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Human Intestinal Organoids and Potential Application for PBPK Modeling

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OSP Community Conference 2025, Paris

Life forward

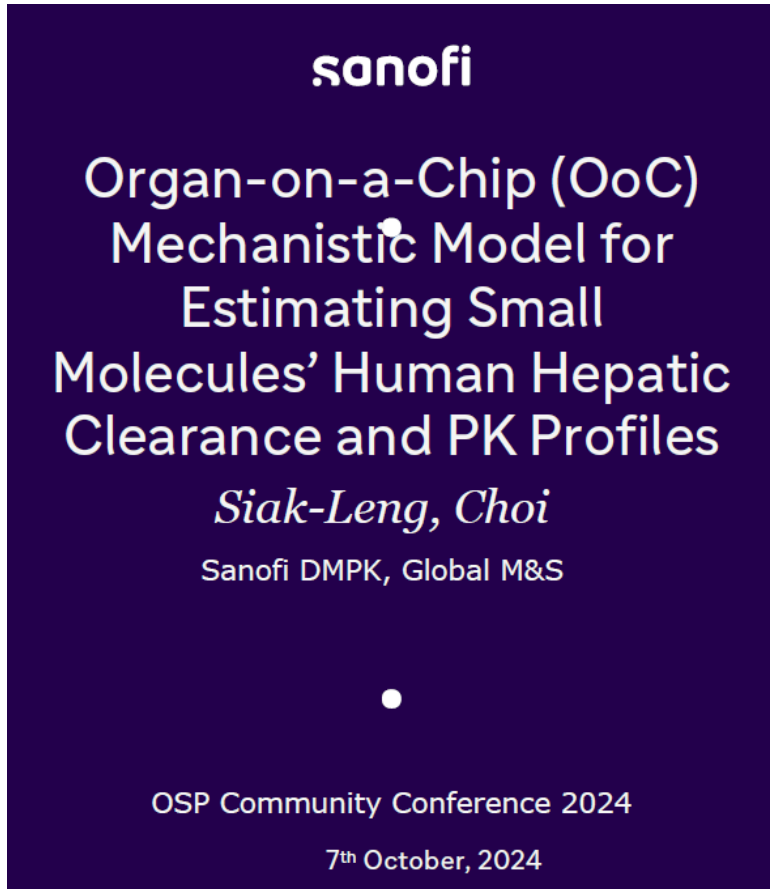
Microphysiological systems as alternative for animal testing

FDA NEWS RELEASE

FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

For Immediate Release: April 10, 2025

- In Vitro Human-Derived Systems (Organoids and Microphysiological Systems)
- In Silico Tools and Computational Modeling (PBPK modeling, AI/ML, QSP, etc.)



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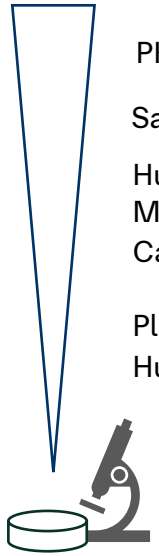
ADME Screening in Drug Discovery

