U.S. FOOD & DRUG ADMINISTRATION

M-CERSI Workshop

Drug Permeability: Best Practices for BCS-based Biowaivers

Method Suitability of Caco-2 Cell Models for Drug Permeability Classification

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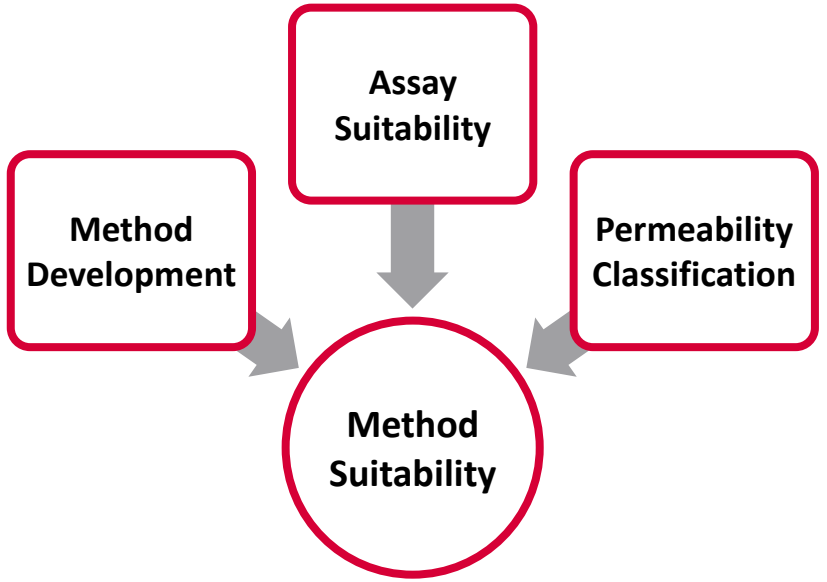
Outline

- Method Suitability
- Model Drugs
- Reference Standards
- Acceptance Criteria
- Use of Permeability Assay



Method Suitability

- Generalized approach to standardize and validate a drug permeability model
- Establishes a correlation between experimental permeability values and human intestinal absorption (f_a)
- Allows for the use of different permeability assays within a laboratory whether they involve human studies, intact animals, intestinal tissue, or epithelial cells
- Accounts for intralaboratory variability
- Relies on assay standardization and validation, reference standards, and acceptance criteria to improve the consistency of experimental data to predict a drug's intestinal permeability

Volpe AAPS J. 2010; 12:670-678

Method Suitability Components

Method Development

- Establish assay protocol
- Optimize and standardize assay parameters
- Set acceptance criteria

Assay Suitability

- Rank order relationship for model drugs between experimental permeability values and human intestinal absorption
- Define the high-permeability internal standard (HP-IS)

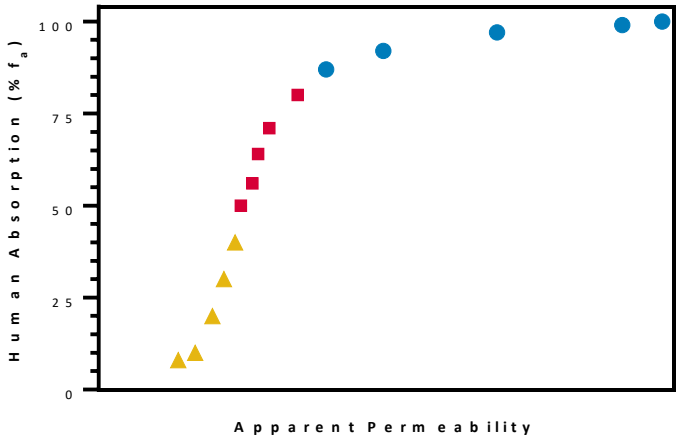
Permeability Classification

- Reference standard to demonstrate assay reproducibility
- Molecular markers for cell monolayer integrity
- Utilize HP-IS to classify new compound

