


The Suitability of the In situ Perfusion Method for BCS Permeability Classification

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Outline

- Permeability – Definitions and Models
 - In Situ Single Pass Perfusion
 - Description
 - Model compounds
 - Confidence Interval Analysis
 - Permeability Characterization for Drug Development – Description and Examples
 - Positional
 - Mesenteric
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Definition of High Permeability


..... a drug substance is considered to be *highly permeable* when the extent of absorption in humans (Fraction Absorbed) is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose¹.

¹*BCS Guidance, August 2000*

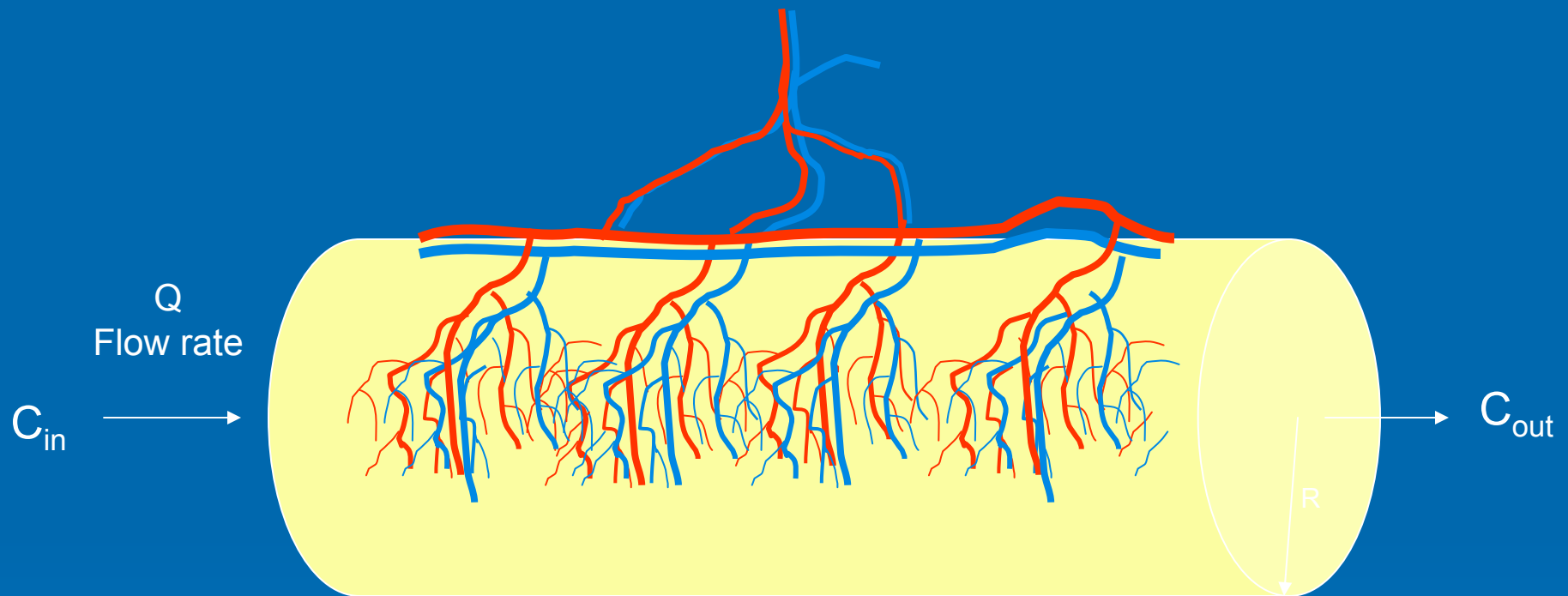
BCS Guidance..... Suggested Methods for Determining Drug Substance Permeability Class

- Clinical
 - Mass balance
 - Absolute BA
 - Intestinal perfusion.
- Non clinical
 - Monolayers of suitable epithelial cells.
 - In vivo or in situ intestinal perfusion in a suitable animal model (e.g., rats)
 - In vitro permeability methods using excised intestinal tissues

Commonly Used Permeability Models

- Caco-2 cell cultures
 - In situ perfusion
 - Excised Tissue models (Ussing)
 - Artificial Membrane (PAMPA)
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In Situ Single Pass Perfusion



- Intact Rat Intestine – blood flow remains intact throughout experiment
- Good Correlation with Human Permeability

In Situ Perfusion - Mass Balance



- $(QC)_z - (QC)_{z+dz} = (2\pi R dz) P_{eff} (C)_z$

- $P_{eff} \text{ (cm/sec)} = \frac{Q \ln (C'_{out}/C'_{in})}{2 \pi R L}$

In situ Perfusion

- Fasted Rats
- Cannulated Jejunal Intestinal Segment (~ 10 cm)
- Range of Concentrations
 - 1x (highest dose strength/250 mls), 0.1X and 0.01X
 - Solubility limited
- Co-perfuse with:
 - C-14 PEG 4000 (water transport marker)
 - High Permeability Internal Standard (e.g., metoprolol)
 - Low Permeability Internal Standard (e.g., atenolol, ranitidine)
- Measure C_{out} and C_{in} over a 60 to 90 minute time course
- Performed under GLP conditions

In Situ Single Pass Perfusion

- Water Transport (WT)

$$WT = \frac{(A_{in} - A_o) - (A_{out} - A_o)}{A_{out} - A_o} \times 100$$

- Corrected C'_{out}/C'_{in}

$$\frac{C'_{out}}{C'_{in}} = \frac{C_{out}}{C_{in}} \times \frac{A_{in} - A_o}{A_{out} - A_o}$$

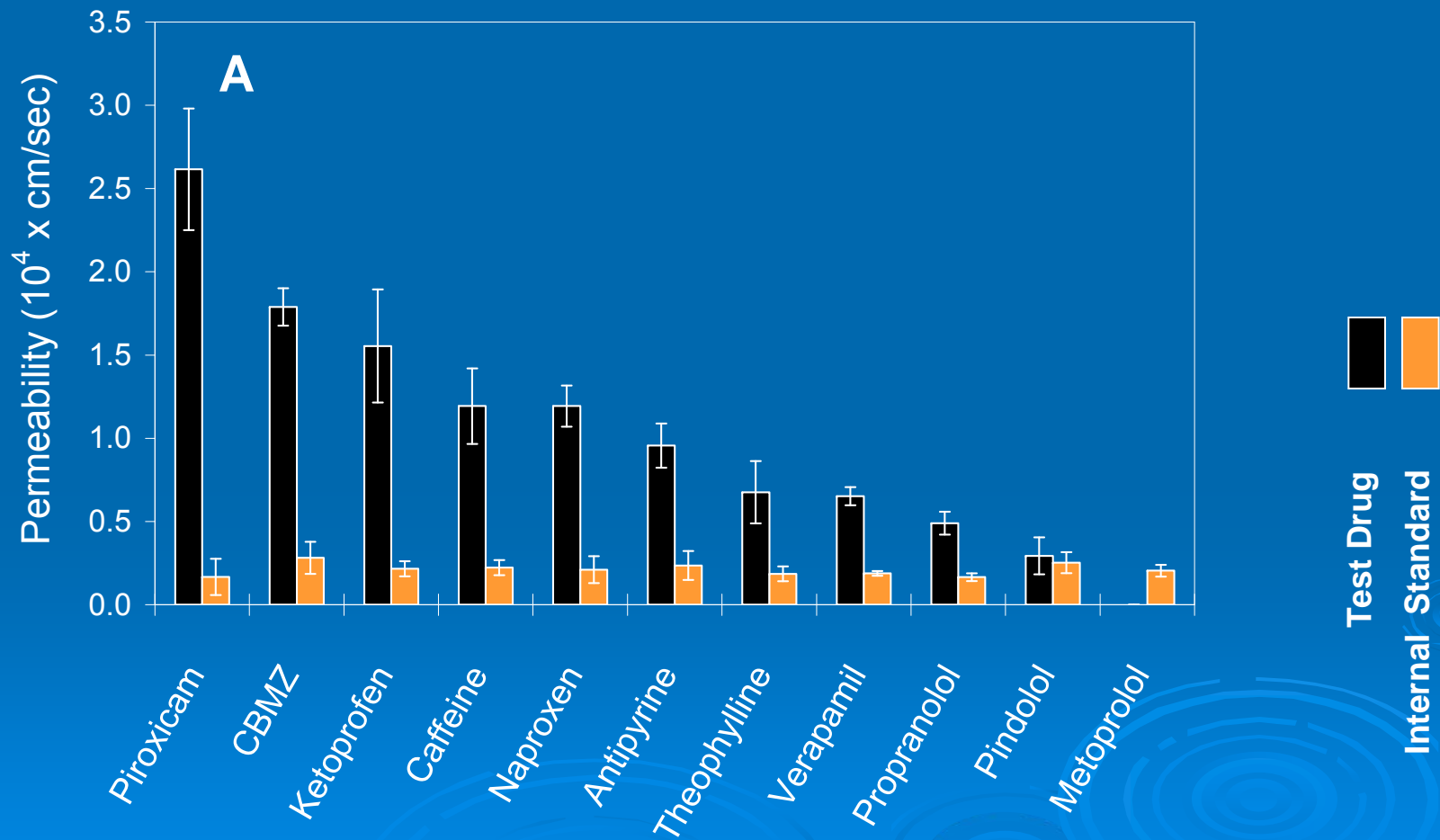
- Calculation of effective permeability (P_{eff})

