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Drug Permeability: Best Practices for BCS-based Biowaivers

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M-CERSI workshop
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Agenda

- Establishment of in-house validated *in vitro* models for permeability
  - Caco-2
  - Intestinal animal tissue
- Case studies

Biowaiver guideline changes

	2015	2017	2021
BCS	1 & 3		Different salts for BCS1 allowed
Dosage form	IR solid oral Not for narrow therapeutic window Not for products absorbed in oral cavity		Suspensions Only when mode of administration includes water
Solubility	Highest strength Soluble in 250 mL or less @ pH 1 - 6.8 @ 37° ± 1°C; 3 replicates or more pH check (start/end) Shake-flask, acid-base titrations Degradation of drug should be reported	Additional information needed when the highest single does is not the same as the highest strenght – linearity of PK	Highest single therapeutic dose Solubility maintained over relevant timeframes to accomodate expected duration of absorption. Smaller volumes allowed NMT 10% degraded
Papp	F_{abs} in humans Rate&mass transfer through human intestinal membrane Perfusion studies on humans Alternative models for passive permeation: in situ/in vivo animal, in vitro epithelial cell cultures Model suitability demonstration More than one method may be used when conflicting evidence F_{abs} ≥ 85 % or ≥ 85 % unchanged drug recovered in urine/recovered in urine as drug + metabolites	Mass recovery for in vitro models > 80 %	F_{abs} ≥ 85 % ≥ 85 % unchanged drug recovered in urine/recovered in urine as drug + Phase I, phase II metabolites For mass balance: metabolites from feces, if they were formed after absorption; unchanged drug secrected by biliary route, intestinal secretion or from unstable metabolite converted back to drug by microbiota. Only Caco-2 in vitro model allowed.
Stability	Stability in the GI tract unless ≥ 85 % uncganged drug recovered in urine In vivo/in situ perfusion methods that are based on measuring the loss of drug from application site Aspirate HGF/HIF or SGF/SIF, incubate @ 37°C for 1h/3h; NMT 5% degradation		Required for mass balance studies and for Caco-2 studies. Degradation NMT 10%.
Disso	AP1, 100 rpm or AP2, 50 rpm (75 rpm when justified, coning) 500 mL or less (0.1N HCl or SGF, pH 4.5, pH 6.8 or SIF) Rapidly dissolving: NLT 85% @ 30 min Very rapidly dissolving: NLT 85% @ 15 min f_2		900 mL or less; AP1, 100 rpm or AP2, 50 rpm, Coning BCS 1: test and reference both either ≥ 85% @ 15 min or @ 30 min, or use f_2
Excipients	Do not affect rate or extent of absorption for BCS 1, new/atypical excipients, high quantities, surfactants, sweeteners BCS 3: qualitatively the same, quantitatively very similar	Criteria of BCS 3 quantitative changes in excipients listed based on excipient role	Amount of excipient, the mechanism by which it could affect absorption and drug absorption propeprties. Slowly absorbed BCS 1 Qualitative and quantitative differences allowed (± 10 %)
Prodrugs	P_{app} determined for species that is absorbed Solubility for both important		Only when absorbed as prodrugs

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Validation of in-house Caco-2 (and rat intestine model)

Caco-2

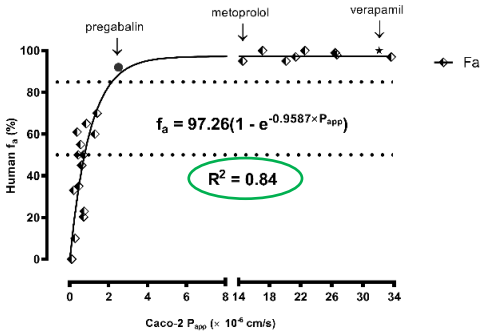

Figure 5. Correlation of human fraction absorbed (fa) and apparent permeability coefficients (Papp) determined in Caco-2 cell model. Dotted line represents cutoff value for fa 50% and 85%.