Volpe AAPS J. 2010; 12:670-678

Model Drugs

- For *in vitro* cell culture methods, at least 5 model drugs per group are recommended to establish method suitability
- Model drugs have known intestinal absorption in humans and are passively absorbed
- Model drugs should represent a range of *in vivo* human intestinal absorption:
  - "Low" permeability (f_a < 50%)
  - "Moderate" permeability (f_a = 50-84%)
  - "High" permeability ($f_a \geq 85\%$)



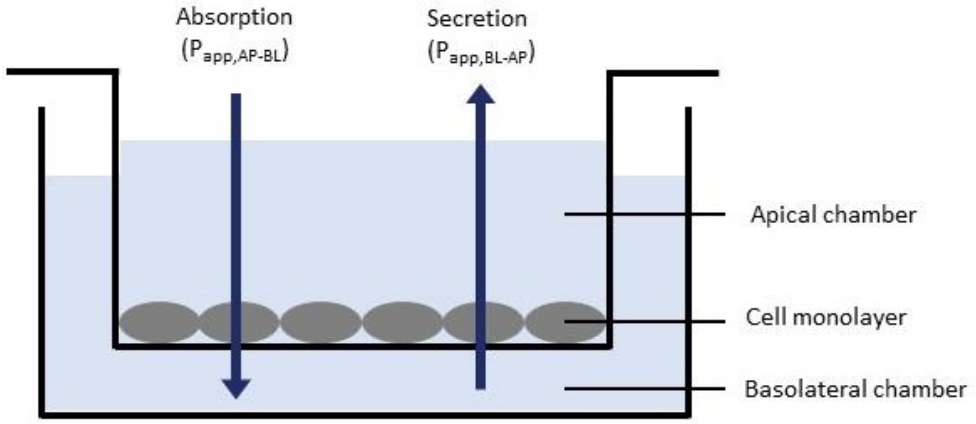
M9 BCS Guidance, 2021. [https://www.fda.gov/media/148472/download]

Reference Standards

- Use of reference standards in permeability experiments:
  - High (HP) and low (LP) permeability standards to monitor experimental variations
  - Zero-permeability standard to monitor cell monolayer integrity (*e.g.*, FITC-dextran, PEG-4000, Lucifer yellow, inulin)
  - HP internal standard (HP-IS) to classify drugs
- Permeability values of the standards should not differ significantly between different experiments, including those conducted to demonstrate method suitability
- Experimental options:
  - measure test drug together with reference standards
  - evaluate standards in the same monolayers after experiment with the test drug
  - use additional cell monolayers for standards

Acceptance Criteria

- Acceptance criteria are defined for selected reference standards and measurements for the permeability assays:
  - Measure of monolayer integrity
  - Apparent permeability (P_{app}) of zero, HP ($f_a \geq 85\%$) and LP ($f_a < 85\%$) permeability standards
  - Efflux of probe substrate compound
- Demonstrate functionality of assay
- Reference standards ensure reproducibility