$$P_{eff} \text{ (cm/sec)} = -\frac{Q \ln\left(\frac{C'_{out}}{C'_{in}}\right)}{2 * 60 * \pi RL}$$

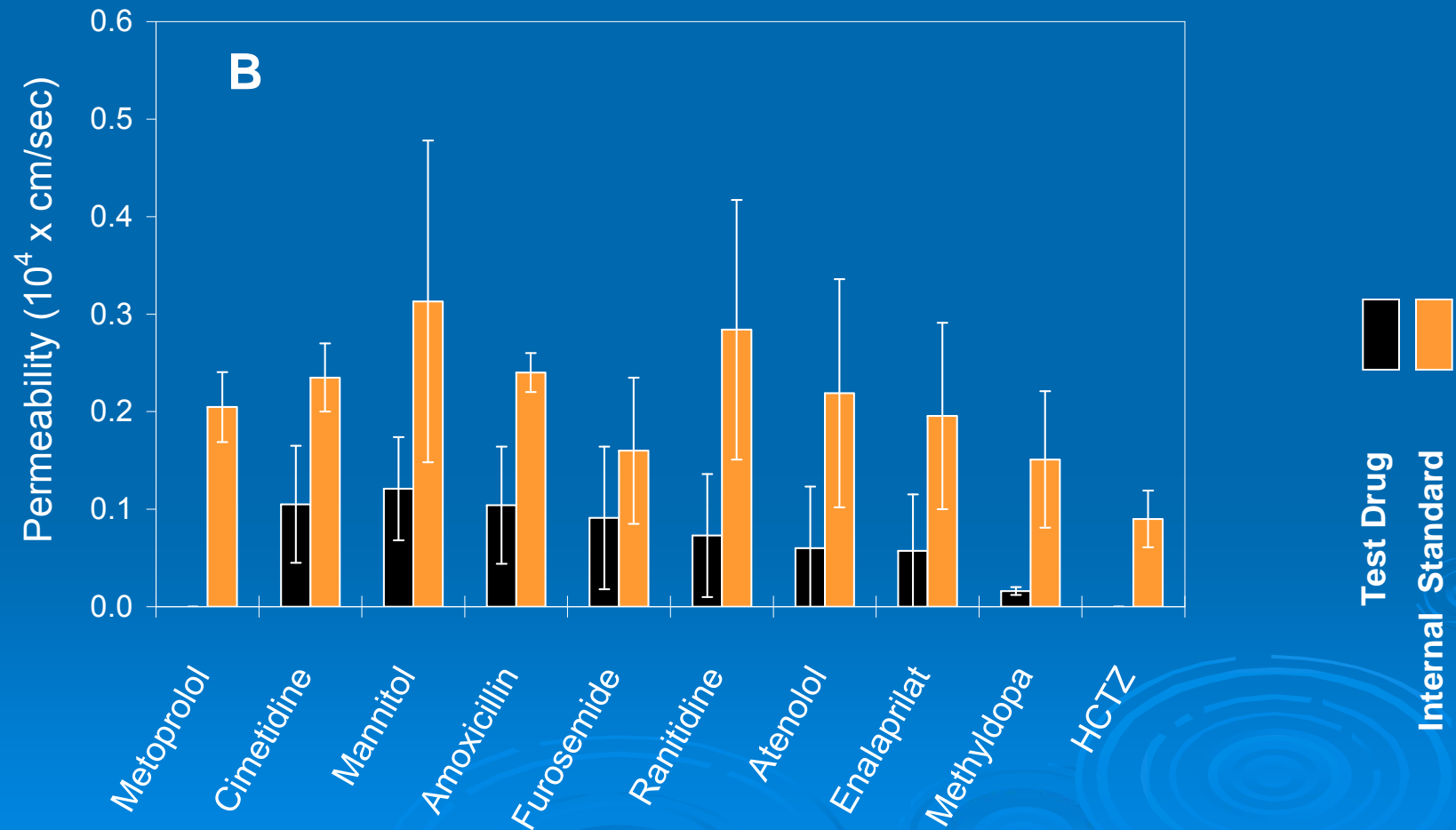
- Determine T/R permeability ratio

$$P_{eff - \text{Test}} / P_{eff - \text{metoprolol}}$$

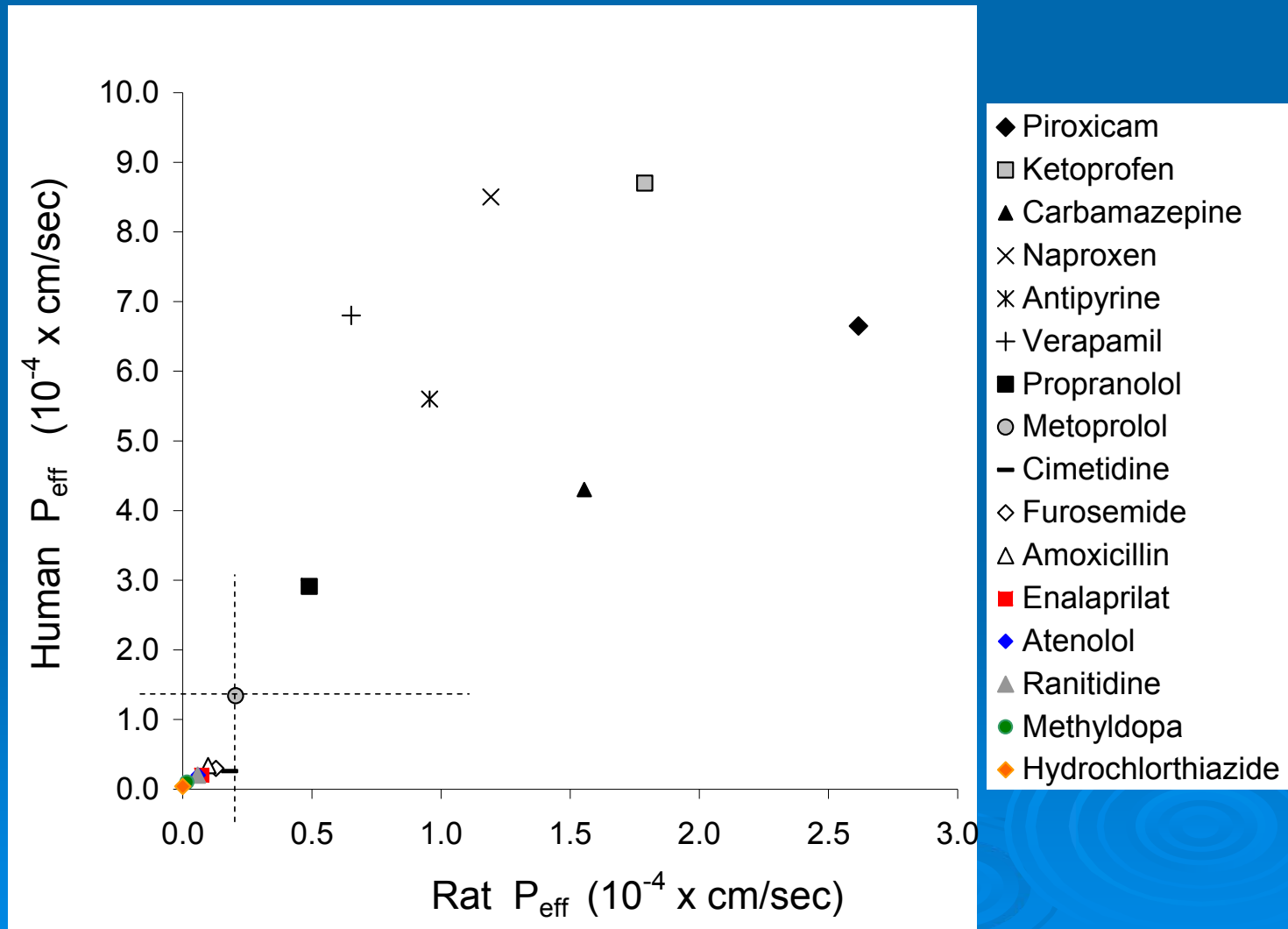
High Permeability Compounds



Low Permeability Compounds



Rat versus Human Permeability



Comparison of Human P_{eff} and %FA with Rat P_{eff}

Compounds (Permeability Class)	Dose (mg)	Human Permeability ^a (10^{-4} cm/sec)	Fraction Absorbed ^a %	Rat Permeability (10^{-4} cm/sec)	Rat P_{eff} Ratio Test/IS
Piroxicam (H)	20	10.4 ± 5.9	100	2.62 ± 0.37	22.9
Ketoprofen (H)	75	8.4 ± 3.3	100	1.55 ± 0.34	7.9
Carbamazepine (H)	200	4.3 ± 2.7	100	1.79 ± 0.11	7.1
Naproxen (H)	500	8.3 ± 4.8	100	1.19 ± 0.12	10.1
Caffeine (H)	200	nd	100	1.19 ± 0.23	5.5
Antipyrine (H)	188	5.6 ± 1.6	100	0.96 ± 0.13	4.5
Theophylline (H)	300	nd	96	0.68 ± 0.19	3.8
Verapamil ^b (H)	120	6.7 ± 2.9	100	0.65 ± 0.05	3.9
Propranolol (H)	80	2.8 ± 1.3	100	0.49 ± 0.07	3.2
Pindolol (H)	10	nd	89	0.293 ± 0.11	1.2
Metoprolol (H)	100	1.5 ± 0.9	96	0.20 ± 0.04	1.0