Rat intestine

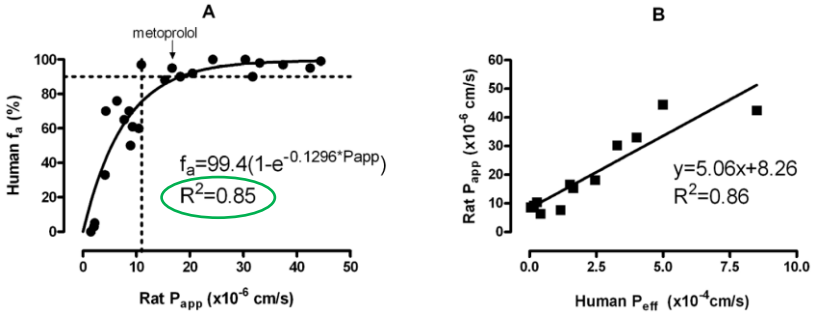

Figure 2. (a) Correlation of human fraction absorbed (f_a) and apparent permeability coefficients (P_{app}) determined in isolated rat jejunum tissue. Dotted line represents cutoff values for $f_a = 90\%$ and $P_{app} = 11 \times 10^{-6}$ cm/s. (b) Correlation of rat P_{app} and human P_{eff} values. Results are shown as mean values; refer to Table 1 for sample size.

Requirements:

Min 5 low, moderate and high permeability model drugs

Zero permeability marker

Min 3 cell assay replicates

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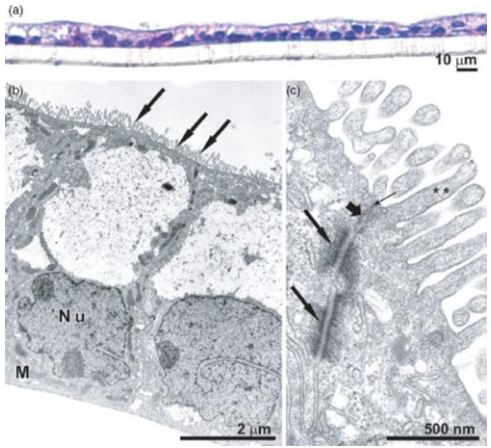
Suitability of Isolated Rat Jejunum Model for Demonstration of Complete Absorption in Humans for BCS-Based Biowaiver Request, *Journal of Pharmaceutical Sciences*, 2012.
Demonstrating suitability of the Caco-2 cell model for BCS-based biowaiver according to the recent FDA and ICH harmonised guidelines, *Journal of Pharmacy and Pharmacology*, 2019.

Validation of in-house Caco-2 (and rat intestine model)

Integrity/vitality criteria

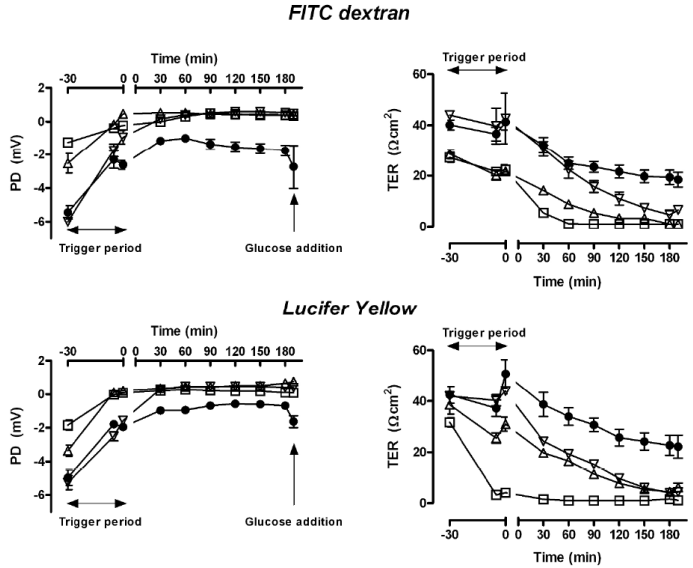
Caco-2

TEER @21 day of growth
TEER start of exp
TEER end of exp
Lucifer yellow



TEER – transepithelial electrical resistance
PD – potential difference

Rat intestine



TEER start of exp
TEER end of exp
PD start of exp
PD end of exp
PD after addition of glucose
FITC dextran