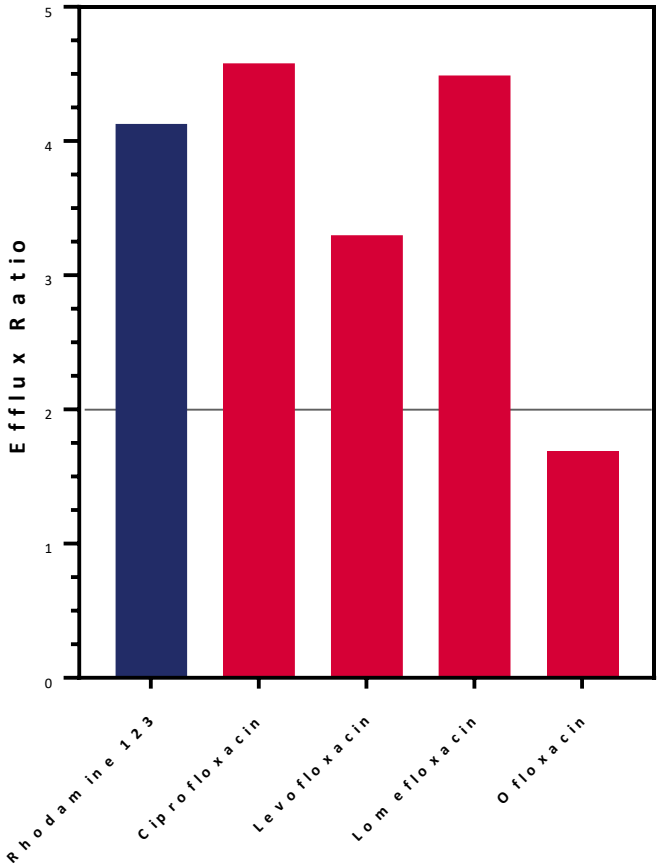


Efflux

- Functional expression of efflux mechanism demonstrated in bidirectional experiments with a probe transporter substrate
- Efflux revealed by higher rate of substrate's permeability in BL-to-AP (secretion) direction as compared to AP-to-BL (absorption) direction
- A drug with an efflux ratio (ER) ≥ 2 is considered as an efflux transporter substrate

$$ER = P_{app,BL-AP}/P_{app,AP-BL}$$

- Rhodamine 123, digoxin, vinblastine, paclitaxel or quinidine can be used as probe substrates for demonstrating presence of P-gp transporter



Volpe. AAPS PharmSci. 6 (2):1-6, 2004

Use of Permeability Method

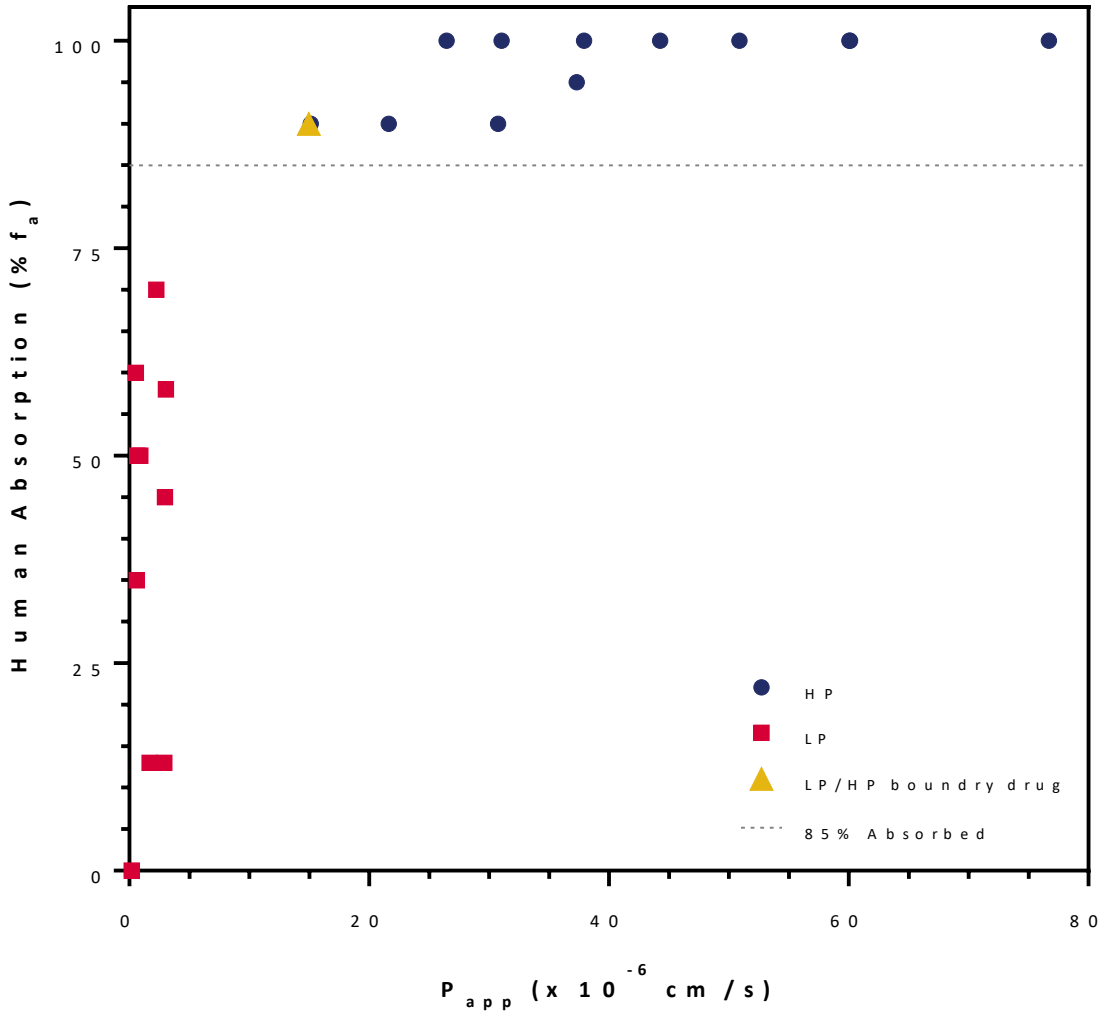
- Maintain same protocol from method suitability experiments to classify drug substance permeability
- Reference standards for classification and reproducibility
- Demonstrate passive transport of test drug:
  - Evaluate test drug at several concentrations (*e.g.*, 0.01×, 0.1× and 1× the highest strength in 250 mL), and
  - Measure bidirectional permeability of test drug (*i.e.*, AP-to-BL vs. BL-to-AP)
- Classify a test drug as highly permeable when its P_{app} is equal to or greater than that of the HP-IS

