M-CERSI Workshop: Drug Permeability: Best Practices for BCS-based Biowaivers

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Typical Deficiencies relating to permeability assessment supporting BCS Biowaiver

Disclaimer: The views expressed here are personal and do not represent those of the FDA
A quality product of any kind consistently meets the expectations of the user – drugs are no different.

Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is assuring every dose is safe and effective, free of contamination and defects.

It is what gives patients confidence in their next dose of medicine.
Outline

➢ Background on BCS classification criteria
➢ Permeability measurements
➢ Criteria and support of permeability
➢ Case studies
➢ Typical deficiencies related to permeability measurements
BCS Classification Criteria

The BCS is a scientific approach based on the aqueous solubility and intestinal permeability characteristics of the drug substances, resulting in four classes:

- **Class I:** high solubility, high permeability
- **Class II:** low solubility, high permeability
- **Class III:** high solubility, low permeability
- **Class IV:** low solubility, low permeability
Permeability Measurements

➢ Extent of absorption from Human PK studies
  • Absolute bioavailability
  • Mass balance studies

➢ Human In vivo data from published literature (product knowledge and bioavailability studies) may be acceptable*

➢ In vitro methods using Caco-2 cells#

*Peer reviewed articles may not contain the necessary details of the testing to make a judgement regarding the quality of the results.

#Acknowledges other in vitro/in situ methods but agreement to rely on method with most experience. Other methods are for future consideration upon standardization.
Criteria and support of permeability

➢ A drug substance is considered highly permeable if

• absolute BA ≥ 85%.

• ≥ 85% of the administered dose is recovered in urine as unchanged drug (parent) or sum of parent drug, phase 1 oxidative and Phase 2 conjugative metabolites.

• Metabolite in feces – only oxidative and conjugative metabolites.

  (Metabolites formed through reduction or hydrolysis should not be included (unless it is demonstrated that it is not produced prior to absorption – microbial action); Unchanged drug in feces cannot be included for extent of absorption (unless data supports biliary excretion, intestinal secretion).

➢ Results indicating high permeability from validated in vitro Caco-2 permeability assays.

• Discuss Caco-2 results in context of available human PK data.

• Limited to passively absorbed compounds.
Stability in the GI tract

➢ If mass balance or Caco-2 studies are used, data to support drug substance stability in the GI tract should be provided.
➢ However, stability data are not required if mass balance study shows ≥ 85% of the administered dose recovered as unchanged drug in urine.
➢ GI stability: Documented using compendial or simulated gastric and intestinal fluids.
➢ Other relevant methods with appropriate justification.
➢ Drug solution incubated at 37°C (1 h in gastric fluid and 3 h in intestinal fluid).
➢ Significant degradation (> 10%) of a drug suggest potential instability.
BCS Class I Classified Products

- Reviewed: 97
- Classified: 66
- Not classified: 31

BCS CLASS I SUBMISSIONS RECEIVED BY THE COMMITTEE

- Classified: 68%
- Not classified: 32%

PERMEABILITY STUDIES

- RLD labeling/literature: 13
- In situ rat intestine perfusion: 2
- Caco-2: 28
- Absolute BA: 14
- Mass Balance: 19
Case Studies
Case Study 1-IND (Mass Balance Study)

- Applicant submitted solubility, dissolution, Caco-2 in vitro permeability studies, mass balance studies, and gastric stability studies
- Compound A demonstrated high solubility and rapid dissolution
- However, permeability based on Caco-2 in vitro cell culture system was moderate (in comparison to model compounds), saturable transport and active efflux.
- Cmax was dose proportional within the range of doses tested
- Demonstrated adequate gastric stability