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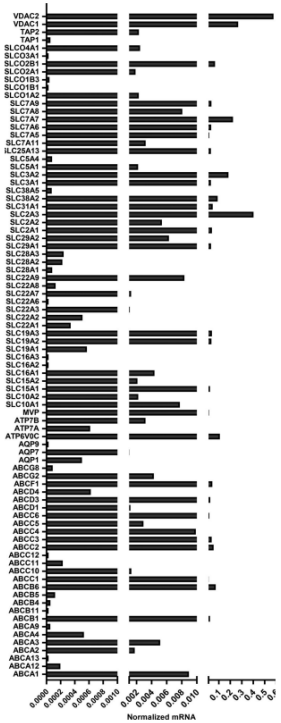
Validation of in-house Caco-2 (and rat intestine model)

Caco-2



Gene expression

Gene expression for key absorptive and secretory transporters known.

Rat intestine

Number	Gene symbol	Protein name	Rat jejunum	Human[a] jejunum	Comparison
1	*Abcb11*	MDR11			Different
2	*Abcb1b*	MDR1B			Different
3	*Abcb4*	MDR4			Same
4	*Abcc1*	MRP1			Different
5	*Abcc2*	MRP2			Different
6	*Abcc3*	MRP3			Different
7	*Abcc4*	MRP4			Same
8	*Abcc5*	MRP5			Same
9	*Abcc6*	MRP6			Same
10	*Abcg2*	WHITE2			Same
11	*Slc10a1*	NTCP			Different
12	*Slc10a2*	ISBT			Different
13	*Slc15a1*	PEPT1			Same
14	*Slc15a2*	PEPT2			Same
15	*Slc16a1*	MCT1			Same
16	*Slc22a1*	OCT1			Different
17	*Slc22a2*	OCT2			Same
18	*Slc22a3*	OCT3			Same
19	*Slc22a6*	OAT1			Same
20	*Slc22a7*	OAT2			Same
21	*Slc22a8*	OAT3			Same
22	*Slc22a9*	OAT4			Same
23	*Slc28a3*	CNT3			Same
24	*Slco2b1*	OATP-B			Different
25	*Slco3a1*	OATP-D			Same
26	*Slco4a1*	OATP-e			Same

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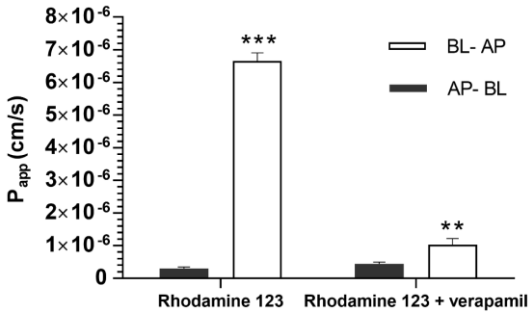
Validation of in-house Caco-2 (and rat intestine model)

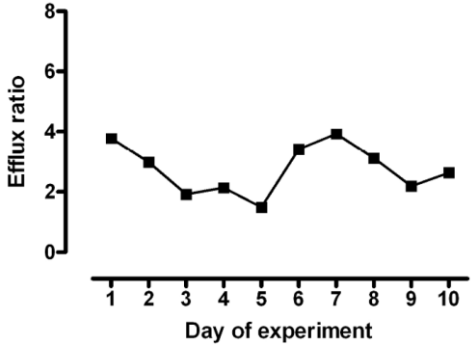
P-glycoprotein

Caco-2



Rat intestine

pH		$P_{app} \times 10^{-6}$ (cm/s)		
Donor (Mucosa)	Acceptor (Serosa)	MS	SM	Efflux Ratio
7.0	7.4	6.2 ± 0.7 ($n = 15$)	$16.9 \pm 1.1^{**}$ ($n = 17$)	2.7
7.4	7.4	5.5 ± 0.4 ($n = 4$)	$19.0 \pm 0.8^{**}$ ($n = 4$)	3.5
7.4	7.0	6.6 ± 0.8 ($n = 6$)	$14.3 \pm 0.5^{**}$ ($n = 6$)	2.2



P-glycoprotein expressed & functional.
Inter-subject variability in ER on rat determined to be at least 2.