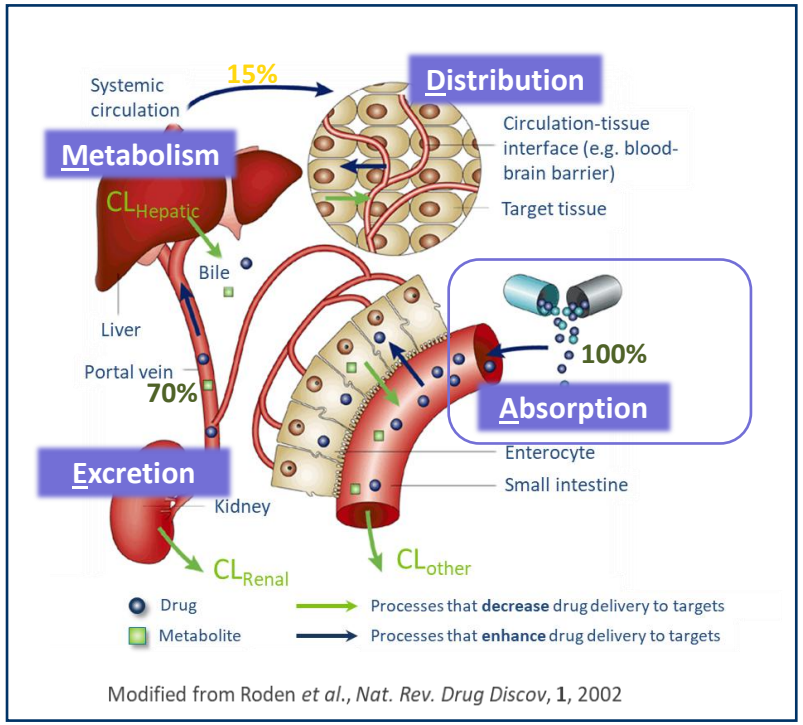


PK studies in animals

Sandwich-cultured human hepatocytes

Human cryo. hepatocytes
MDCK-MDR1 permeability
Caco-2 permeability

Plasma protein binding
Human liver microsomes



Oral Bioavailabiliy

$$F = F_a \times F_g \times F_h$$

Permeability
Transporter

GI metabolism

Hepatic extraction

Oral Drug Absorption: Caco-2 doesn‘t tell the whole story

- Advantages of Caco-2 model
  - Immortalized cell line
  - Automated cell culture, automated permeability assay
  - Tight monolayer, relevant P-gp expression level
  - Good in vitro/in vivo correlation regarding fraction absorbed in human intestine

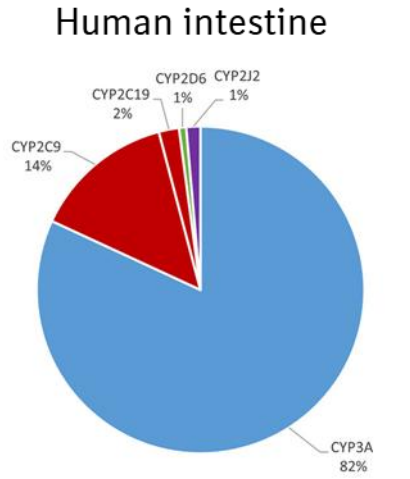
sanofi

Prediction of human fraction absorbed from in vitro Caco-2 permeability – are we there yet?

OSP Community Conference, Basel, Oct 7-8, 2024
Session II: In Vitro-In Vivo Extrapolation

Denise Feick, DMPK Modeling & Simulation, Sanofi, Frankfurt

- Disadvantages of Caco-2 model
  - CYP3A4 activities missing
  - Potentially different expression of additional drug metabolizing enzymes and transporters



Schelstraete et al. Sci Rep 9:9233 (2019)

- MPS models in evaluation
  - Static tissue models: Intestinal organoids as monolayer culture on Transwell inserts
  - Human intestinal organoids as organ-on-chip model

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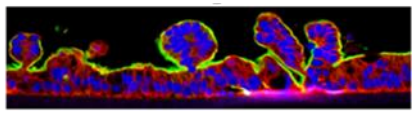
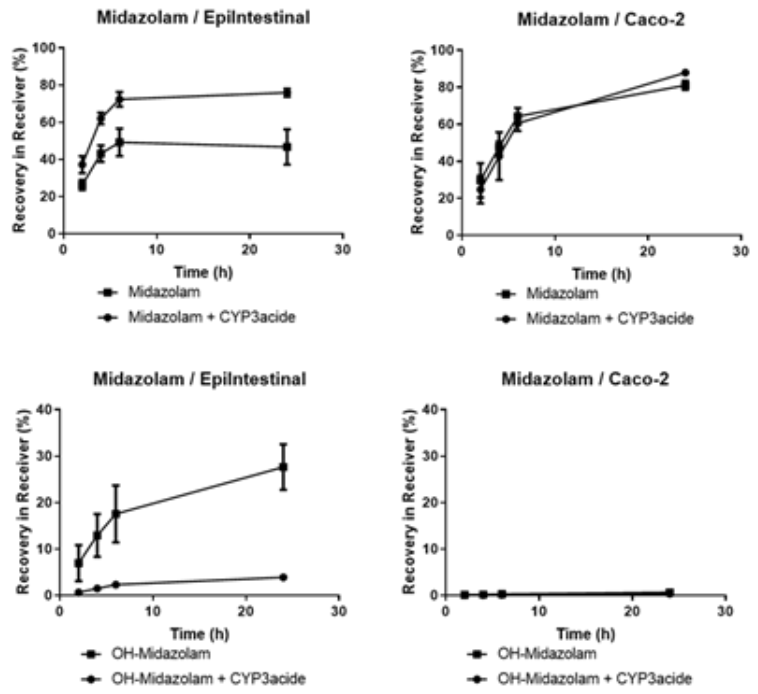
Intestinal Organoids as Monolayer Culture on Transwell Inserts: EpiIntestinal

