Industry Case Studies #2: Successful Permeability Studies supporting BCS Biowaiver in ANDA (Caco-2)

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

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Virtual Workshop, December 6, 2021

Laboratory Services

Chemistry, Manufacturing and Control

Radiolabelled Sciences

Clinical Development

Biologics & CGT

Case Study 1 – Importance of Recovery

Recovery

- Solution recovery – donor and receiver compartments
- Mass balance – solution recovery + cell lysate and rinses; Acceptance criteria typically >80%

Non-specific Binding to Cell-free Transwell Device

Incubation Concentration (μM)	A-to-B
	Recovery (%)*
0.508	51.2 ± 1.41
*The result is expressed as mean ± SD, n=3	

Recovery from Cell-free Transwell Device

Analyte	Chamber	Nominal Conc. (nM)	Mean C_{final} (nM)	Measured C_{dosing} (nM)	Mean Recovery (%)	% CV
Drug Substance	Top (Insert)	500	333	483	69.0	1.91
	Bottom (Well)	500	376	483	77.7	2.33

Case Study 1 – Recovery Related Procedures

1) Question:

"…..a 10-minute pre-incubation step with test compound was performed for the permeability experiment to reduce the non-specific binding. However, the concentration and volume of drug substance used for the pre-incubation were not clearly mentioned in the permeability study report. Please provide…."

Response: The pre-incubation step was performed with the **same respective concentration** level of drug substance (i.e., x, 10x, or 100x µM) and the **same dosing volumes** as **the dosing solution for the permeability experiments**. Pre-incubation solution or dosing solution (0.6 mL) in HBSSg, pH 7.4 was added to the apical chamber for A-to-B assessment (with 1.5 mL HBSSg, pH 7.4 in the basolateral chamber), or to the basolateral chamber (1.6 mL) for B-to-A assessment (with 0.5 mL HBSSg, pH 7.4 in the apical chamber). **After the 10 minute pre-incubation period**, all solutions from the pre-incubation step (both apical and basolateral sides) were **removed and replaced with fresh dosing solution** (for donors) or buffer (for receivers) to **initiate the permeability assay**…..

Case Study 1 – Recovery Expectations

2) Question:

"It is noticed that in the permeability study, the recovery rate (87.3%) is higher when the concentration of dosing solution is 5.08 µM than the recovery rate (80.1%) when the concentration of dosing solution is 50.8 µM. You have mentioned in your submission that the recovery rate would be higher as concentration of testing compound in the dosing solution increases if non-specific binding exists. However, your recovery data mentioned above from the bidirectional permeability study do not support this finding. Please provide the reason/explanation on this discrepancy."

Response: The **nominal dosing concentration** was used in the recovery equation based on the criteria for analytical acceptance of measured dosing concentrations (measured concentration within 85.0 to 115% of nominal, per Absorption Systems SOP). However, using the nominal concentration for compounds such as ……., which **tend to associate with the test system may create the appearance of atypical differences in recovery**.

For each test concentration (i.e., low to high), the actual initial concentration available for permeation (D_0) was **not equally biased due to differences in dosing solution preparation, non-specific binding and cell accumulation**. The mean values for basolateral-to-apical % recovery, when **normalized to the initial available donor concentration D_0**….

Case Study 1 – Recovery Expectations

Nominal Dosing Concentration	Average % Recovery (using D_0)	Average % Mass Balance (using D_0)
5.08 µM	87.7	99.4
50.8 µM	89.0	102

Response Continued: The mean values for basolateral-to-apical % recovery, when normalized to the **initial available donor concentration D_0, were 87.7% and 89.0% at the 5.08 µM and 50.8 µM dosing concentrations, respectively.** Additionally, **the mass balance** for the same treatments becomes complete when D_0 is used in the calculation; **99.4% and 102%** mass balance for the 5.08 and 50.8 µM concentrations, respectively.

Case Study 1 – Recovery Expectations

3) Question: In the bidirectional permeability study, the average recoveries of drug substance from basolateral to apical direction were higher when compared to recoveries from apical to basolateral direction, at multiple concentrations and at different studies. (e.g. Table 2 in the study report #493). Please explain this difference in recoveries between basolateral to apical and apical to basolateral directions.

Response: With compounds such as ……. that have physicochemical properties (e.g., log P: 4.36), which result in association with the cell monolayer, a considerable fraction of the compound is recovered, post-experiment in the cell lysate.

An assessment of recovery from all compartments of the test system was performed, and the results are detailed. These results indicate that there was greater accumulation in the **cell monolayer compartment for the apical-to-basolateral direction (average 28.2%) vs. the basolateral-to-apical direction (8.43%)**, while non-specific binding to the device itself (donor and receiver compartments) was similar in both directions.