M9 BCS Guidance, 2021. [www.fda.gov/media/148472/download]

Example

- Caco-2 cells in 12-well plate format
- 18- to 22-day monolayer age
- HBSS pH 6.8 in AP chamber and pH 7.4 in BL chamber
- Initial drug concentration based on highest dose strength in 250 mL
- LP/HP boundary drug = Labetalol

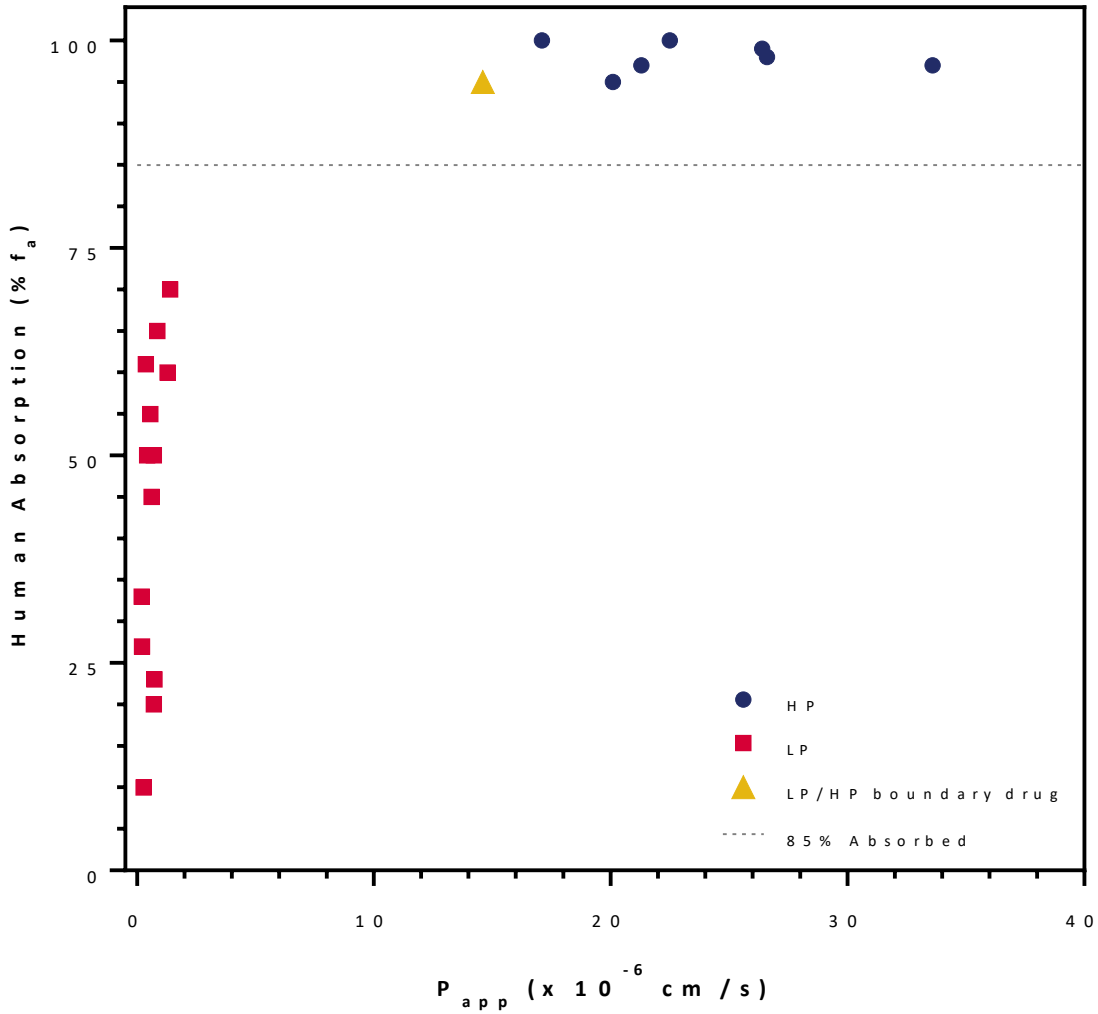
Volpe *et al*. Clin Res Reg Affairs. 2007; 24:39-47.