**AB permeability and BA Permeability (pH 6.5/7.4)**

<table>
<thead>
<tr>
<th>Compound A</th>
<th>Permeability ($10^{-6}$ cm/s)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>0.6 µM</td>
<td>3.34</td>
<td>3.75</td>
</tr>
<tr>
<td>6 µM</td>
<td>1.36</td>
<td>1.28</td>
</tr>
<tr>
<td>60 µM</td>
<td>1.38</td>
<td>1.33</td>
</tr>
</tbody>
</table>

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<tr>
<th>Compound A</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>0.6 µM</td>
<td>27.06</td>
<td>22.94</td>
</tr>
<tr>
<td>6 µM</td>
<td>3.84</td>
<td>3.62</td>
</tr>
<tr>
<td>60 µM</td>
<td>3.59</td>
<td>3.39</td>
</tr>
</tbody>
</table>

**Gastric Stability**

<table>
<thead>
<tr>
<th>System</th>
<th>Initial pH</th>
<th>End pH</th>
<th>HPLC Purity (%) and Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>FaSSGF</td>
<td>1.5</td>
<td>8.41</td>
<td>99.92%</td>
</tr>
<tr>
<td>FeSSIF</td>
<td>5.0</td>
<td>8.36</td>
<td>99.81%</td>
</tr>
<tr>
<td>FaSSIF</td>
<td>6.5</td>
<td>8.83</td>
<td>99.91%</td>
</tr>
</tbody>
</table>

**Dissolution Data**

<table>
<thead>
<tr>
<th>USP Apparatus</th>
<th>Speed (RPMs)</th>
<th>Medium/Temperature</th>
<th>Volume (mL)</th>
<th>Sampling points</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (Paddle)</td>
<td>50</td>
<td>Hydrochloric acid solution (0.1 M); Acetate buffer pH 4.5; Phosphate buffer pH 6.8; Water /37.0 ± 0.5°C</td>
<td>500</td>
<td>10, 15, 30, and 45 min</td>
</tr>
</tbody>
</table>

**Solubility (mg/mL) versus pH**

**Log Cmax versus Log Dose**
Case Study 1-IND Contd....

- **Mass balance study:** Open-label, randomized single-dose crossover Phase 1 study conducted in healthy subjects. Plasma and urine samples were collected pre-dose and up to 48 hrs post-dose. 13 subjects were randomized, 1 subject withdrew and 12 subjects completed the study.

- **Total amount of Compound A excreted in the urine was greater than 85% of the administered dose.**

- However, though the total amount of compound A excreted in the urine was greater than 85% of the administered dose, the variability of the measured concentrations in urine was very high based on the available data in the study report.

- The applicant was, therefore, requested to submit the entire urine datasets as well as provide their explanation regarding this high variability.

- The Applicant noted that of the 12 subjects, only 2 individual subjects had less than 85% urine recovery: one subject was close at 82.8% recovery, and another was a clear outlier who had only 50.9%.

- When the excretion outcomes are recalculated excluding outlier, the mean excretion is 2.78 mg (representing 92.7% of the administered dose of 3 mg), with a standard deviation of 0.210, yielding a CV% of 7.5%. This reduction in the SD and CV% shows that the variability was heavily influenced by the data from the outlier.

- Compound A was classified as BCS Class I drug substance and BCS class I drug product.

**PK parameters**

<table>
<thead>
<tr>
<th>Parameter (mg/mL)</th>
<th>Geo. Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>32.9</td>
<td>0.790</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>4.71</td>
<td>0.47</td>
</tr>
<tr>
<td>AUC(0-24h)</td>
<td>0.147</td>
<td>3.11</td>
</tr>
<tr>
<td>AUC(0-12h)</td>
<td>173</td>
<td>170</td>
</tr>
<tr>
<td>AUC(0-24h)%</td>
<td>3.11</td>
<td>3.11</td>
</tr>
<tr>
<td>CL/F (mL/h)</td>
<td>16700</td>
<td>11000</td>
</tr>
<tr>
<td>Vd/F (L)</td>
<td>11000</td>
<td>11000</td>
</tr>
</tbody>
</table>

**Cumulative percentage Ae-time profiles of individuals**

**Cumulative Urinary Excretion with and without the outlier subject**

- Calculated using the PK set, with the exception of total amount excreted (Ae) and total Ae% calculated using the urine excretion set.