The differences in cell accumulation may be attributable to **a) the direct placement of the dosing solution on the apical surface of the cell monolayer when dosed in the apical-to-basolateral direction and b) the morphology of the differentiated Caco-2 monolayer, where the apical side of the cells contains microvilli, which amplify the surface area**. Therefore, the drug substance has a greater tendency to accumulate in (or associate with) the cell monolayer when dosed in the apical-to-basolateral direction and solution recovery (i.e., recovery from only the donor and receiver compartments) was higher in the B-to-A direction vs. the A-to-B direction.

Case Study 1 – Recovery Expectations

Response Continued:

A to B	Solution Recovery (%)	Lysate Recovery (%)	Donor Rinse Recovery (%)	Receiver Rinse Recovery (%)
R1	54.2	27.1	27.6	1.99
R2	54.2	29.4	21.2	<1.23
R3	49.9	28.1	29.4	1.34
Mean	**52.8**	**28.2**	**26.0**	**1.67**
SD	2.46	1.15	4.31	N/A

B to A	Solution Recovery (%)	Lysate Recovery (%)	Donor Rinse Recovery (%)	Receiver Rinse Recovery (%)
R1	66.9	8.67	27.9	0.640
R2	68.5	8.12	27.5	0.652
R3	62.2	8.49	26.0	0.573
Mean	**65.9**	**8.43**	**27.1**	**0.622**
SD	3.26	0.282	0.977	0.0424

Case Study 2 – Permeability Procedures

1) Question: your [redacted], "Procedure for BCS Permeability Measurement", if the recovery of the test compound is less than 85%, then a pre-incubation step may be employed during the permeability experiment." However, pre-incubation was not conducted during method suitability validation ([redacted]. Please conduct the pivotal experiment for the permeability classification of the test compound in the same manner as the method suitability study; alternatively, please validate your modified method.

Response: In order to unequivocally demonstrate that pre-incubation does not impact permeability classification outcomes when using the validated Caco-2 model for a drug substance transported by passive mechanisms, a **bridging study was performed (using Caco-2 monolayers from different days in culture and passage numbers) with a subset of low and high permeability model compounds from the validation** (antipyrine, metoprolol, as well as the internal standards from the pivotal study, atenolol and minoxidil) **with and without a 10-minute pre-incubation.**

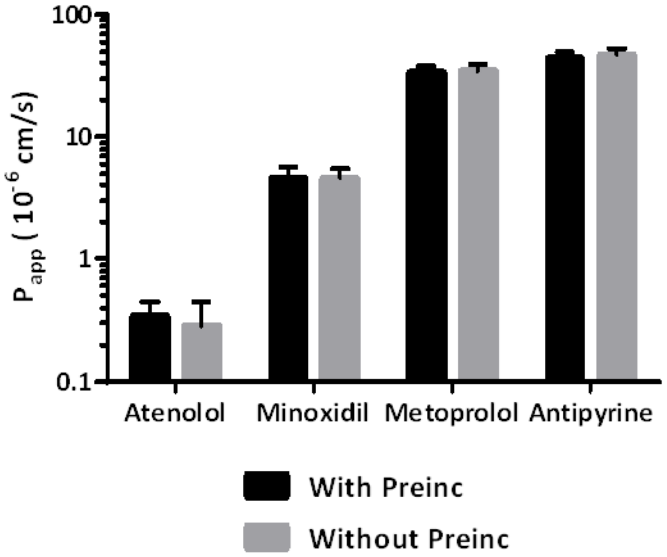
Case Study 2 – Permeability Procedures

Response Continued:

Summary of Model Compound Permeability across Multiple Days in Culture and Passages

Analyte	With Pre-inc.		Without Pre-inc.	
	Mean P_{app} (10^{-6} cm/s)	SD	Mean P_{app} (10^{-6} cm/s)	SD
Atenolol	0.344	0.110	0.289	0.158
Minoxidil	4.54	1.17	4.60	0.976
Metoprolol	33.7	4.26	35.0	4.14
Antipyrine	44.6	4.80	47.8	4.85

Permeability of Model Compounds with and Without Pre-incubation



Case Study 2 – Permeability Procedures

The results of this study demonstrate that the pre-incubation step:

1. Does not impact the ability of the Caco-2 cell monolayer model to clearly discriminate a low-permeability compound such as atenolol from a high-permeability compound such as minoxidil.
2. Does not impact, the permeability rank order. The four model compounds behave as expected, i.e., permeability of antipyrine > metoprolol > minoxidil > atenolol, both with and without a 10 minute pre-incubation.
3. Does not impact the measured in vitro permeability value(s) because regardless of the pre-incubation conditions, the slope of the linear portion of the cumulative receiver concentration vs. time (i.e., the permeability time course profile), on which the P_{app} calculation is based, remains unchanged.
4. These results were consistent across a sample of Caco-2 batches and days in culture, i.e., within the established method suitability assay window.

