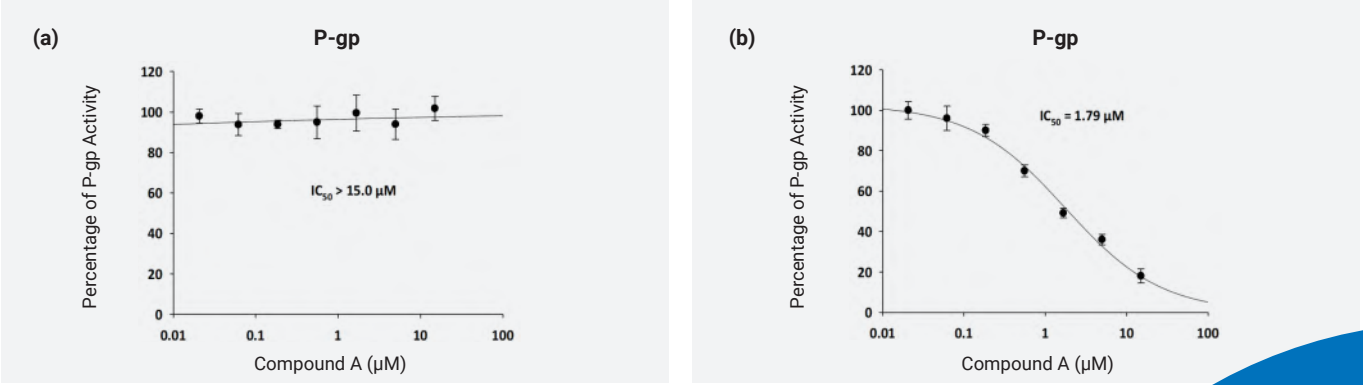

Fig. 5. Comparison of the P-gp inhibition assessment for a low permeable compound in MDR1-MDCK II cell model (a) and P-gp vesicle model (b)

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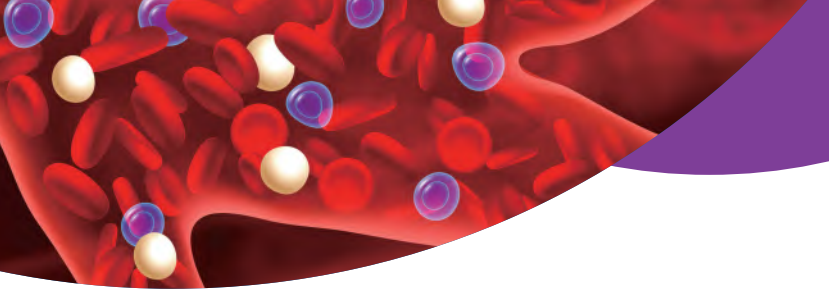


Permeability & Transporter Services

Part III: SLC Transporters

Highlights

- Substrate and inhibition evaluation as well as K_m and V_{max} determination
- Hepatic uptake and hepatic uptake clearance assessment in cryopreserved hepatocytes from different species
- Customized approaches to improve solubility and reduce non-specific binding
- Differentiated protocols for discovery and IND filing

Introduction

SLC transporters are found in the small intestine, liver, kidney, and other tissues. They play important roles in the uptake of various drugs into cells, as well as endogenous nutrients, and thus may influence the absorption, distribution, metabolism, and excretion (ADME) properties of drugs. The interaction of a drug with SLC transporters as a substrate or inhibitor may result in drug interactions and hence impact its efficacy, toxicity, or both.

Available Models

The SLC transporters recommended by FDA, EMA, NMPA, PMDA, and ICH are all available in WuXi AppTec DMPK as shown in the following table:

Transporter	Models	Agency Requirement
OATP1B1	HEK293-OATP1B1	FDA, EMA, NMPA, PMDA, and ICH
OATP1B3	HEK293-OATP1B3	
OAT1	HEK293-OAT1	
OAT3	HEK293-OAT3	
OCT2	HEK293-OCT2	
MATE1	HEK293-MATE1	
MATE2-K	HEK293-MATE2-K	
OATP2B1	HEK293-OATP2B1	ICH
OCT1	HEK293-OCT1	EMA, PMDA, and ICH
PEPT1	HEK293-PEPT1	Not mentioned
PEPT2	HEK293-PEPT2	
NTCP	HEK293-NTCP	

Validation Data

NTCP Uptake and Inhibition Assessment

NTCP is one of the major transporters mediating drug-induced liver injury (DILI), and it is also identified as the receptor responsible for the cellular entry of HBV and HDV. WuXi AppTec DMPK validated NTCP substrate and inhibition assays using the probe substrate deuterated sodium thiocholate (TCA-d4) and the reference inhibitor cyclosporin A (CSA) (Figs. 6 and 7).