Furosemide (L)	80	0.3 ± 0.3	61	0.117 ± 0.08	0.74
Amoxicillin ^{b, c} (L)	875	0.3	45-75	0.120 ± 0.11	0.50
Cimetidine (L)	800	0.3 ± 0.05	60	0.105 ± 0.06	0.44
Enalaprilat (L)	20	0.1 ± 0.3	8	0.057 ± 0.06	0.41
Mannitol (L)	-	nd	16	0.121 ± 0.05	0.39
Ranitidine (L)	10	0.2 ± 0.06	50	0.073 ± 0.06	0.29
Atenolol (L)	100	0.2 ± 0.2	50	0.060 ± 0.06	0.06
Methyldopa (L)	500	0.1	45	0.016 ± 0.004	0.10
Hydrochlorothiazide (L)	50	0.04 ± 0.05	67	0.001 ± 0.001	0.002

Borderline Permeability

- High Permeability – T/R permeability ratio > 1
- Workable for high permeability drugs
 - Piroxicam (22.9)
 - Ketoprofen (7.9)
 - Naproxen (7.1)
- Borderline (T/R permeability ratio \sim or < 1) problematic
 - Variability
 - Propose Confidence Interval Analysis

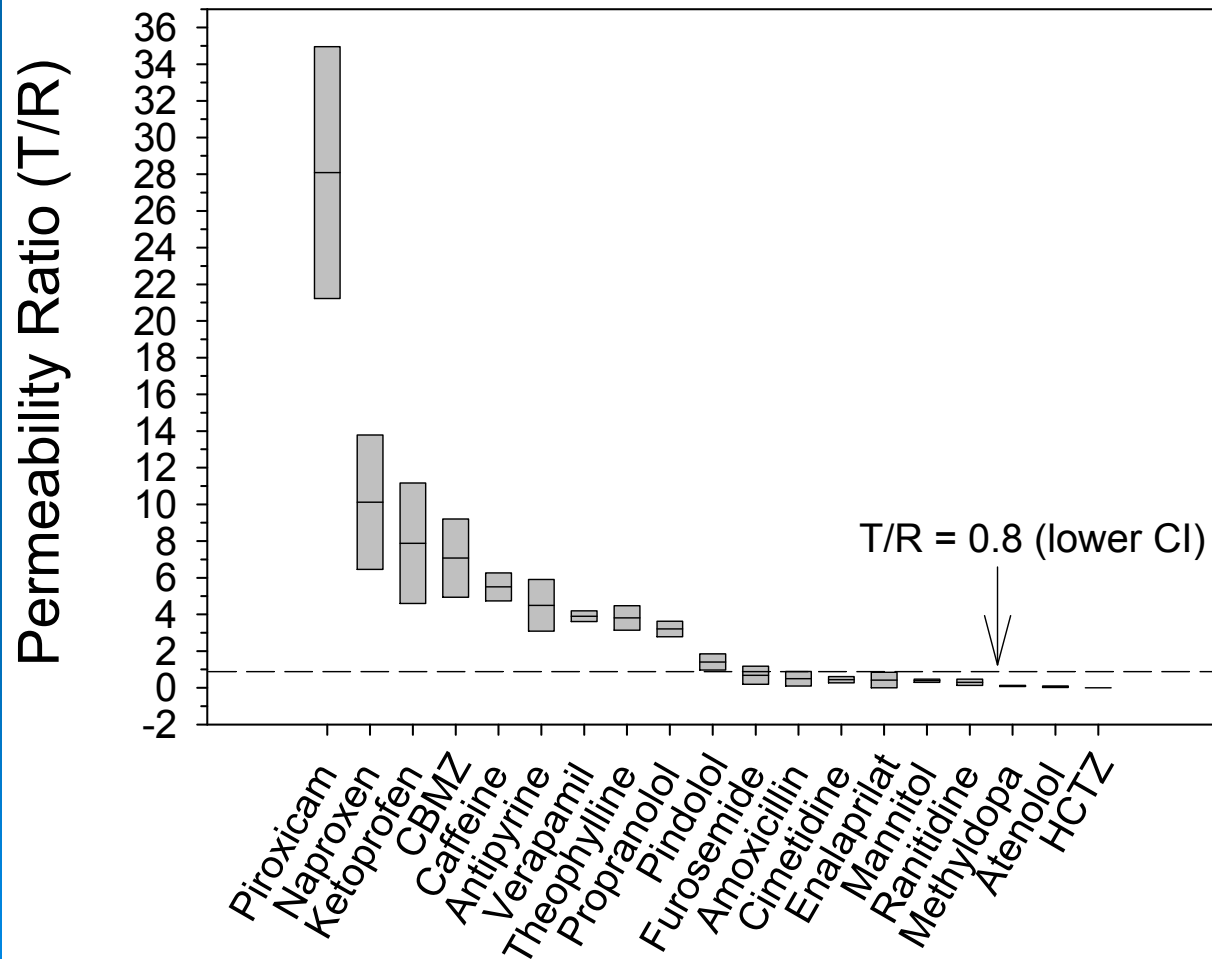
Current Acceptance Criteria for *In Vitro* and *In Vivo* Bioequivalence Studies

Study Type	Parameters	Acceptance Criteria
In Vitro BE	Langmuir Isotherms, k_1 , k_2 (Bile Acid Sequestrant)	$0.8 < 90\%CI \text{ of } k_2 < 1.2$ $0.8 < k_1 \text{ ratio} < 1.2$
In Vivo BE	C_{max} , AUC	$0.8 < 90\%CI < 1.25$

Proposed In situ BE.