CV = coefficient of variation, PK = pharmacokinetic, SD = standard deviation
Case Study 2-ANDA (First Pass Effect)

- Compound B demonstrated high solubility in the range of pH 1-6.8.
- Rapid dissolution was observed with ≥ 85% of the labeled amount dissolved in 30 minutes.
- RLD labeling indicated that absolute bioavailability of compound B when compared to IV infusion is 25% due to extensive first-pass metabolism. Also there exists linear relationship across a dose range.
- High permeability is supported by in vitro permeation studies across Caco-2 cell monolayers and stability data in the GI tract. Compound B showed greater permeability than high permeability model compound minoxidil at all the tested concentrations.
- Deficiencies pertaining to method validation in initial submission.
- No significant degradation was observed from the stability studies.
- Compound B was classified as a BCS class I drug substance and BCS class I drug product.

### Solubility Data

<table>
<thead>
<tr>
<th>No.</th>
<th>Buffer</th>
<th>Initial Drug Concentration (mg/mL)</th>
<th>Mean Drug Solubility (mg/mL)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH 1.0 KCl/HCl</td>
<td>5</td>
<td>4.53</td>
<td>4.66</td>
</tr>
<tr>
<td>2</td>
<td>pH 3.0 potassium biphthalate buffer</td>
<td>5</td>
<td>4.31</td>
<td>6.71</td>
</tr>
<tr>
<td>3</td>
<td>pH 5.0 potassium biphthalate buffer</td>
<td>5</td>
<td>3.23</td>
<td>4.72</td>
</tr>
<tr>
<td>4</td>
<td>pH 6.8 potassium phosphate buffer</td>
<td>5</td>
<td>1.30</td>
<td>8.78</td>
</tr>
</tbody>
</table>

### Permeability Data

<table>
<thead>
<tr>
<th>Nominal Dosing Concentration</th>
<th>Basis of Normalization</th>
<th>A-to-B</th>
<th>B-to-A</th>
<th>Effect Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>52.9 μM</td>
<td>Nominal Dosing</td>
<td>13.2 ± 1.39</td>
<td>105 ± 5.56</td>
<td>25.3 ± 5.76</td>
</tr>
<tr>
<td>329 μM</td>
<td>Nominal Dosing</td>
<td>14.8 ± 0.615</td>
<td>116 ± 3.55</td>
<td>17.9 ± 1.82</td>
</tr>
<tr>
<td>2.47 mM</td>
<td>Measured Donor</td>
<td>11.8 ± 0.870</td>
<td>91.9 ± 6.16</td>
<td>15.1 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>Nominal Dosing</td>
<td>18.8 ± 0.450</td>
<td>91.2 ± 0.723</td>
<td>18.2 ± 1.47</td>
</tr>
</tbody>
</table>

### Dissolution Data
Case Study 3-ANDA (Food Excipient Interaction)

- Compound C was classified as BCS class I based on solubility, dissolution and mass balance of RLD application.
- Later in the year few other ANDA applications did not pass 90% criteria for fed BE studies.
- Data indicated excipient-food interactions.
- High proportion of microcrystalline cellulose and swelling of MCC matrix under fed conditions could lower the rate of absorption.
- Waiver of fasting BE study was granted but fed BE study was required.

**Solubility Data**

<table>
<thead>
<tr>
<th>Highest Strength</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required conc. to be considered highly soluble</td>
<td>0.04 mg/mL</td>
</tr>
</tbody>
</table>

**Dissolution Data**

- USP apparatus II (Paddle)
- 50 rpm
- 900 ml of 0.1 N HCl, pH 4.5 Acetate buffer and pH 6.8 Phosphate buffer
- Very rapid dissolving

**Mass Balance Study**

- Single dose study in 6 healthy male subjects.
- Mass balance following IV and oral administration of radioactive compound.
- Total radioactivity was measured in blood, urine and feces for up to 7 days.
- Following oral dose 93% was recovered in urine.
- Highly permeable drug substance and drug product.