Case Study 2 – Permeability Procedures

2) Question: Summary Table 4: Material and Methods for Validation of Permeability Study in Module 2.7.1 indicated that the plates containing the cell monolayers were shaken using a Lab-Line Instruments Titer Plate Shaker set at a speed of 2.0. However, Study Report No. [redacted] states that the plates were shaken using a VWR Model 3500 Orbital Plate Shaker set to a speed of 1.0 (~50 rpm). Please clarify this discrepancy.

Response: The **Lab-Line Instruments Titer Plate Shaker from the validation was replaced with VWR Model 3500 Orbital Plate Shaker as an equipment update**.

To address the lack of impact of shaking speed on permeability classification of co-dosed compounds, a study **was performed using model compounds with different permeability values over multiple cell passages and days in culture**.

The study was performed using the VWR Model 3500 Orbital Plate Shaker on **both shaking speed setting 1 and shaking speed setting 2**.

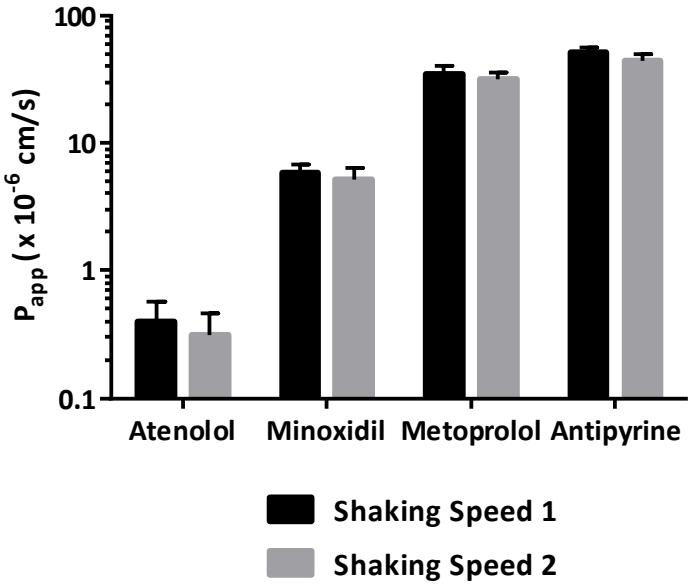
Case Study 2 – Permeability Procedures

Response Continued:

Summary of Model Compound Permeability across Multiple Days in Culture and Passages

Analyte	Shaking Speed 1		Shaking Speed 2	
	Mean P_{app} (10^{-6} cm/s)	SD	Mean P_{app} (10^{-6} cm/s)	SD
Metoprolol	35.1	4.85	31.9	4.47
Minoxidil	5.77	1.10	5.14	1.36
Antipyrine	52.2	5.17	44.0	6.54
Atenolol	0.396	0.177	0.309	0.157
Minoxidil/ Atenolol	14.6		16.6	

Permeability of Model Compounds at Two Shaking Speeds



Case Study 2 – Permeability Procedures

The results of this study demonstrate that:

1. The two tested shaking speeds do not impact the ability of the Caco-2 cell monolayer model to clearly discriminate the permeability of atenolol from a high-permeability compound such as minoxidil as shown by their mean P_{app} values and P_{app} ratios (minoxidil P_{app}/atenolol P_{app}).
2. The permeability rank order of the four model compounds was as expected from the validation, i.e., antipyrine > metoprolol > minoxidil > atenolol, at both shaking speeds.
3. At the shaking speed conditions (Setting 1 and Setting 2) evaluated, the slope of the linear portion of the cumulative receiver concentration vs. time profile (i.e., the permeability time course), on which the P_{app} calculation is based, remains similar.
4. These results were consistent across a sample of batches and days in culture, i.e., within the established method suitability assay window.

Case Study 3 – Test System or Method Suitability

1) Question: Per the BCS Guidance, you have not evaluated the expression of multidrug resistance-associated protein 2 (MRP2) in your Caco-2 cell model. Please evaluate the expression of MRP2 in your Caco-2 cell model and submit the data for review.

Response: The permeability of the **MRP2 substrate, 5(6)-carboxy-2,'7'-dichlorofluorescein (CDCF)** was monitored with the batch quality control compounds **for 7 batches of Caco-2 monolayers.** The chosen batches cover a range of passages and days in culture within the **established assay window for BCS permeability classification.**

The results are summarized and **demonstrate robust MRP2 functional activity in the Caco-2 monolayers** along with **satisfactory batch QC results** for all other co-dosed control compounds.