EpiIntestinal® / MatTek

Villi Structures -
Brush Borders (Villin, green) -
Columnar Epithelium (K19, red) -

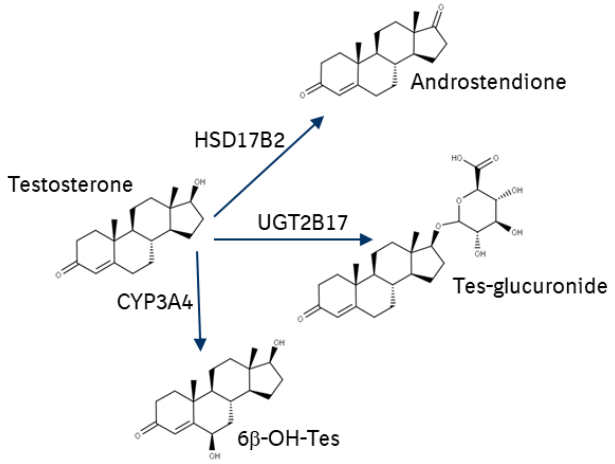


Midazolam metabolism



Cui, et al. Pharmaceutics, 12:405 (2020)

Testosterone metabolism



	Hepatocyte (% of Met)*	Enterocyte (% of Met)*	EpiIntestinal (% of Met)
Androstenedione	60	89	98
Tes-glucuronide	20	7	2
6β-OH-Tes	11	3	Trace

*: Taken from Zhang et al., Biochem Pharmacol, 156:32 (2018)

Unpublished results

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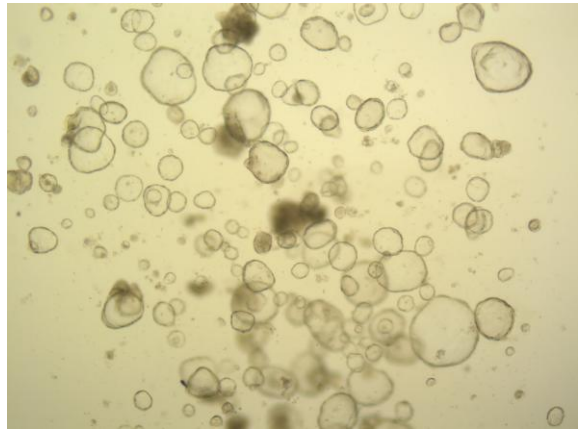
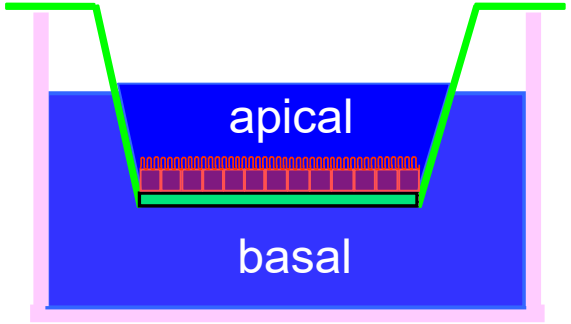
Differentiation of Intestinal Organoids to Intestinal Epithelial Monolayer

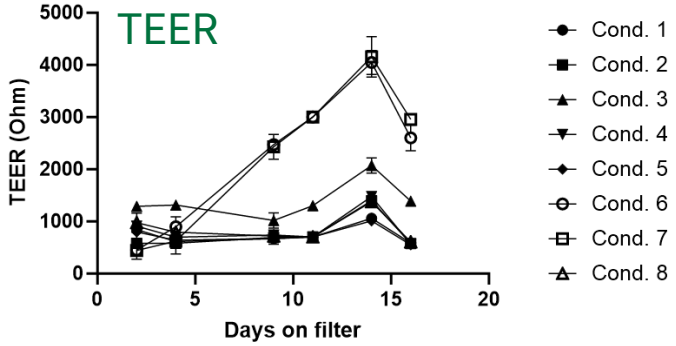


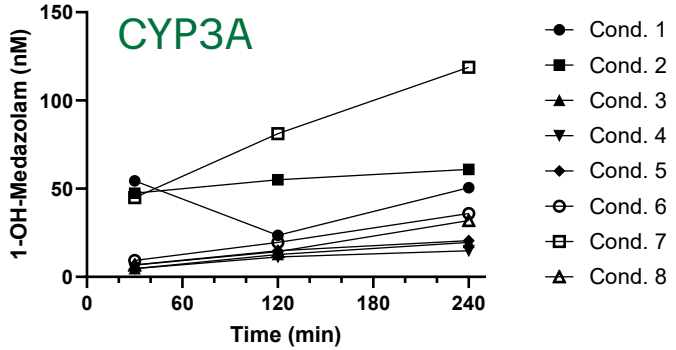
Human intestinal organoids
(HUB Organoids)