Example

- Caco-2 cells in a 24-well plate format
- 21-day monolayer age
- HBSS pH 7.4 in AP and BL chambers
- Initial drug concentration based on maximal clinical dose in 250 mL
- LP/HP boundary drug = Metoprolol

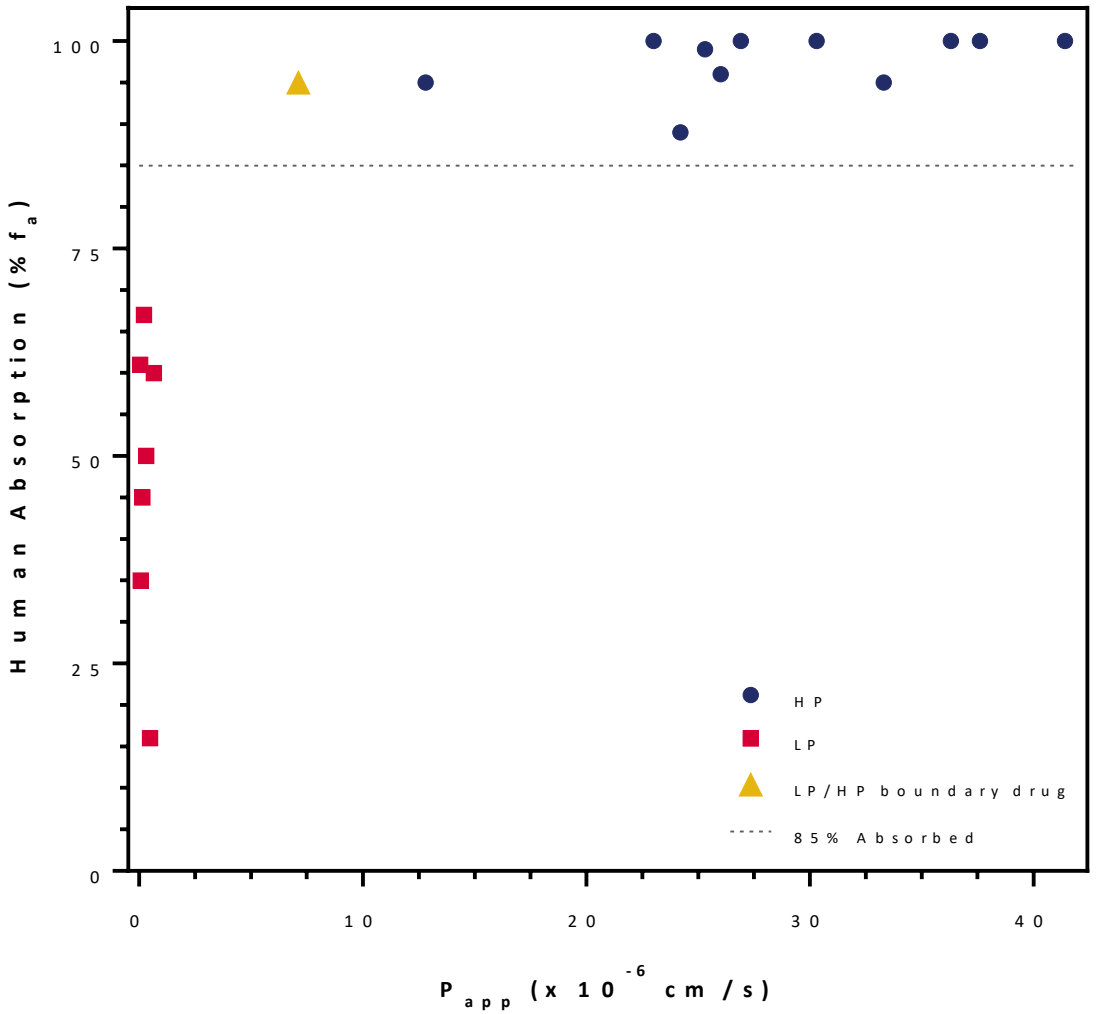
Jarc *et al*. J Pharm Pharmacol. 2019; 71(8):1231-1242