Analyte	Mean P_{app} (10^{-6} cm/s)	SD	Efflux Ratio
Atenolol	0.218	0.0800	N/A
Propranolol	14.2	1.62	N/A
A-to-B Digoxin	0.530	0.186	39.9
B-to-A Digoxin	21.1	8.93	
A-to-B E3S	0.359	0.0930	93.2
B-to-A E3S	33.5	5.49	
A-to-B CDCF	2.44	0.435	**10.9**
B-to-A CDCF	26.7	4.84	

Case Study 3 – Test System or Method Suitability

2) Question: Your model compound validation study [redacted] reported that the P_{app} (unit: $x10^{-6}$ cm/s) values of the drugs atenolol, furosemide, hydrochlorothiazide, and ranitidine were 0.19 ± 0.05, 0.18 ± 0.01, 0.55 ± 0.14, and 0.28 ± 0.04, respectively. The respective P_{app} values of the said four drugs were similar to the other low permeability model drugs that you used (in the range of 0.07 ± 0.00 to 0.77 ± 0.15). However, the four model drugs are classified as moderate permeability drugs in the Attachment A of the current BCS Guidance. Given these observations, there is a concern about your cell model to adequately distinguish among low, moderate, and high permeability drugs. Please clarify and provide the necessary data to address this concern.

Response: The **model compound data in question** (other than atenolol, which was averaged from the test system ruggedness evaluation) **was from a single unidirectional experiment with four replicates**.

Different validation compounds were assessed using cell batches from different passages and days in culture, which may explain the overlapping measurements as each of these calculated apparent permeability values have intrinsic test system variability associated with their determinations. **An example of this intrinsic variability is data cited in the report "Minoxidil Criteria", where the minoxidil P_{app} acceptance ranges over a ten year period provide insights** (within a year and across multiple years) **on variability for a highly permeable compound. It is reasonable to expect that the variability of a moderate to low permeability compound is similar or likely greater.**

Case Study 3 – Test System or Method Suitability

Therefore, to obtain a clearer sense of the test system's ability to adequately distinguish, **we typically co-dose compounds with different permeability values as a cassette** over multiple experimental days using cell monolayer passages and days in culture across the assay window. This is in-line with the conduct of a BCS permeability classification study where the test compound is co-dosed with permeability markers.

These **results clearly demonstrate that the Caco-2 cell model can adequately discriminate and rank compounds** with different permeability values consistent with the expectations of the "Method Suitability" section of the BCS Guidance (FDA, December 2017) which calls for "accurate differentiation between drug substances of low and high intestinal permeability attributes" using model drugs that represent a range of zero, low (e.g., < 50 percent), moderate (e.g., 50 – 84 percent), and high (≥ 85 percent) absorption.

Analyte	pH 6.5		pH 7.4	
	Mean P_{app} (10^{-6} cm/s)	SD	Mean P_{app} (10^{-6} cm/s)	SD
Antipyrine	28.5	3.92	32.0	3.34
Minoxidil	5.17	1.23	5.35	0.557
Hydrochlorothiazide	0.408	0.0756	0.464	0.145
Atenolol	0.109	0.0328	0.182	0.117
Lisinopril	0.0572	0.0180	0.0595	0.0256
FITC-D	<0.0180	0.000	0.0243	0.0255

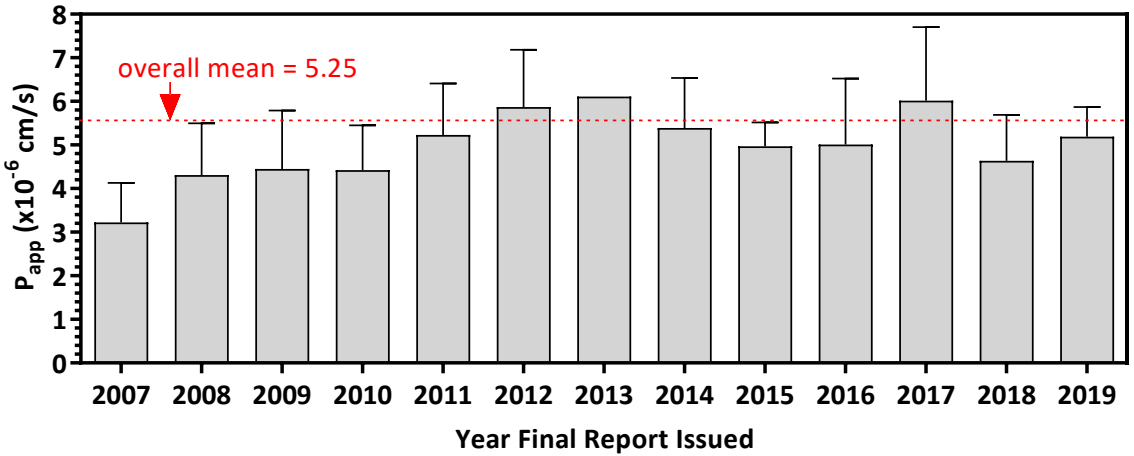
Case Study 3 – Test System or Method Suitability

3) Question: It is noted that the Papp of drug substance is substantially different in the permeability studies with (unidirectional study) and without internal standards (bidirectional study). For example, at concentration of 50.8 µM, the Papp with and without internal standards are 32.1×10-6 and 25.8 ×10-6 respectively. These data indicate that permeability of drug substance may be influenced by the presence of internal standards. Please provide an explanation on this difference in Papp and its potential impact on permeability classification of drug substance?