In Situ BE (BCS Permeability)	Permeability Ratio	$0.8 < \text{Lower Limit of } 90\% \text{ CI}$ (suggested for “borderline” permeability drugs)
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Confidence Interval Analysis



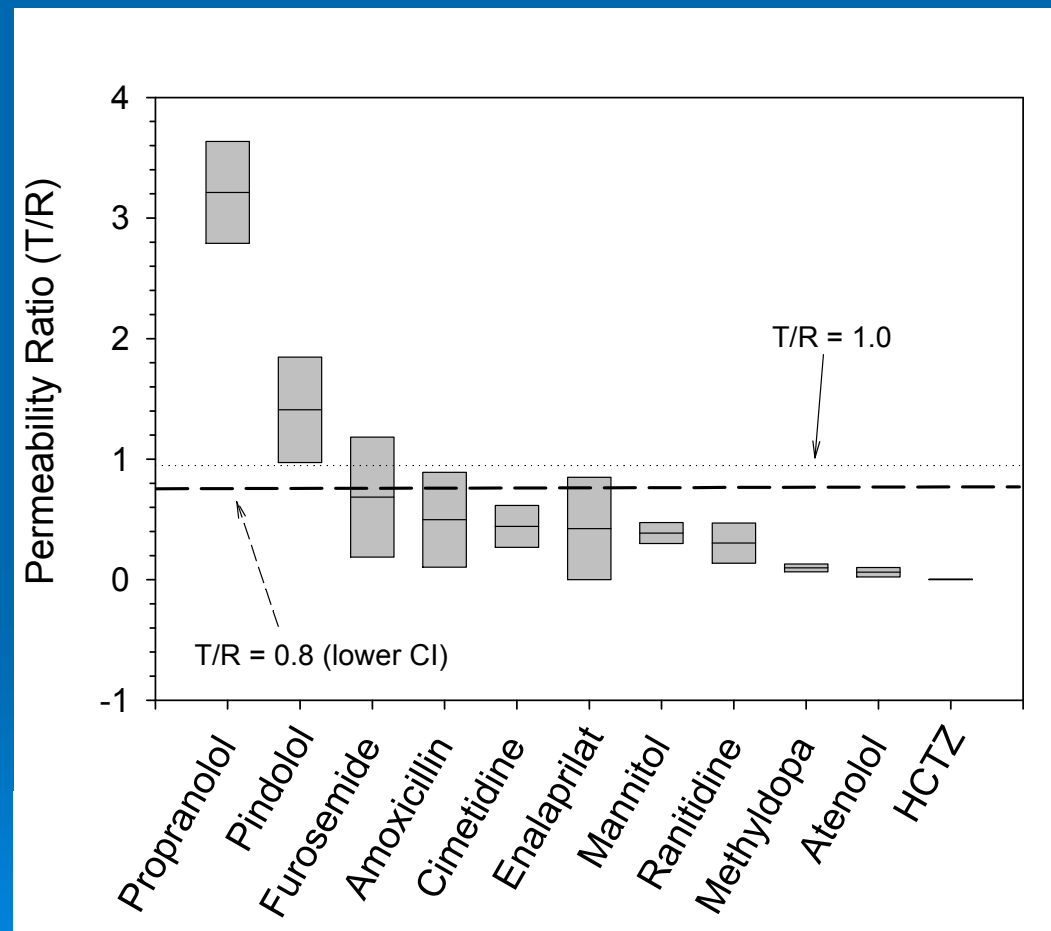
$$\bar{x} \pm 1.645 \left(\frac{\sigma}{\sqrt{n}} \right)$$

\bar{x} = mean permeability ratio

σ = standard deviation

n = sample size

Confidence Interval Analysis... Low Permeability Drugs



Confidence Interval Analysis.....estimating sample size

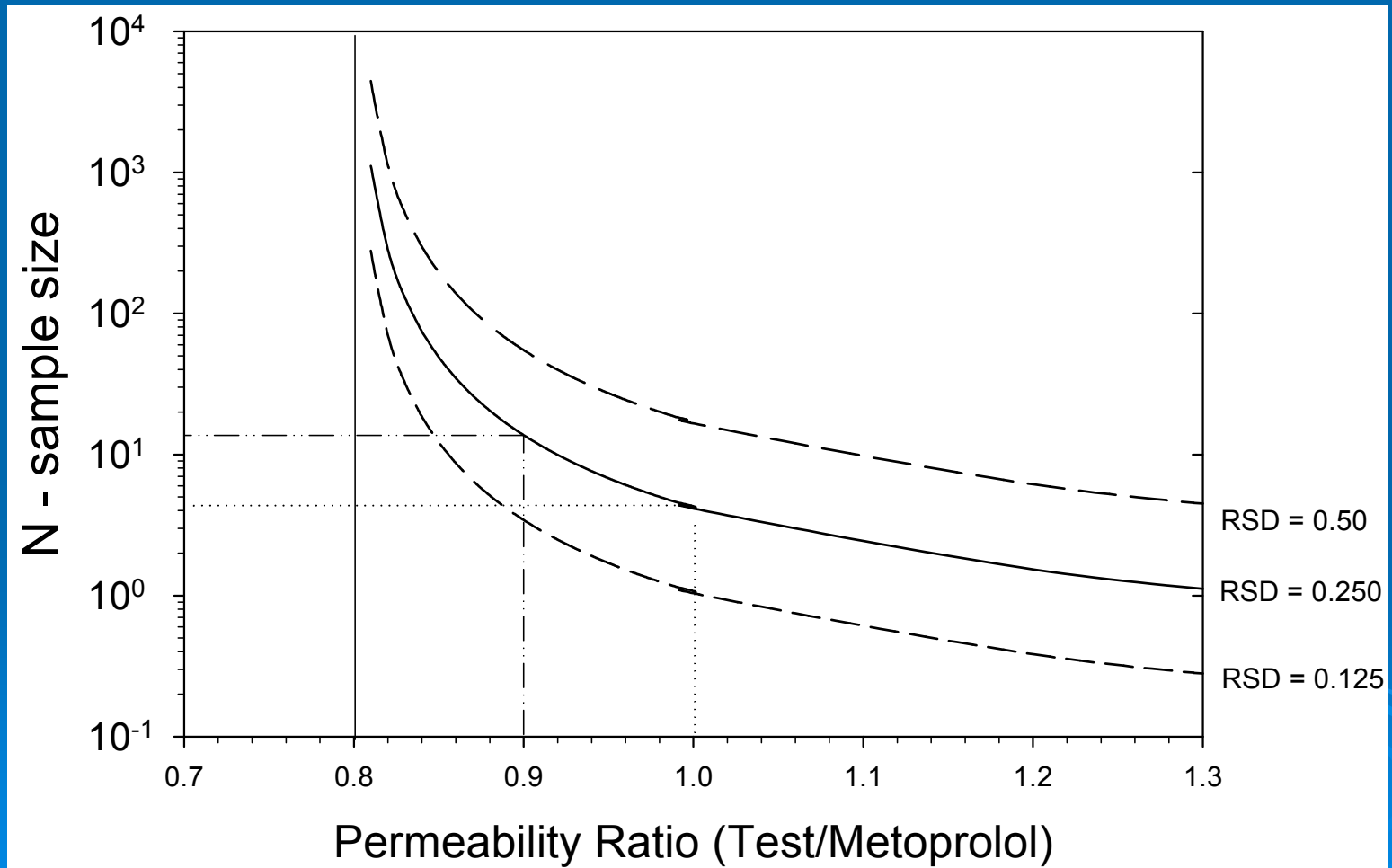
$$n = \left[z_c \times \sigma \times \frac{P_{\text{mean}}}{(P_{\text{mean}} - 0.8)} \right]^2$$

P_{mean} = mean of the permeability ratio

σ = relative standard deviation (RSD) obtained from in situ perfusion study using four rats

z_c = 1.645 - the confidence coefficient at 90% level.

Confidence Interval Analysis..... Estimate of Sample Size



In Situ Perfusion - Bioequivalence

- Allows for High Permeability Classification of a drug when test $P_{\text{eff}} < \text{IS } P_{\text{eff}}$
 - Controlled Variability
 - Balance of Costs
- The 90% Fraction Absorbed (FA) level for a Class 1 drug is Conservative.
 - 80% - 85% FA may be more appropriate.
 - Reduction in the FA level ~ Reduction in ratio of test/ref Permeability ratio.
 - Metoprolol FA = 96%

FA, %	Mean P_{eff} ratio	CI lower limit
90	0.94 (90/96)	0.71
85	0.88 (85/96)	0.67
80	0.83 (80/96)	0.63

Summary

- Rat single pass perfusion method accurately categorizes the selected reference drugs determined in human experiments.
- Suitable for classifying drugs into their appropriate BCS permeability class.
- Performed under GLP conditions
- Proposed “In situ bioequivalence” criteria allows for a regulatory relevant and robust means to assess drug permeability.