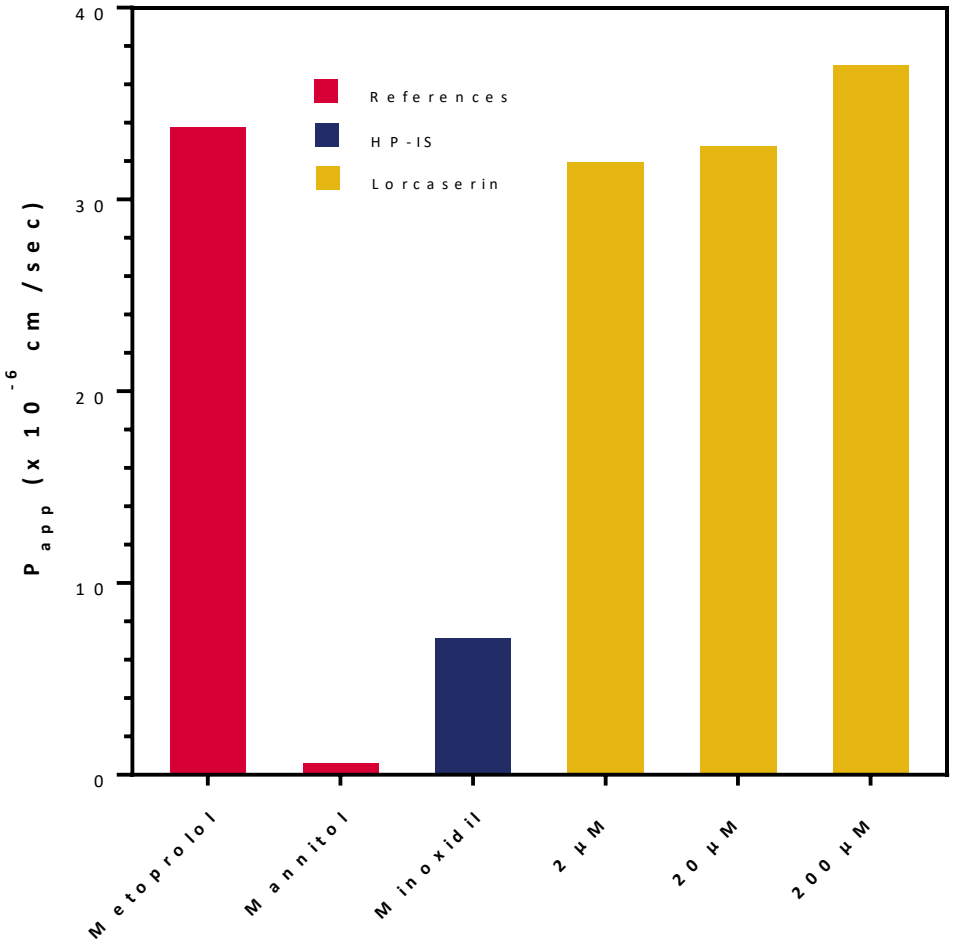
Example

- Caco-2 cells in 12-well plate format
- 22- to 24-day monolayer age
- HBSS pH 6.8 in AP chamber and pH 7.4 in BL chamber
- Initial drug concentration = 10 µM
- LP/HP boundary drug = Minoxidil

Chen et al. Mol Pharm. 2013; 10(12):4739-4745.