Response: The **typical variability of this Caco-2 cell monolayer test system for a high permeability drug substance can be evaluated using minoxidil data, which is collated periodically to update the minoxidil permeability acceptance criteria used for BCS permeability studies**. Because this data set represents numerous batches of Caco-2 cell monolayers over 10 years, it can be used as a benchmark for the expected variability of the test system both; across the years and for within a year. The coefficient of variation (CV) for the high-permeability internal standard, **minoxidil, was 26.1% over the 10-year period from 2007-2017 and the median annual variability across the same 10-year period was 23.2%.**

Case Study 3 – Test System or Method Suitability

Response Continued:



Data from a total of **128 separate BCS studies and 1431 valid determinations** (i.e., those in which the value was not rejected objectively due to poor linearity for minoxidil or failure of the low-permeability internal monolayer integrity standard), the mean minoxidil P_{app} **was 5.25 × 10^{-6} cm/s, with a median of 5.19 × 10^{-6},** standard deviation (SD) of 1.45 × 10^{-6}, and standard error of the mean (SEM) of 0.0370 × 10^{-6}.

Case Study 3 – Test System or Method Suitability

The **inherent variability for a sticky compound is expected to be higher than minoxidil, which does not have a tendency to associate** with the test system.

As the P_{app} results being compared were performed using different batches of Caco-2 monolayers on different days, variation similar to or greater than that observed historically for minoxidil, may be expected. **The relative percent difference in apical-to-basolateral P_{app} values for drug substance dosed at 50.8 µM with and without internal standards is 21.8%, which is well within the expected degree of variability for this test system.** Additionally, there is no impact on permeability classification because the permeability of drug substance even inclusive of inherent test system variability, is much higher (> 3.2 fold) than that of co-dosed HPIS minoxidil.

Furthermore, the **average P_{app} values for drug substance** from all experiments in this study are 19.4 × 10^{-6} cm/s and 20.1 × 10^{-6} cm/s for unidirectional and apical to basolateral bidirectional experiments.

GLP and Non-GLP (A-to-B Direction)	Average P_{app} (10^{-6} cm/sec)
Unidirectional with internal standards	19.4
Bidirectional without internal standards	20.1

Case Study 4 – Impact of Excipients

1) Question: As per the guidance per industry: Waiver of ln Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System "When new excipients or atypically large amounts of commonly used excipients are included in an IR solid dosage form, additional information documenting the absence of an impact on BA of the drug may be requested by the Agency". Your test formulation contains surfactant, sodium lauryl sulfate. Please provide justification with supporting evidence that the amount of sodium lauryl sulfate used in the test formulation does not have any impact on drug absorption and also dissolution of the drug from the test product.

Response: In the test product that is the subject of ANDA, the formulation **contains 2.32% (w/w) of the surfactant sodium lauryl sulfate (SLS), i.e. 10 mg of SLS in a total weight of 430 mg per capsule**. According to the FDA Inactive Ingredients Database, SLS can be present in an oral capsule in an amount as high as **25.8 mg and in an oral tablet in an amount as high as 51.69 mg.** Additionally, based on a recent bioequivalence clinical study, the recommended maximum amount of SLS that BCS Class 3 biowaivers can accommodate is 50 mg. The SLS content of the test product is in-line with the FDA Inactive Ingredients Database and much lower than the maximum allowable amount of 50 mg for a Class 3 biowaiver. This class of drug products is more sensitive to the effect of excipients on their absorption when compared to a BCS Class 1 product such as the test article. **Therefore, SLS at the amount present in the test product is not expected to affect its absorption. Additionally…..**

Case Study 4 – Impact of Excipients (Transwell)