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Case 1: Levetiracetam

Literature data
- Highly soluble
- Highly permeable
- Not a narrow therapeutic window drug
- In 2015, 282 levetiracetam IR products available

Highest strength: 1000 mg
Max single dose: 3000 mg

Aim of the Study	Observed Effect	Comment
Extent of absorption	Rapid absorption, T_{max} = 1.3 h, 95% recovery in urine	Absorption is almost complete (>95%)
Food Interaction	T_{max} delayed, C_{max} unaffected	Levetiracetam can be ingested with or without meals
Protein binding	Less than 10%	Protein binding displacement interactions with other highly protein bound drugs is unlikely to occur
Distribution	V_d = 0.5–0.7 L/kg	Value close to total body water, that is, rapidly distributes into tissue with concentration approximating the one in blood.
Interactions with active transport/elimination	C_{max} and AUC are dose proportional in doses up to 5000 mg	Absorption/elimination is most probably not modulated via active transporters.
Metabolism	Non-hepatic, in blood	Unlikely to produce clinically relevant interactions through induction/inhibition of CYP reactions
Plasma elimination $t_{1/2}$	6–8 h (healthy adults)	Dosage adjustments are not necessary when patients are co-medicated with other AEDs; adjustment needed in children and elderly population.
	6–8 h (adults with epilepsy)[a] 10–11 h (elderly) 5–7 h (children with epilepsy)	

Suitability of Isolated Rat Jejunum Model for Demonstration of Complete Absorption in Humans for BCS-Based Biowaiver Request, *Journal of Pharmaceutical Sciences*, 2012
Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Levetiracetam, *Journal of Pharmaceutical Sciences* 104:2676–2687, 2015

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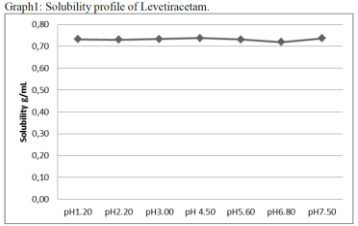
Case 1: Levetiracetam

Solubility

High solubility in pH range 1.2 – 7.5 (0.72-0.74 g/ml) (shake-flask method).

Graph1: Solubility profile of Levetiracetam.



Solubility (g/mL)		pH1.20	pH2.20	pH3.00	pH 4.50	pH5.60	pH6.80	pH7.50
Flask 1	1	0.734	0.730	0.734	0.737	0.732	0.721	0.736
	2	0.731	0.733	0.735	0.737	0.731	0.717	0.736
Flask 2	1	0.732	0.729	0.732	0.738	0.732	0.719	0.737
	2	0.731	0.733	0.736	0.737	0.732	0.719	0.737
Flask 3	1	0.732	0.730	0.734	0.739	0.732	0.721	0.737
	2	0.734	0.729	0.734	0.738	0.732	0.719	0.736
Mean		**0.73**	**0.73**	**0.73**	**0.74**	**0.73**	**0.72**	**0.74**
SD		0.001	0.002	0.001	0.001	0.000	0.001	0.000
RSD		0.18	0.22	0.16	0.11	0.07	0.17	0.06
D/S (1000 mg) mL		**1.4**	**1.4**	**1.4**	**1.4**	**1.4**	**1.4**	**1.4**

Dissolution

	Dissolution Study	
	% Dissolved of levetiracetam from 1000 mg tablets in 15 min [mean values ($n = 12$) ± SD[a]]	
Medium	Reference-UCB	Test-Sandoz
0.1 M HCl	100.9 ± 1.3	94.8 ± 3.1
Acetate buffer pH 4.5	100.0 ± 2.1	100.9 ± 1.9
Phosphate buffer pH 6.8	104.9 ± 1.3	99.7 ± 4.7
	% Dissolved of levetiracetam from 1000 mg tablets in 30 min [mean values ($n = 12$) ± SD[a]]	
0.1 M HCl	102.3 ± 1.7	99.1 ± 1.2
Acetate buffer pH 4.5	102.3 ± 2.3	102.3 ± 2.6
Phosphate buffer pH 6.8	104.4 ± 1.2	102.1 ± 1.6