**Dissolution Study**
Case Study 4-ANDA
(In situ rat intestine perfusion method)

➢ Compound D demonstrated high solubility in aqueous medium over pH range of 1-6.8.
➢ Multimedia dissolution data demonstrated rapid dissolution with ≥85% dissolved in 30 minutes
➢ Data from Caco-2 study shows moderate permeability (previous NDA).
➢ In vivo human pharmacokinetic information and scientific literature indicated that the bioavailability is high.
➢ In this ANDA, applicant conducted in vitro permeability using in-situ rat intestine perfusion method.
➢ Cytotoxic causing loss of integrity and hence Caco-2 is not an appropriate model.

### In-situ rat intestine perfusion method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>20.0 µg/mL (263 µM)</th>
<th>200 µg/mL (2.63 mM)</th>
<th>2.00 mg/mL (26.3 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Compound</td>
<td>P_{eff} (mean ± SD)*</td>
<td>0.690 ± 0.121</td>
<td>0.459 ± 0.125</td>
<td>0.657 ± 0.134</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>High Internal Standard</td>
<td>P_{eff} (mean ± SD)*</td>
<td>0.573 ± 0.141</td>
<td>0.370 ± 0.0913</td>
<td>0.506 ± 0.155</td>
</tr>
<tr>
<td>(Metoprolol, 50 µM)</td>
<td>Recovery (%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Instability in the Gastrointestinal Tract

<table>
<thead>
<tr>
<th>Medium</th>
<th>Time of Incubation (min)</th>
<th>Incubation Temperature (°C)</th>
<th>Concentration (mg/mL) Before Incubation</th>
<th>% Recovery After Incubation</th>
<th>*% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulated Gastric Fluid</td>
<td>60</td>
<td>37.0</td>
<td>2.00</td>
<td>99.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Simulated Intestinal Fluid</td>
<td>180</td>
<td>37.0</td>
<td>2.00</td>
<td>98.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Typical Deficiencies: Absolute bioavailability and Mass Balance studies

- Lack of study reports, validation reports and other procedural documents
- Unexplained high variability in the measured urine concentrations, request for complete datasets along with calculations.
- Missing GI stability data to support <85% of the unchanged parent drug excreted in urine.
Typical deficiencies: Caco-2 studies

- **Pre-study Model compound Validation studies**
  - Missing Standard operating procedures
  - Missing validation data with model compounds
  - Missing calibration curve and quality control data including linearity, slope, precision, and accuracy for all analytical runs used in the permeability study.
  - Missing data on zero permeability model drug

- **Permeability study**
  - Missing non-specific binding (cell free) permeability study
  - Missing procedures to ensure pH 7.4 was maintained during the course of the study.
  - Missing data to demonstrate stability of stock solutions
  - High recovery rates of internal standards and differences in recoveries between A-B/B-A directions.
  - Permeability measurements influenced by presence of internal standards.
  - Difference in permeability measurements of internal standards between validation and pivotal studies
Typical Deficiencies/concerns: Literature based, labeling

- Lack of necessary testing details for peer reviewed Journals
- Unreliable RLD label data (large variability, fewer subjects etc)

Typical deficiencies: Instability in GI Tract

- Missing procedural SOPs (study conduct, preparation of buffers etc).
References

➢ M9 Biopharmaceutics Classification System-Based Biowaivers, Guidance for Industry, May 2021 (FDA, ICH)
Acknowledgements

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➢ Office of Bioequivalence, Generic Drugs
➢ Office of Clinical Pharmacology/OTS/CDER/FDA
Thank you!