Excipient	Conc., mg/ml	Apparent permeability coefficient (P_{app}) (10^{-6} cm/s)				
		Antipyrine	Acyclovir	Atenolol	Ganciclovir	Nadolol
Control[a]		39.9 ± 2.14	0.28 ± 0.06	0.23 ± 0.05	0.25 ± 0.06	0.21 ± 0.06
Control[b]		43.6 ± 0.89	0.28 ± 0.06	0.25 ± 0.05	0.22 ± 0.05	0.21 ± 0.04
D-Lactose	0.024[a]	42.3 ± 5.53	0.18 ± 0.04	0.19 ± 0.05	0.14 ± 0.04	0.14 ± 0.04
	0.13[a]	41.6 ± 0.58	0.32 ± 0.11	0.57 ± 0.46	0.48 ± 0.42	0.44 ± 0.37
	0.24[a]	39.8 ± 0.55	0.31 ± 0.20	0.27 ± 0.19	0.28 ± 0.19	0.27 ± 0.20
	1.0[b]	33.3 ± 0.85	0.20 ± 0.05	0.16 ± 0.06	0.17 ± 0.05	0.14 ± 0.05
	2.0[b]	33.1 ± 1.32	0.17 ± 0.14	0.14 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
Povidone	0.024[a]	44.5 ± 0.91	0.17 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
	0.042[a]	40.4 ± 0.96	0.21 ± 0.03	0.15 ± 0.02	0.18 ± 0.03	0.13 ± 0.02
	0.06[a]	41.9 ± 0.30	0.14 ± 0.004	0.11 ± 0.003	0.12 ± 0.002	0.09 ± 0.02
	0.1[b]	38.5 ± 1.56	0.19 ± 0.05	0.13 ± 0.03	0.16 ± 0.04	0.12 ± 0.03
	0.2[b]	36.3 ± 0.56	0.23 ± 0.06	0.16 ± 0.05	0.19 ± 0.05	0.15 ± 0.05
HPMC	0.012[a]	40.8 ± 0.99	0.15 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
	0.036[a]	37.3 ± 1.33	0.18 ± 0.04	0.14 ± 0.04	0.15 ± 0.04	0.14 ± 0.03
	0.06[a]	39.2 ± 1.63	0.17 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	0.14 ± 0.05
	1.0[b]	33.2 ± 1.51	0.20 ± 0.06	0.15 ± 0.05	0.16 ± 0.05	0.13 ± 0.05
	2.0[b]	33.5 ± 0.29	0.17 ± 0.03	0.13 ± 0.02	0.15 ± 0.03	0.11 ± 0.02
SLS	0.01[a]	46.9 ± 2.50	0.25 ± 0.07	0.24 ± 0.07	0.21 ± 0.07	0.23 ± 0.07
	0.02[a]	40.9 ± 1.11	0.34 ± 0.04	0.32 ± 0.05	0.30 ± 0.04	0.31 ± 0.05
	0.04[a]	45.9 ± 0.56	0.35 ± 0.11	0.39 ± 0.12	0.30 ± 0.10	0.31 ± 0.10
	0.1[a,c]	36.6 ± 1.23	2.48 ± 1.08	2.14 ± 0.96	2.23 ± 0.98	1.98 ± 0.87
	0.17	Not determined due to disruption of monolayer integrity				
PEG 400	0.015[b]	40.7 ± 0.96	0.34 ± 0.04	0.29 ± 0.04	0.30 ± 0.04	0.27 ± 0.04
	0.06[a]	38.9 ± 0.49	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
	0.18[a]	37.2 ± 0.53	0.22 ± 0.06	0.18 ± 0.05	0.20 ± 0.05	0.14 ± 0.04
	0.30[a]	37.2 ± 0.68	0.19 ± 0.06	0.16 ± 0.05	0.18 ± 0.05	0.12 ± 0.04

The Effect of Excipients on the Permeability of BCS Class III Compounds and Implications for Biowaivers

Alan Parr[1] • Ismael J. Hidalgo[2] • Chris Bode[2] • William Brown[3] • Mehran Yazdanian[4] • Mario A. Gonzalez[5] • Kazuko Sagawa[6] • Kevin Miller[1] • Wenlei Jiang[7] • Erika S. Stippler[3]

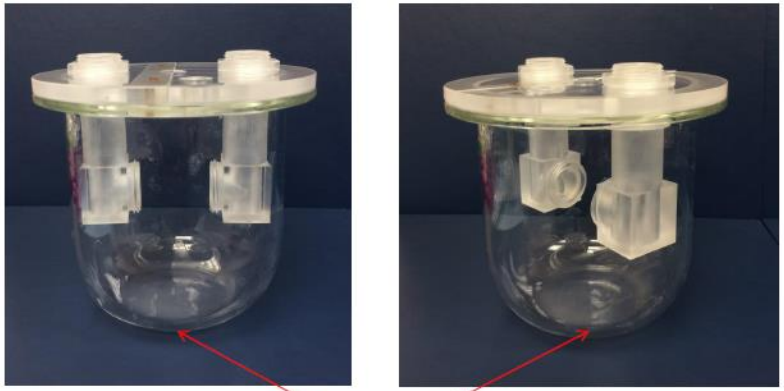
Data presented as mean ± SEM ($n = 4$)

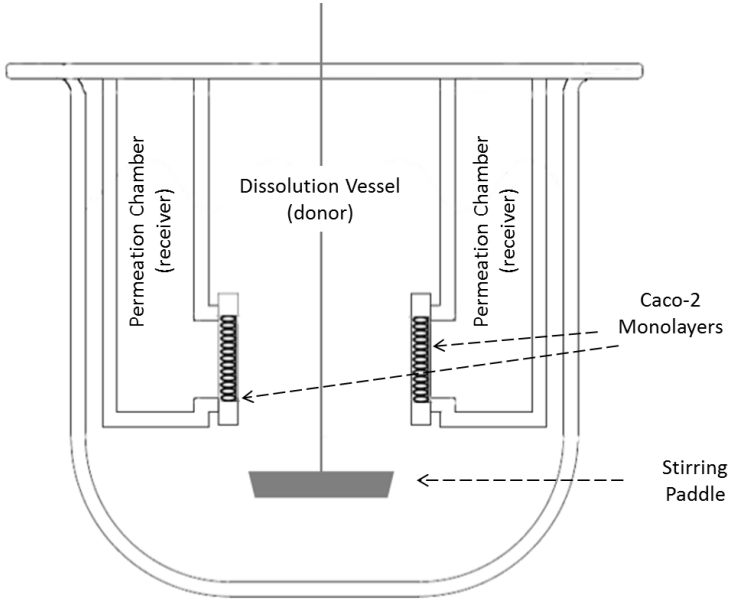
[a,b] Two separate sets of controls were run, each in parallel with a batch of test wells with excipients