Very fast drug release:
> 85% @ 15 min

Permeability

Permeability Method and used Media	Compound	pH Donor/Acceptor	Permeability ($\times 10^{-6}$ cm/s)
Isolated rat intestine S to M (Ringer's buffer solution)	Levetiracetam	7.4/7.4	25.8 ± 5.2
Isolated rat intestine M to S (Ringer's buffer solution)	Levetiracetam	7.4/7.4	30.6 ± 1.5
	Atenolol (low permeability marker)	7.4/7.4	8.9 ± 1.4
	Metoprolol (high permeability marker)	7.4/7.4	22.0 ± 1.4

High permeability
ER ≈ 1 (no active transport)

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Case 1: Levetiracetam

Published bioequivalence studies:

Population	Dosage	Pharmacokinetic Parameters			
		C_{max} (µg/mL)	90% CI	AUC_t (µg h/mL)	90% CI
Healthy adults ($N = 24$),	500 mg tablets versus 500 mg capsules	15.74 ± 3.93 versus 15.33 ± 4.49	–	115.87 ± 27.81 versus 115. 21 ± 27.64	–
Healthy adults ($N = 22$), fed conditions	750 mg tablets test versus reference[a]	19.64 ± 3.85 versus 20.05 ± 4.13	94.5–102.0	176.27 ± 31.91 versus 174.73 ± 33.26	98.1–104.0
Healthy adults ($N = 22$), fasting conditions	750 mg tablets test versus reference[a]	21.62 ± 5.70 versus 23.50 ± 6.25	85.0–100.0	173.93 ± 32.44 versus 174.27 ± 31.78	96.2–103.0
Healthy adults ($N = 21$), fed conditions	1000 mg tablets test versus reference[a]	26.32 ± 5.06 versus 24.93 ± 4.43	98.6–112.0	249.26 ± 44.18 versus 252.52 ± 39.86	95.9–101.0
Healthy adults ($N = 29$), fasting conditions	1000 mg tablets test versus reference[a]	30.28 ± 7.76 versus 31.51 ± 8.84	92.4–101.0	269.17 ± 47.01 versus 272.50 ± 48.08	97.2–100.0
Healthy adults ($N = 17$), fasting conditions	1500 mg oral tablets versus i.v. solution	47.70 ± 13.50 versus 50.50 ± 15.89	91.6–117.4	414.70 ± 88.86 versus 378.60 ± 73.20	88.3 – 95.3
Healthy adults ($N = 24$),	750 mg oral tablets versus 10% oral solution	20.30 ± 3.9 versus 21.10 ± 4.0	–	195.20 ± 35.0 versus 193.21 ± 35.3	–

In-house bioequivalence study:

Parameter	Bioequivalence Study ($N = 21$)		
	Ratio LSM[b] (Test/Reference)	90% Confidence Intervals	Intrasubject CV[c]
AUC_t	100.07%	97.44%–102.78%	5.0%
AUC_i	100.03%	97.38%–102.75%	5.0%
C_{max}	99.95%	93.61%–106.71%	12.2%

Possible effects of excipients unlikely (several published studies, 282 products with various compositions found BE)

Low variability of PKs.
Low intrasubject CV.
Narrow CI.

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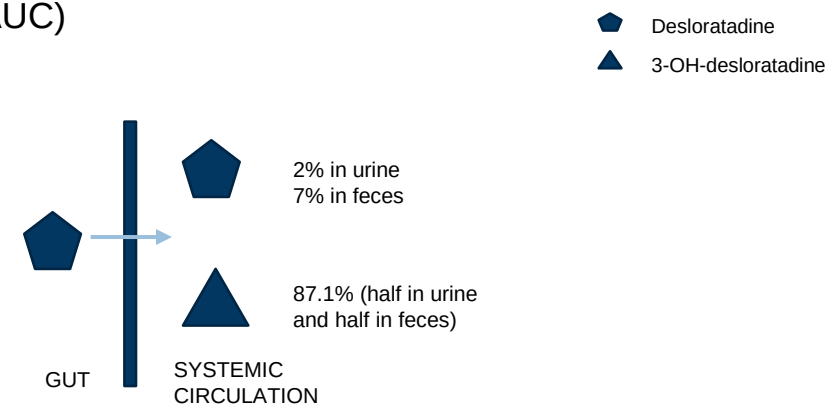
Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Levetiracetam, *Journal of Pharmaceutical Sciences* 104:2676–2687, 2015