Value of BCS Assessments

➤ Discovery

- Series Characterization – Oral Delivery Potential
- Series Selection

➤ Lead Selection

- Best candidate
- Delivery Strategy – formulation considerations

➤ Clinical Development

- NDA
 - Complement / strengthen mass-balance data
 - SUPAC – BA/BE biowaivers
- ANDA – BA/BE biowaivers

Mechanistic Studies_ Example 1

➤ Background

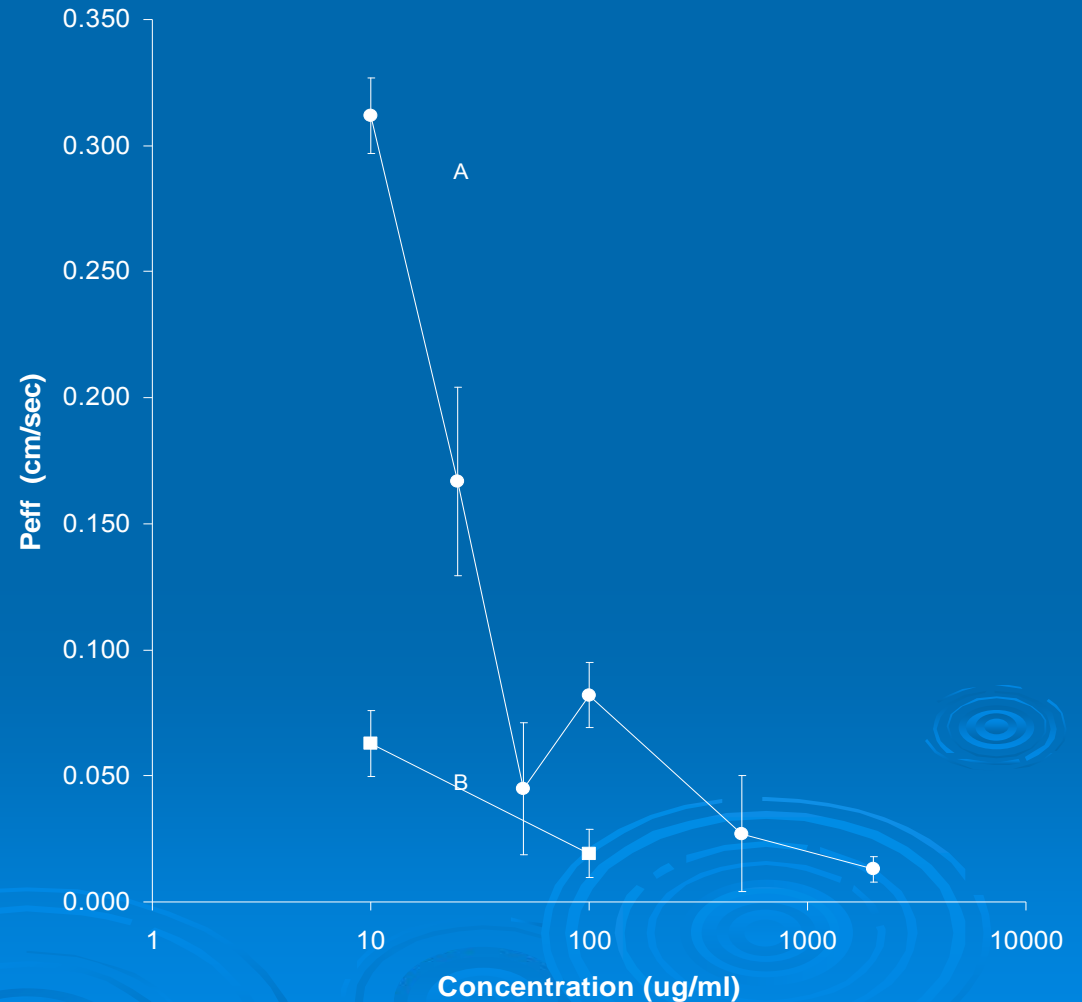
- Anti-angiogenesis series of compounds
- Plasma $t_{1/2} > 130$ hrs
- Bioavailability $\sim 15\%$

➤ In situ perfusion studies

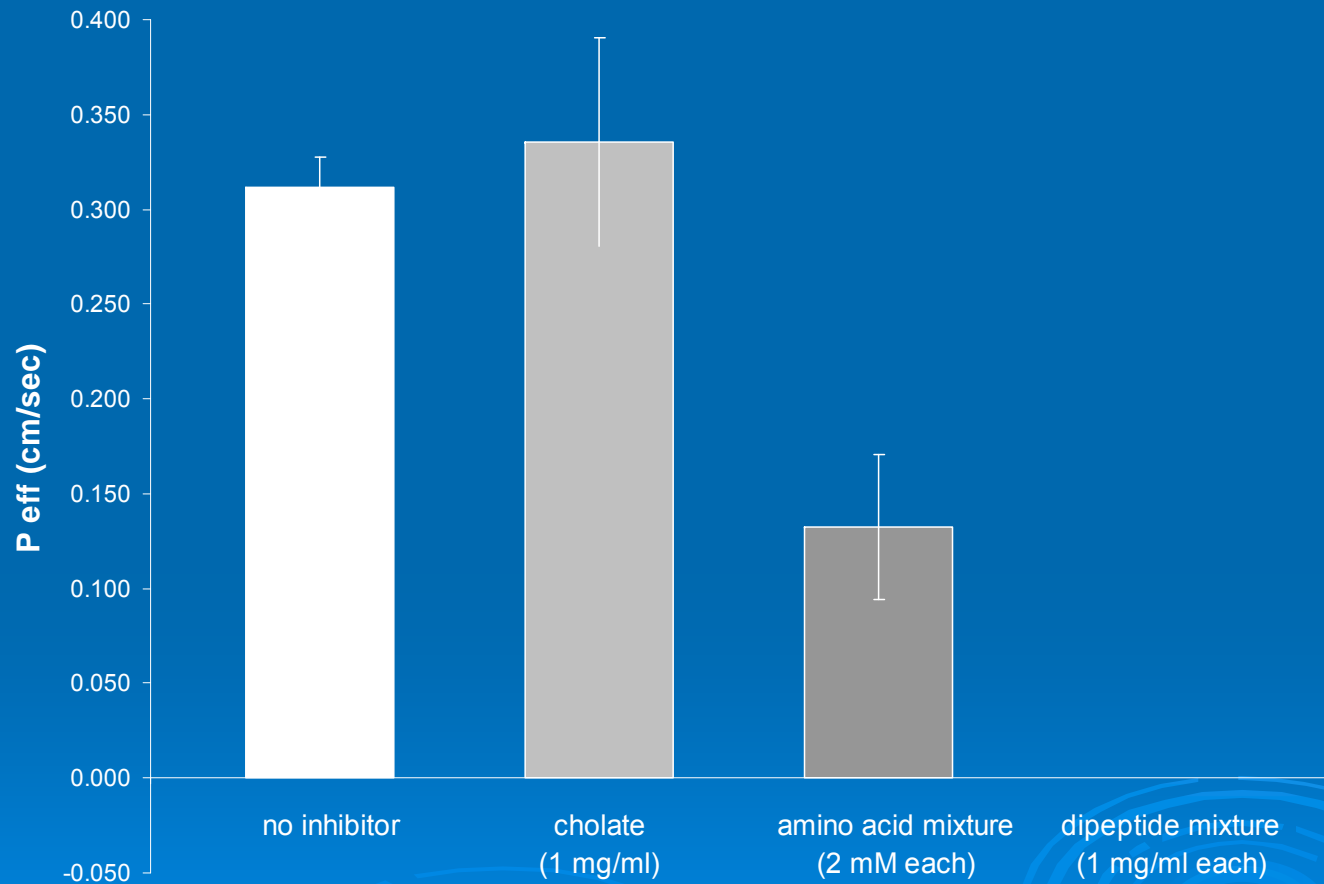
- Concentration
- Inhibitor studies
- Determined permeability rank order of series