Permeability Classification



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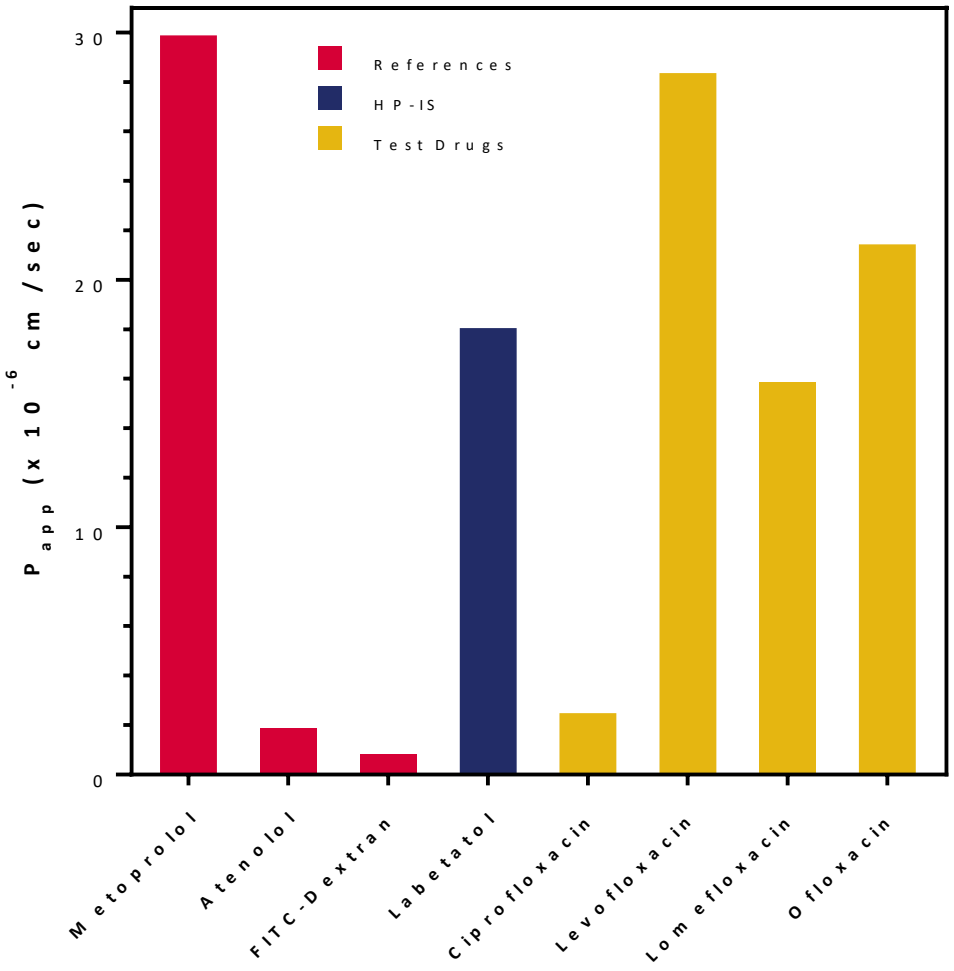

Volpe DA. AAPS PharmSci. 6 (2):1-6, 2004

Summary

- Method suitability for intestinal permeability assays is comprised of three phases (method development, assay suitability, permeability classification)
- Each laboratory should set its own acceptance criteria for cell measurements and reference standards used in the initial experiments with the model drugs and those to classify drug permeability
- Method suitability, with its reliance on assay standardization and validation, reference standards, and acceptance criteria, enhances the consistency of experimental data to predict a drug's intestinal permeability


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