Case 2: Desloratadine

- Antihistamine drug
- IR tablets (1 tbl of 5 mg/day)
- Linear pharmacokinetics 5 - 20 mg in healthy
- Pgp substrate (grapefruit no impact on C_{max}, AUC)
- Literature suggests BCS 1 (?)

	Solubility (mg/mL)	Dissolved in 250 mL
HCl	39.7	> 9 g
Water	0.1	25 mg
Phosphate pH 7.4	1.5	375 mg



Solubility
Intestinal stability
Permeability
Dissolution

F_{abs} > 90 % (?)
Inconclusive data, no literature

When is fecal fraction of metabolites formed?

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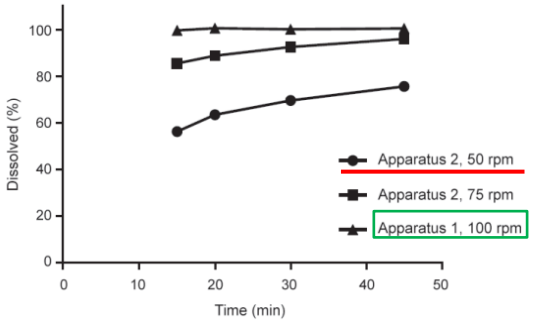
Biopharmaceutical classification of desloratadine – not all drugs are classified the easy way, *Acta Pharm*. 70 (2020) 131–144

Case 2: Desloratadine

Solubility

Media	Equilibrium solubility (mg mL^{-1}) and RSD	Volume of media needed to dissolve 5 mg dose (mL)
0.1 mol L^{-1} HCl	40.49 (0.4 %)	0.123
Acetate buffer pH 4.5	10.65 (1.4 %)	0.469
Sodium phosphate buffer pH 6.8	2.46 (1.2 %)	2.03
Water	0.92 (3.4 %)	5.43

Dissolution



Intraluminal stability

Recoveries:
- 102.6 % in SIF
- 99.6 % in SGF
- 101.1 % in SGF with pepsin

Time (min)	0.1 mol L^{-1} HCl	Acetate buffer pH 4.5	Phosphate buffer pH 6.8
10	100.4	102.5	103.3
15	101.0	102.1	102.6
20	104.7	102.0	102.5
30	101.0	103.0	102.6
45	100.3	102.3	102.8

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Biopharmaceutical classification of desloratadine – not all drugs are classified the easy way, *Acta Pharm*. 70 (2020) 131–144

Case 2: Desloratadine

PAMPA

DONOR pH	ACCEPTOR pH	Average DESLORATADINE permeability ($\times 10^{-6}$ cm s^{-1})	Average METOPROLOL permeability ($\times 10^{-6}$ cm s^{-1})
5.5	7.4	1.13 (n = 8; RSD 32 %)	1.09 (n = 8; RSD 31 %)
6.8	7.4	7.81 (n = 8; RSD 17 %)	4.60 (n = 8; RSD 25 %)
7.4	7.4	16.6 (n = 8; RSD 9.7 %)	9.09 (n = 8; RSD 22 %)

Permeability

High permeability
Not valid for agencies

Rat intestine – side-by-side diffusion chambers

	1 h	2 h
Unexposed standard solution in glassware (*), room temperature	100 %	99.1 %
Carbonated standard solution in glassware, 37 °C	100.4 %	101.3 %
Carbonated standard solution in side-by-side diffusion chambers, 37 °C	90.5 %	90.5 %
Carbonated standard solution incubated with intestinal rings, 37 °C	38.9 %	34.0 %

Too low recoveries to determine P_{APP} (LC-MS no metabolites).
Incubation with intestinal rings show high tissue retention (50% recovered with tissue extraction).
10% plastic binding.