Concentration Dependence

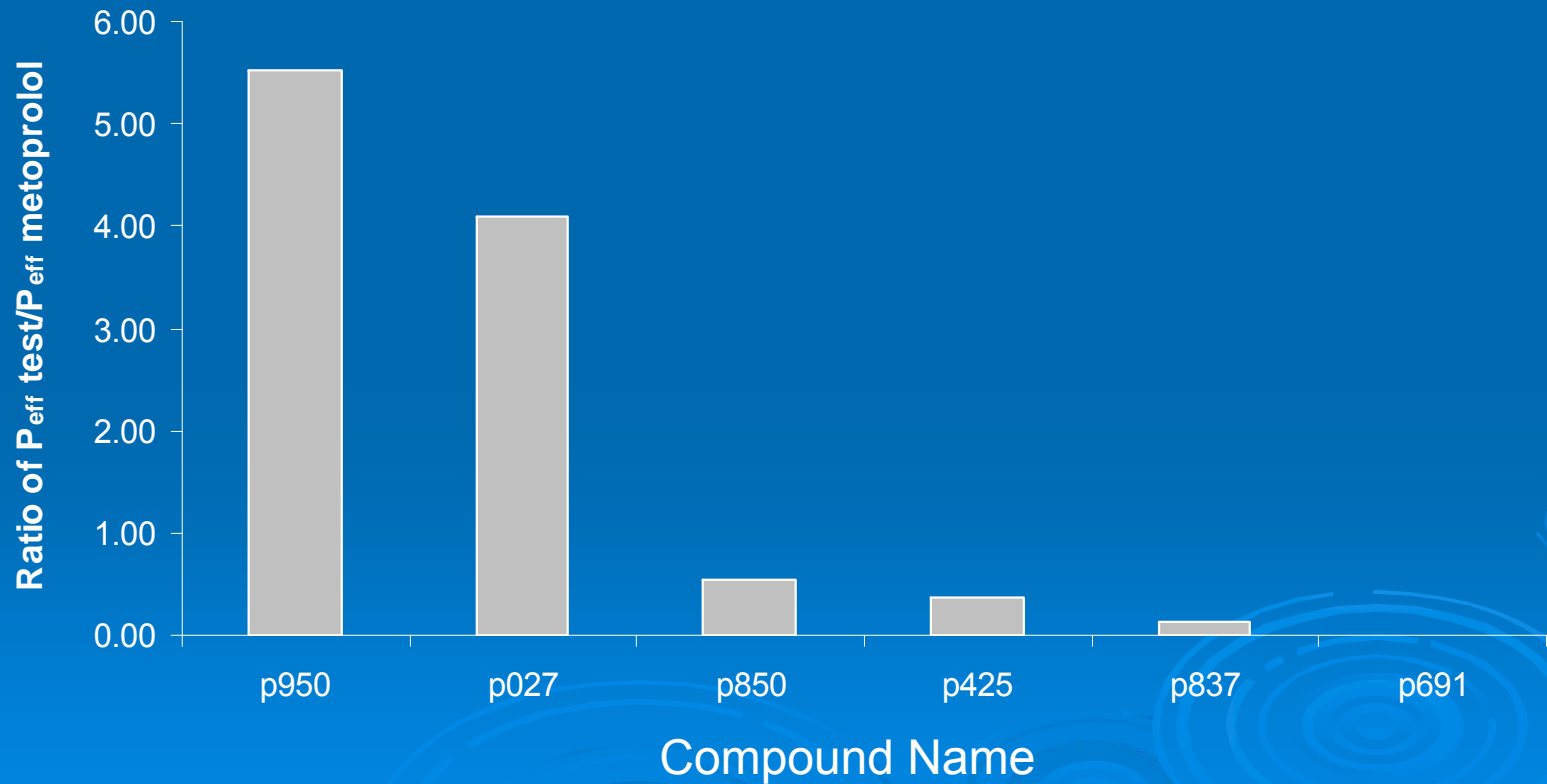
- Ratio P_{eff} test/ref. >1 at low concentration of test drug
- Ratio P_{eff} test/ref. <1 at high concentration of test drug
- Results suggest Carrier-mediated transport.



Inhibitor Studies



Rank Order of Compounds



Example 2

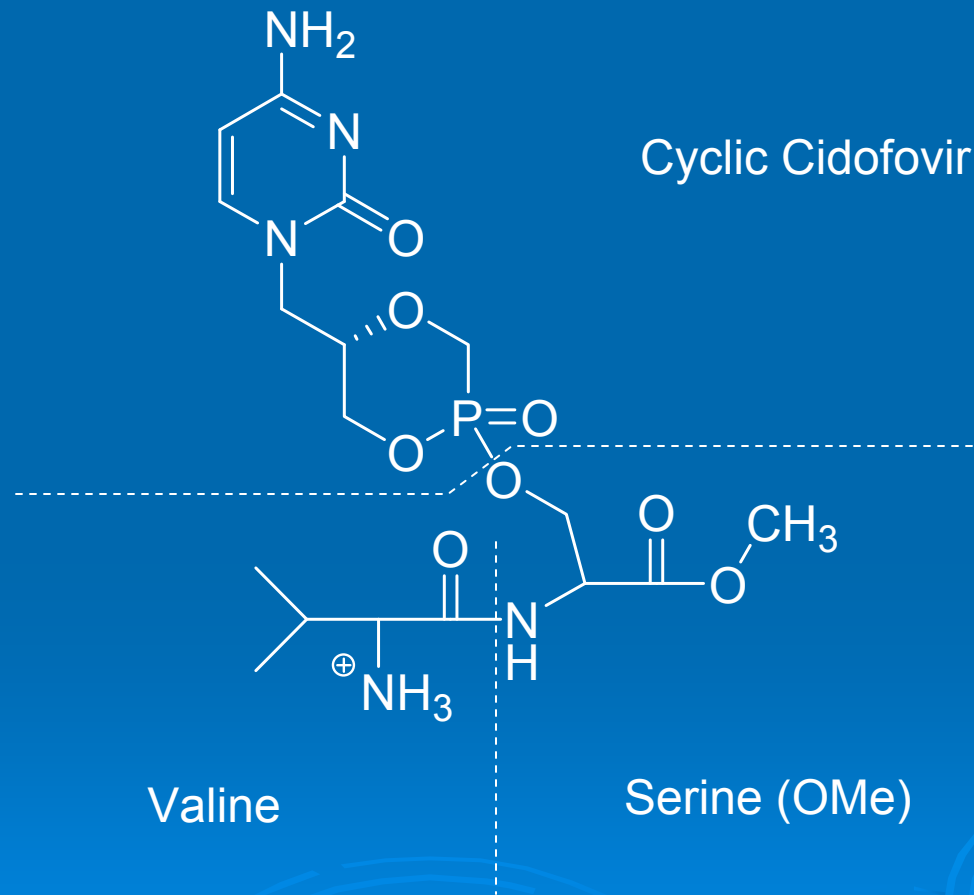
➤ Background

- Prodrugs of antiviral compounds
- Bioavailability of parent – 2%
- Prodrug approach to enhance bioavailability

➤ Studies

- Permeability Studies
 - Caco-2
 - In situ perfusion
 - In situ perfusion with Mesenteric sampling
- Activation of prodrug
- Determine absorption potential of prodrugs