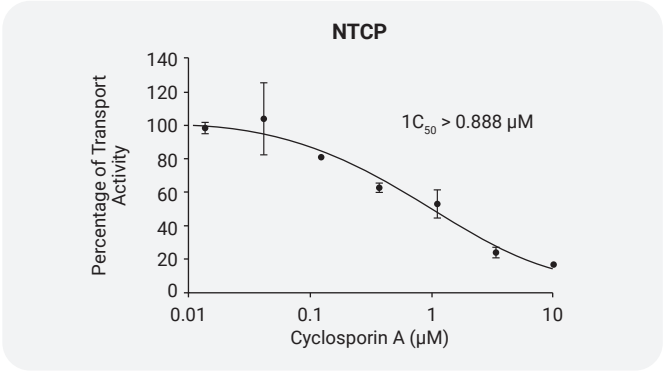


Fig. 6. NTCP mediated TCA-d4 uptake

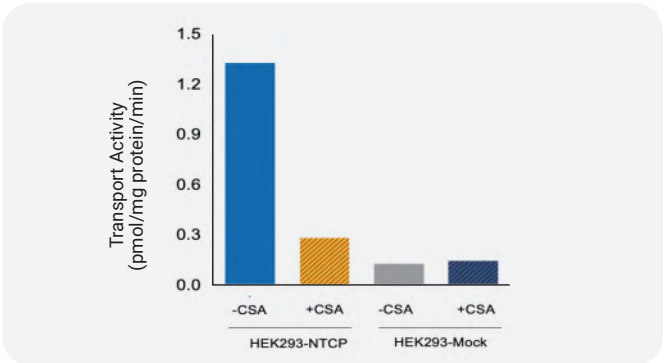


Fig. 7. TCA-d4 uptake in HEK293-NTCP and HEK293-MOCK cells

Hepatic Uptake Clearance Assessment

Based on the extended clearance concept, it has been reported that the hepatic clearance mediated by OATP transporters can be predicted reasonably well regardless of the involvement of metabolism and lipophilicity of test compounds, and the direct extrapolation from intrinsic hepatic uptake clearance could significantly improve the overall prediction of hepatic clearance. WuXi AppTec DMPK has established a hepatic uptake clearance assessment assay by using an oil-spin method in cryopreserved suspension hepatocytes. Pravastatin and cerivastatin were used as low and high-uptake clearance controls, respectively (Figs. 8 and 9).

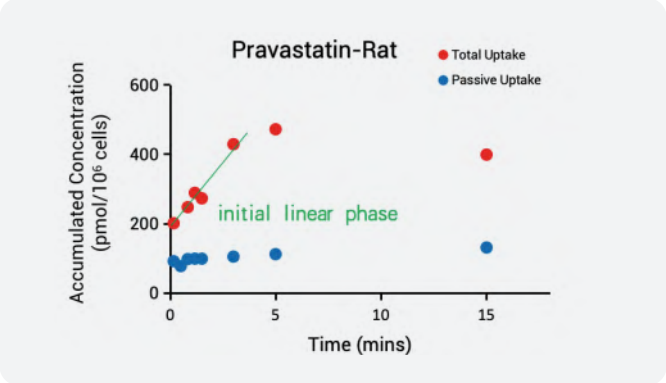


Fig. 8. Pravastatin uptake clearance in rat hepatocytes

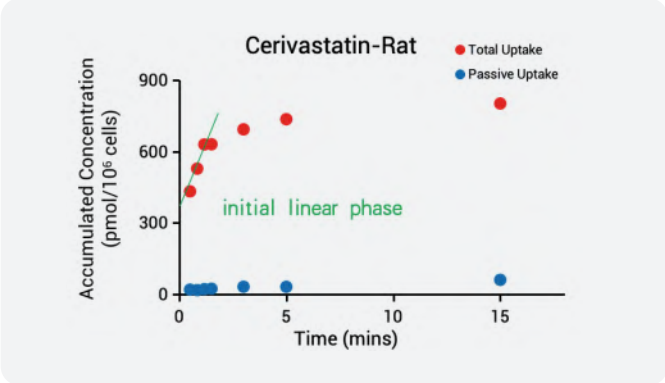
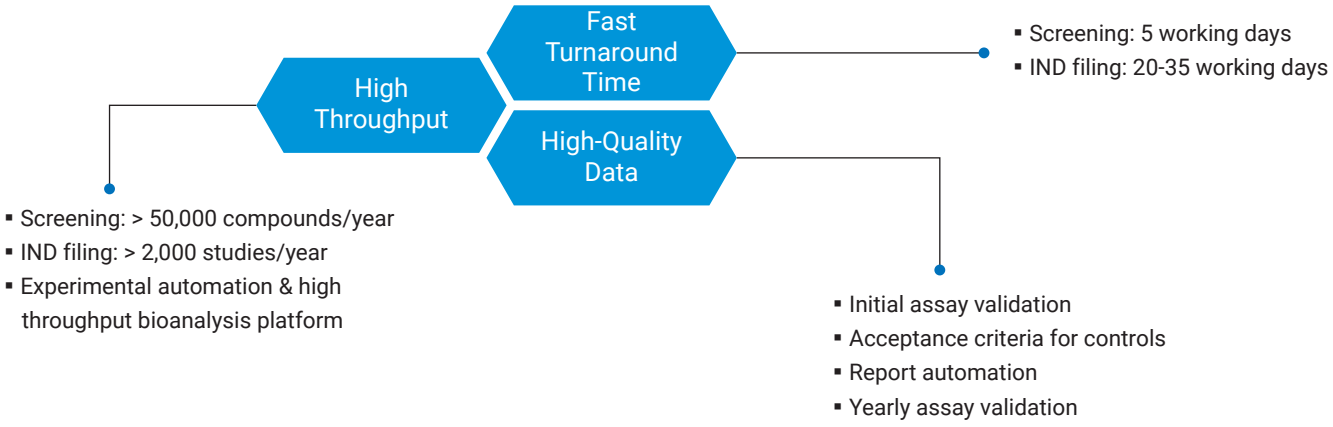


Fig. 9. Cerivastatin uptake clearance in rat hepatocytes

State-of-the-Art Platform



Compound Requirements

- Discovery studies: 20 µL of 10 mM stock per assay
- IND-filing studies: 10-20 mg powder per assay or 80 mg powder for an IND package with substrate and inhibition assays for the 9 transporters

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