Caco-2

Direction	TEER start (Ωcm²)	TEER end (Ωcm²)	Lucifer Yellow (cm/s)	Desloratadine (cm/s)	Metoprolol (cm/s)
AP→BL	1338	706	4.9×10^{-7}	9.5×10^{-6}	9.4×10^{-6}
BL→AP	1202	847	4.7×10^{-7}	16.7×10^{-6}	

Integrity and vitality
ER = 1.7

High permeability

Less biological material available for tissue binding.

Recoveries > 80%.

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Biopharmaceutical classification of desloratadine – not all drugs are classified the easy way, *Acta Pharm*. 70 (2020) 131–144

Case 3: Prodrug permeability classification

FDA guidance 2001; 2017:

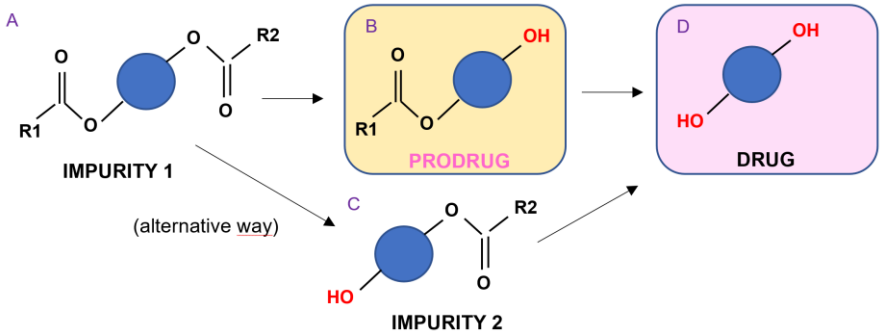
Prodrugs
Permeability of prodrugs will generally depend on the mechanism and (anatomical) site of conversion to the drug substance.
*When the **prodrug-to-drug** (i.e., active moiety) conversion is shown to occur predominantly after intestinal membrane permeation, the **permeability of the prodrug** should be measured.*
*When this conversion occurs prior to intestinal permeation, the **permeability of the drug** should be determined.*
Dissolution and pH-solubility data on both prodrug and drug can be relevant. Sponsors may wish to consult with appropriate review staff before applying the BCS approach to IR products containing prodrugs.

FDA guidance 2021:

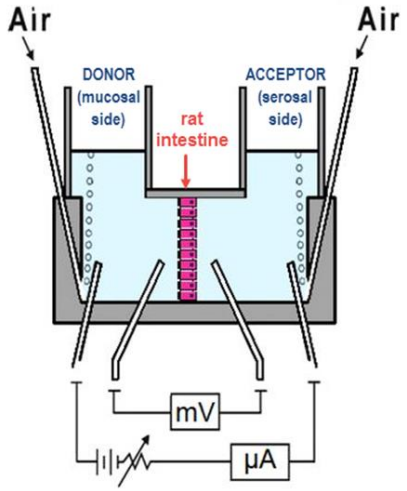
*Pro-drugs may be considered for a BCS-based biowaiver **when absorbed as the pro-drug**.*

Should drug permeability in prodrug cases again be included in the guidelines?

Case 3: Prodrug permeability classification



A - Impurity 1 (or pro-produg?) (0.15% in formulation)
B - Prodrug
C - Impurity 2
D - Drug



Aim #1

To evaluate the rate of hydrolysis of a prodrug at 37°C when in the contact with the rat intestine (and its brush-border enzymes) at donor side (mucosal side) and after permeability (acceptor, serosal side) (Easy Mount diffusion (Ussing) chambers).

Experiments

Prodrug was added to the donor compartments. The samples were withdrawn from donor and acceptor compartments at predetermined time intervals.