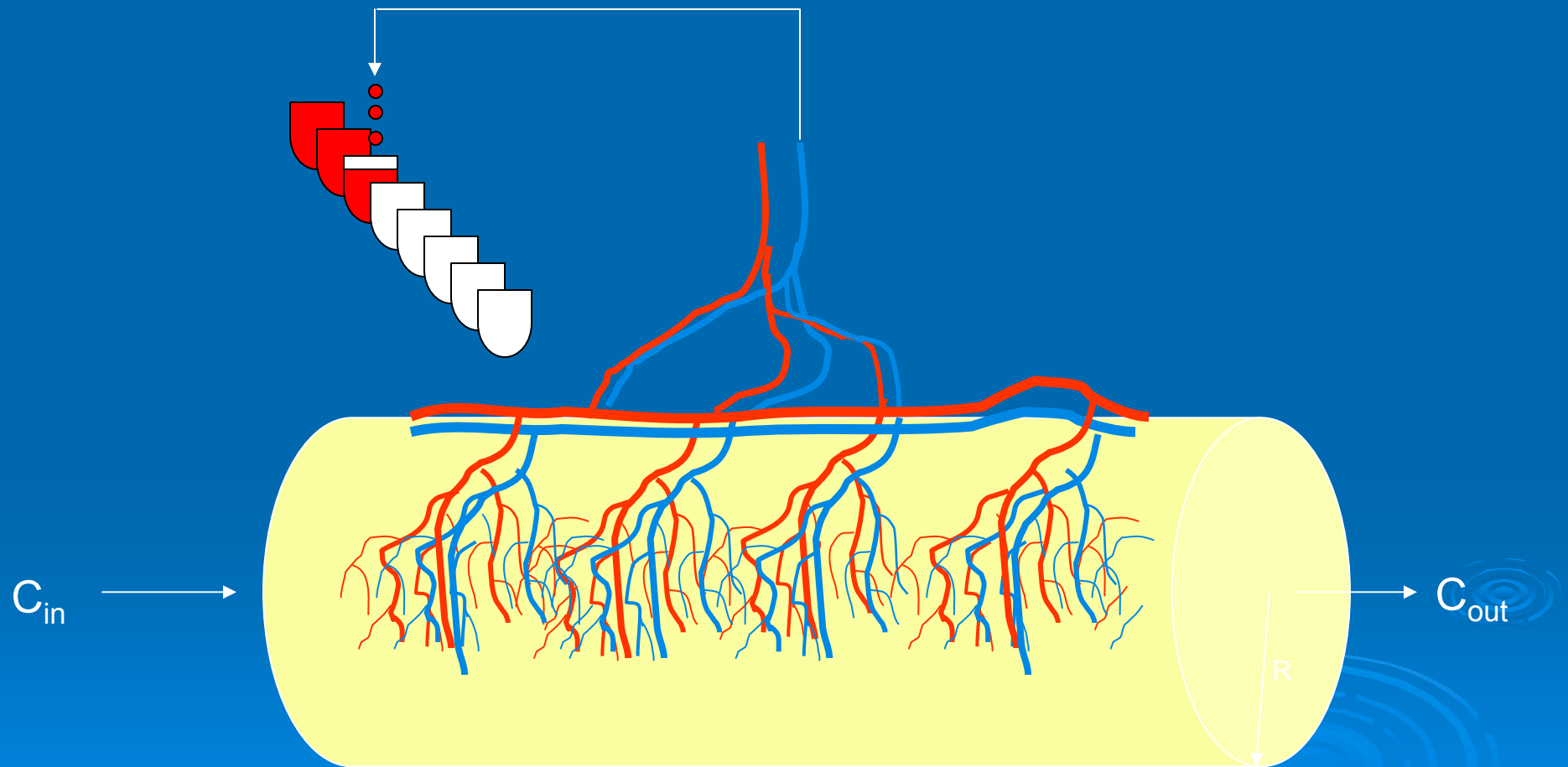
Prodrugs of Cyclic Cidofovir



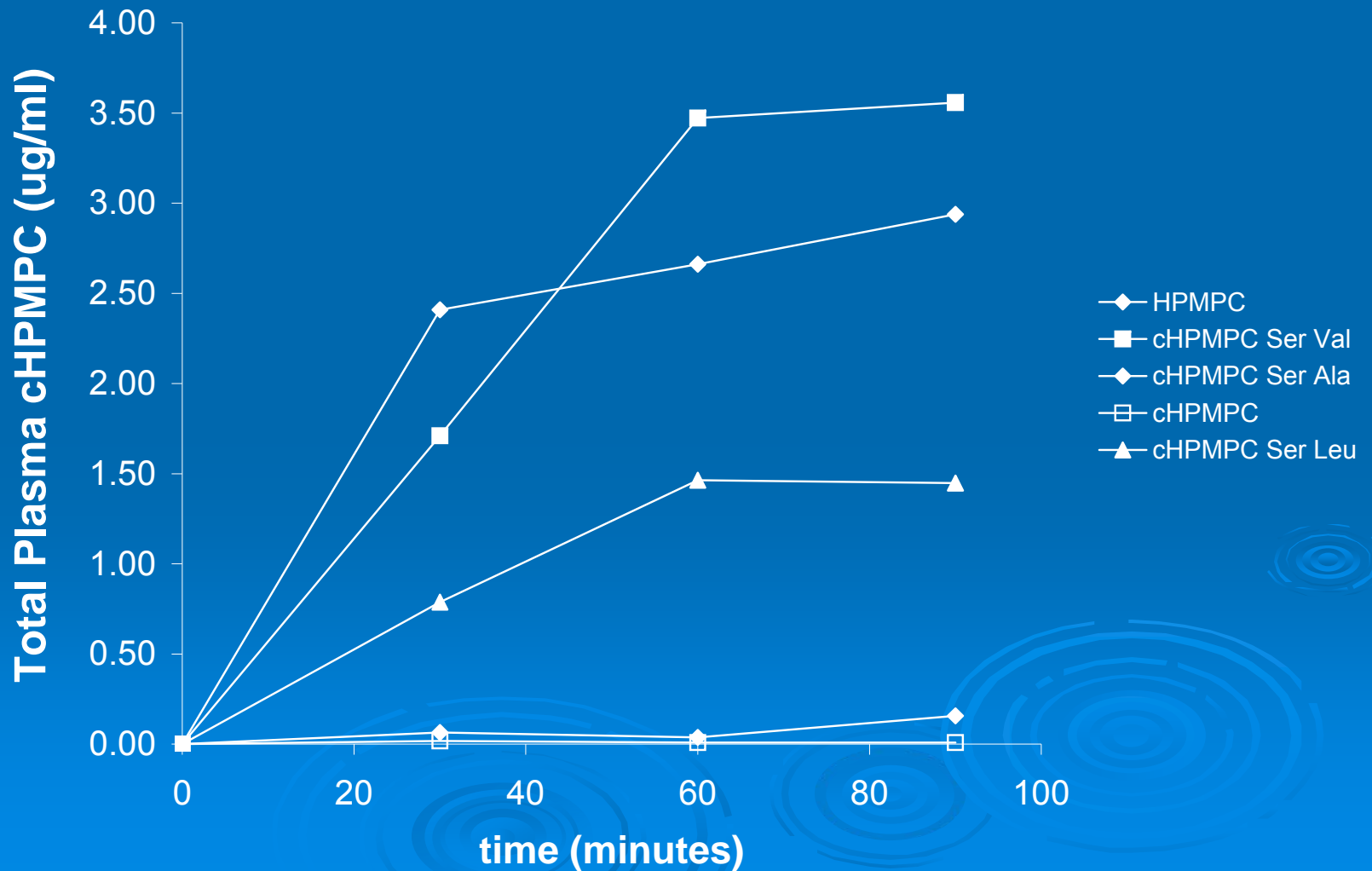
Uptake, Permeability, Absorption Studies of prodrugs cCDV

Antiviral Prodrugs	<i>Caco-2</i> Permeability Cm/sec x 10 ⁴	<i>In Situ</i> Permeability P _{eff} (cm/sec x 10 ⁴)
Cidofovir (C1)	0.001	0.000
Ser-Val cHPMPC	0.0005	High (unstable)
Ser-Ala cHPMPC	0.0007	High (unstable)
cHPMPC	N.D.	N.D.