[c] Integrity of Caco-2 cell monolayers was compromised (LY P_{app} 0.18×10^{-6} cm/s with buffer only; 0.30×10^{-6} cm/s with test compounds only (no excipients); 0.05×10^{-6} cm/s with 0.01 mg/ml SLS; 0.16×10^{-6} cm/s with 0.02 mg/ml SLS; 0.20×10^{-6} cm/s with 0.04 mg/ml SLS; 2.20×10^{-6} cm/s with 0.1 mg/ml SLS; 4.61×10^{-6} cm/s with 0.17 mg/ml SLS)

Furthermore……, Innovation: IDAS

***In Vitro* Dissolution Absorption System** combines traditional dissolution testing with a means to **determine and quantify** interactions with a bio-relevant membrane.





Biopharmaceutics Dissolution with Better *In Vivo* Correlation

Case Study 4 – Impact of Excipients (IDAS)

Funded by a 2-year FDA Contract: "EXPANDING BCS CLASS 3 WAIVERS FOR GENERIC DRUGS TO NON-Q1/Q2 Products"

Aim 1:

System Suitability: Qualification of IDAS for assessment of excipient effects on drug permeation. Effects of individual excipients on permeation: cassette of five model drugs (four Class 3, one Class 1) +/- individual excipients (**total of 15 excipients** × 3 concentrations of each)

Aim 2:

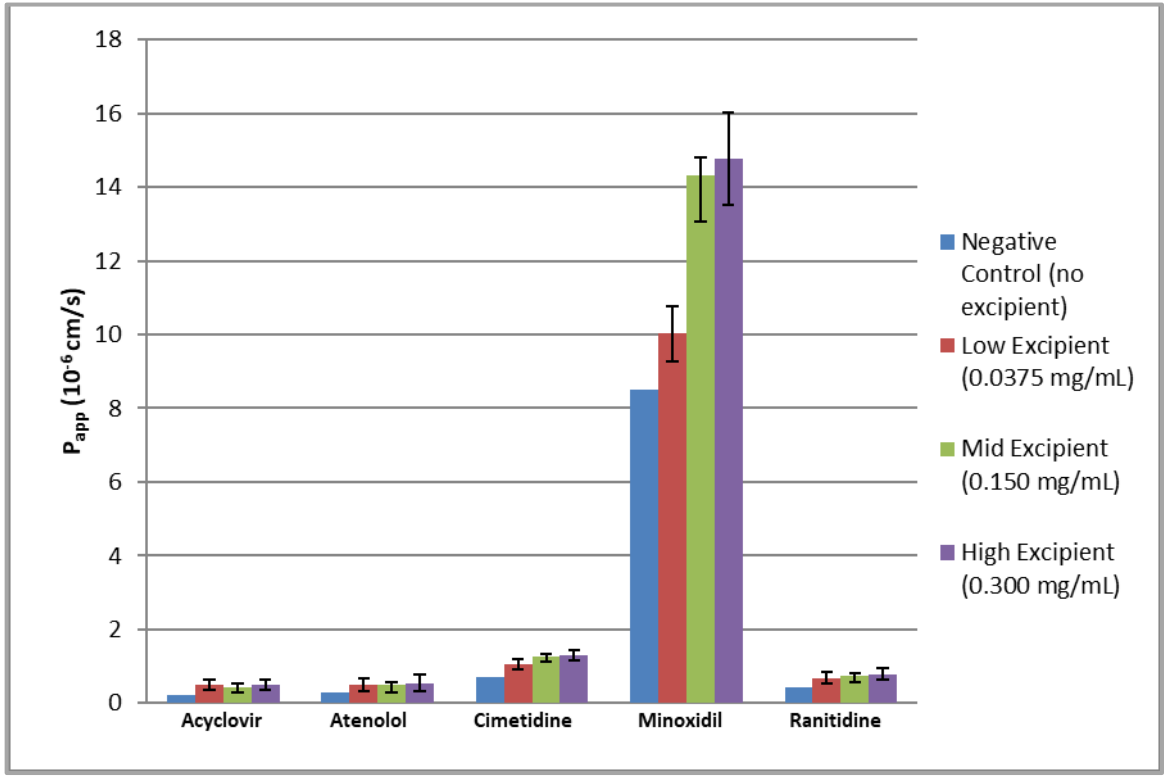
IVIVC: Individual Class 3 drug products with published clinical data on drug absorption with different formulations (i.e., different combinations of excipients) tested in IDAS with the same formulations tested clinically. Retrospective analysis to establish IVIVC with available clinical BE data.