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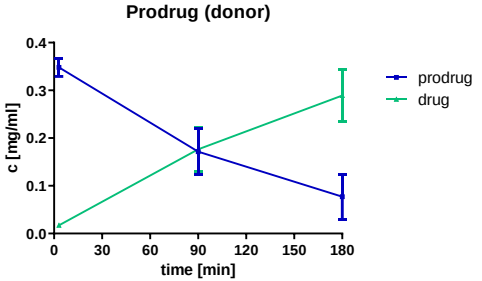
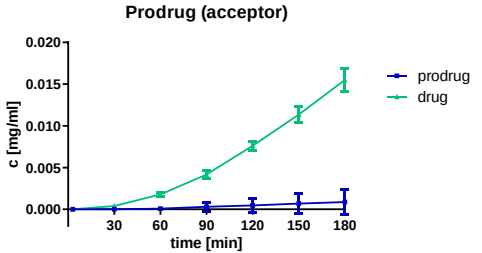
Case 3: Prodrug permeability classification

RESULTS #1

- Prodrug is rapidly hydrolyzed at donor (mucosal) side by intestinal brush border enzymes, even prior drug absorption.
- The major compound detected in acceptor is drug, the hydrolysis most probably occurs also at acceptor side.
- It remains unclear what is the ratio between both forms at absorption/permeation.
- In the guidelines it is clear that prodrugs may be considered for a BCS-based biowaiver when absorbed as the pro-drug. However, *in vivo* very seldom only a prodrug is absorbed.





<u>Literature data:</u> The main site of prodrug-drug conversion is believed to be blood. ✗

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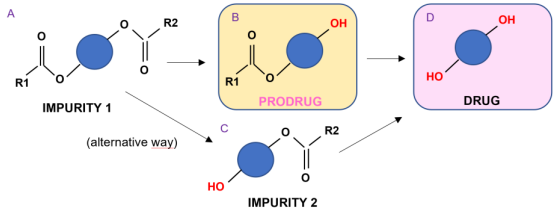
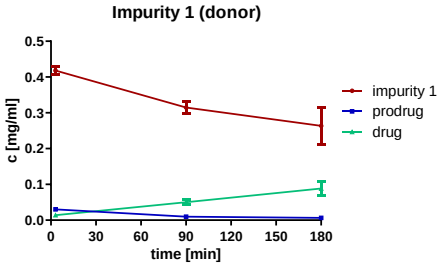
Case 3: Prodrug permeability classification

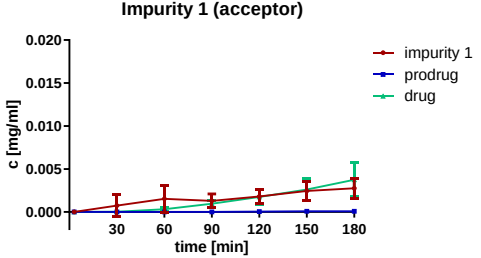
Aim #2
To evaluate the hydrolysis of Impurity 1 (pro-prodrug?) before and after permeation.

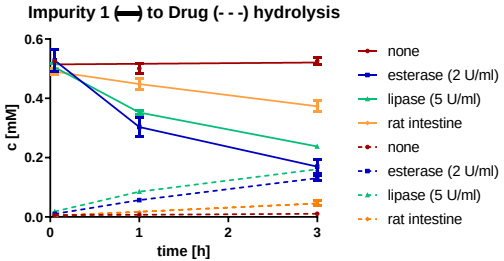


RESULTS #2

- Impurity 1 is formed during prodrug synthesis (process-related impurity).
- It is hydrolyzed at donor (mucosal) side by intestinal brush border enzymes prior drug absorption.
- This was confirmed with commercially available enzymes esterase and pancreatic lipase.
- Due to presence of esterases in GIT and lipases excreted in duodenal fluid it can be concluded that Impurity 1 would hydrolyse rapidly to drug in human GIT.







Literature data:
Approx. 1,000 U of lipases per ml of duodenal fluid of adults. Between 25,000 and 40,000 U is required to digest a typical meal.

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Conclusions

Permeability studies from generic perspective for BCS biowaiver support:

- **BCS biowaiver approach is well accepted by the generic companies, because it can facilitate approval for highly soluble BCS1 and BCS3 drugs.**
- **Guidance could again allow complementary systems for drug permeability evaluation, such as rat intestine and other appropriate cell lines, to capture more complex absorption features (such as metabolism).**
- **BCS biowaiver approach could be applied to IR products containing prodrugs that convert to drugs also in the lumen or during permeation.**

Thank you

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