Mesenteric Sampling during the In Situ Single Pass Perfusion



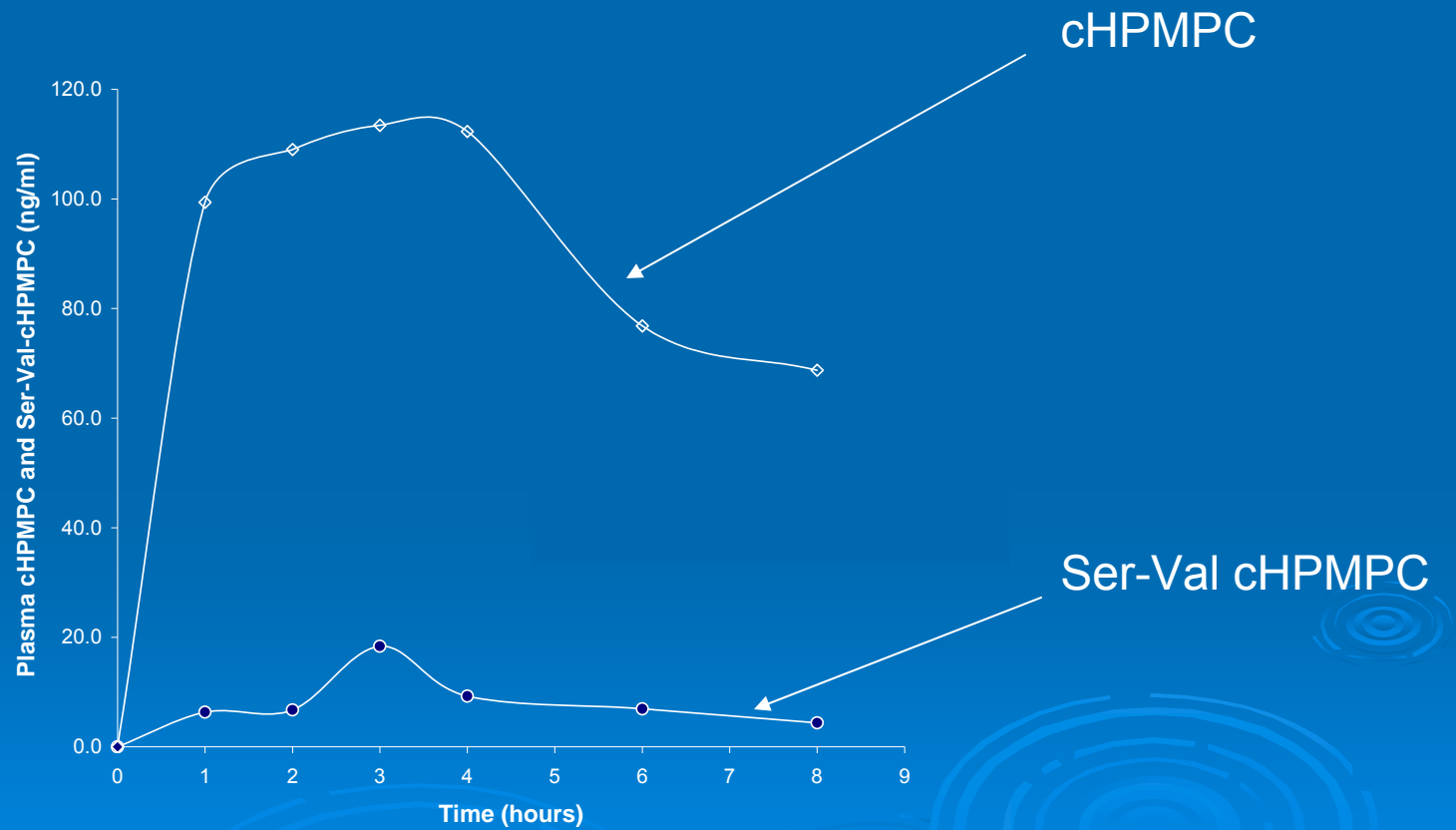
In situ Perfusion with Mesenteric Plasma Sampling



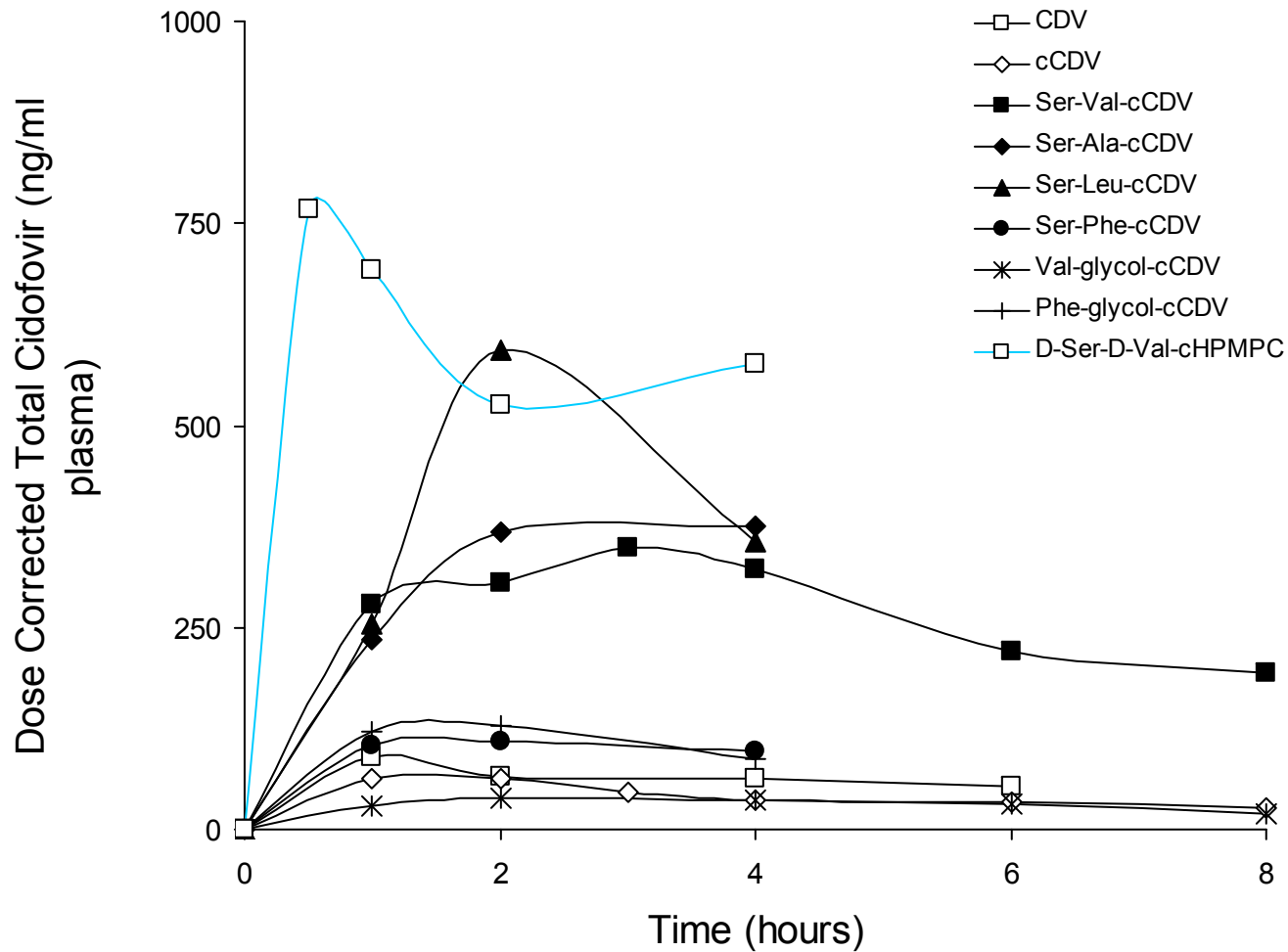
Uptake, Permeability, Absorption Studies of prodrugs cCDV

Antiviral Prodrugs	<i>Caco-2</i> Permeability Cm/sec x 10 ⁴	<i>In Situ</i> Permeability P _{eff} (cm/sec x 10 ⁴)	Mesenteric permeability P _{eff- mes} (cm/sec x 10 ⁵)
Cidofovir (C1)	0.001	0.000	0.052
Ser-Val cHPMPC	0.0005	High (unstable)	1.18
Ser-Ala cHPMPC	0.0007	High (unstable)	0.98
cHPMPC	N.D.	N.D.	0.003

Conversion of Prodrug



Absorption Potential of Cyclic Cidofovir Prodrugs



Summary

- Single pass perfusion method
 - BCS classification – GLP conditions, Biowaiver
 - Transport mechanism studies
 - Flexible system
- Robust and Flexible Methodology
 - Discovery
 - Oral Delivery Potential
 - Rank order/Series Selection
 - Preclinical Development
 - Candidate Selection
 - Delivery Strategy – formulation considerations
 - Clinical Development
 - Complement / strengthen mass-balance data
 - Bioequivalence waivers