FDA BAA-19-00123 (2019-2021)

Functional Class(es) of Test Excipients

Excipient	Functional Class(es)
Povidone K30	Disintegrant, dissolution enhancer, binder
Hydroxypropyl methylcellulose 2910 (4000 mPa·s)	Binder, dispersing agent
Hydroxypropyl methylcellulose 2910 (15 mPa·s)	Binder, dispersing agent
SLS	Anionic surfactant, lubricant
PEG-400	Solvent, lubricant
Lactose monohydrate	Filler/diluent
Microcrystalline cellulose	Filler/diluent, disintegrant
Magnesium stearate	Lubricant
Croscarmellose sodium	Disintegrant
Sorbitol	Filler/diluent
Dibasic calcium phosphate dihydrate	Filler/diluent, binder
Silicon dioxide	Glidant
Pregelatinized starch	Binder, disintegrant, filler/diluent (at higher amounts)
Mannitol	Filler/diluent, sweetener, plasticizer, tonicity agent
Talc	Lubricant, glidant, anti-caking agent, diluent

Effects of SLS on P_{app} of Model Drugs (IDAS)



Improved dynamic range for testing:

- Potentially up to an order of magnitude higher vs. Transwell
- Able to test 0.3 mg/mL of SLS vs. 0.04 mg/mL in the Transwell format

Improved confidence in outcomes (via comparison to clinical correlates):

- Impact at the low excipient SLS concentration is in-line with the Transwell format and supports no impact on permeation at this concentration of SLS

Summary of Results and Implications (IDAS)

1

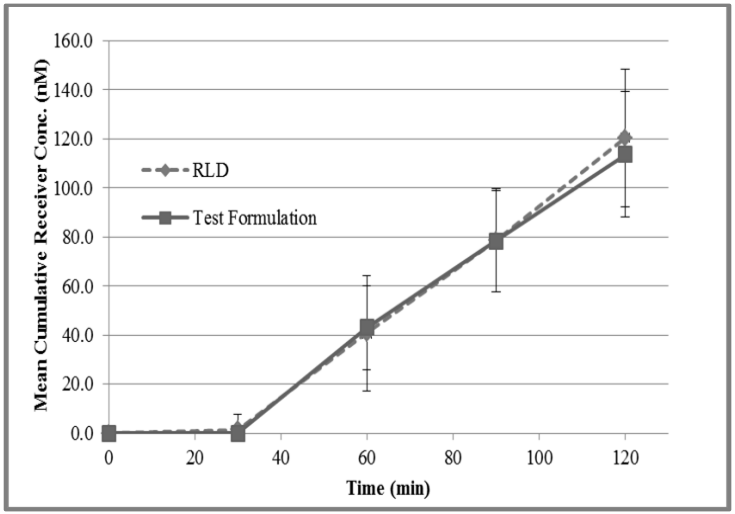
Caco-2 cell monolayers in IDAS were less sensitive to excipients than in Transwells, a format in which the cells are overly sensitive to excipients. This may be due to the geometry (vertically oriented cell monolayers) and more effective mixing (apical surface of the cell monolayer exposed to the dissolution chamber, which is agitated by a paddle).

2

Most of the excipients tested had little or no effect on the permeation of Class 3 drugs, suggesting that expanding biowaivers to non-Q1/Q2 formulations within a certain range for a Class 3 drug biowaiver may be possible. This could have important consequences for the development and regulatory approval of generic drugs.

Case Study 4 – Impact of Excipients (IDAS)

Permeability Comparison of RLD and Test Formulation Tablets using IDAS



Statistical Comparison of RLD and Test Formulation Tablets

Formulation		RLD	Test	P*
Cumulative Receiver Conc. (nM) (Mean ± SD)#	30 min	1.38 ± 6.46	0.00 ± 0.00	N/A
	60 min	40.8 ± 23.5	43.0 ± 17.3	
	90 min	78.7 ± 21.1	78.3 ± 20.8	
	120 min	120 ± 28.1	114 ± 25.6	
AUC (nM × min)		**5433 ± 1616**	**5421 ± 1396**	0.977
AUC/dose (nM × min/nmol)		0.0402 ± 0.0120	0.0401 ± 0.0103	N/A
Flux (nmol/min/cm^2)		9.33E-03 ± 2.20E-03	8.82E-03 ± 1.95E-03	0.415

* Two-tail P, calculated with a two sample t-test assuming equal variances. Two data sets can be considered similar if P > 0.05.

Mean values were calculated with n = 22 replicates for the RLD formulation and n = 24 replicates for the test formulation, depending on individual acceptance.