Seeding density
Growth media
Differentiation media
Extracellular matrix



Monolayer on Transwell





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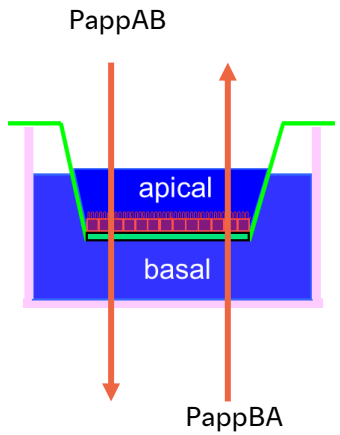
Differentiation of Human intestinal Organoids: Epithelial Barrier functions

Compound	HUB human intestinal orgnoids				Caco-2		Probe characteristics
	Ileum		Duodenum				
	PappAB (10^{-6} cm/s)	Efflux Ratio	PappAB (10^{-6} cm/s)	Efflux Ratio	PappAB (10^{-6} cm/s)	Efflux Ratio	
Atenolol	0.28	2.8			1.2	1.5	Low permeable
BI-1	0.40	1.0	0.17	3.9	0.37	1.2	Low permeable
BI-2	0.089	29	0.068	13	0.30	2.9	Low permeable, potentially P-gp
Rosuvastatin	0.30	8.7	0.22	3.8	0.60	10	Low permeable, BCRP and MRP2
Fexofenadine	0.36	9.4	0.088	13	6.6	1.1	Low permeable, multiple transporters
Apafant	1.5	23	1.4	18	2.4	11	P-gp
Apafant / Zosuquidar	4.3	0.84	2.7	0.80			
Otenzepad	0.45	38	0.16	81	0.10	192	Potentially P-gp
BI-3	1.1	37	2.7	14	1.3	22	Potentially P-gp
Cyclosporin A	8.3	5.3	38	0.81	28	0.70	P-gp



Unpublished results

- Differentiation of human intestinal organoids on Transwell inserts established and optimized
- Intact epithelial barrier functions in organoids as monolayer on Transwell inserts
- Comparable P-gp activities in Caco-2 and intestinal organoids
- Potential difference in activities of additional transporters

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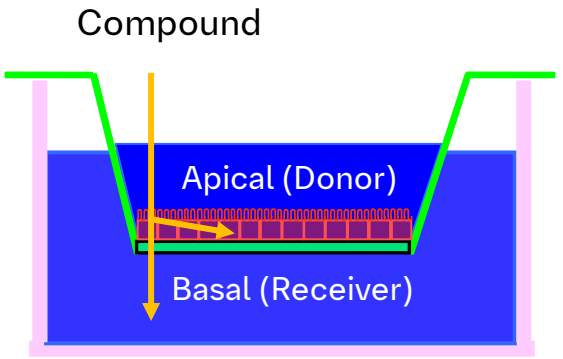
Prediction of first-pass availability of drugs: Assay setup

Firstpass availability



Readouts:

- Time-dependent recovery of parent in Donor and Receiver
- Recovery in lysate at the end of experiment
- Efflux ratio (under steady state)
- Donor and receiver samples stored for MetID

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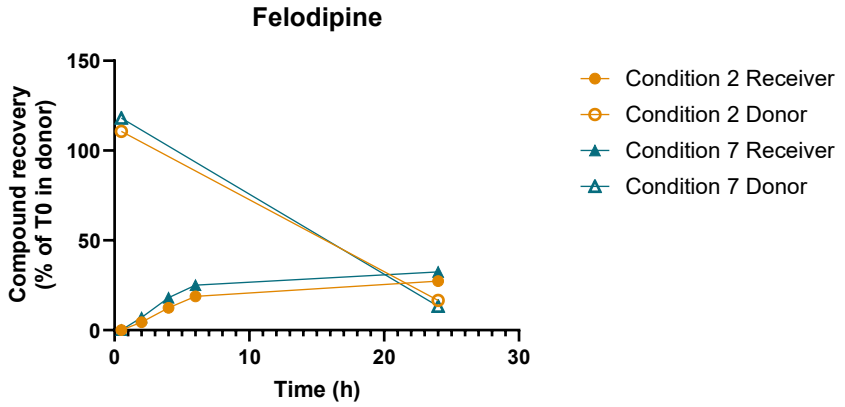
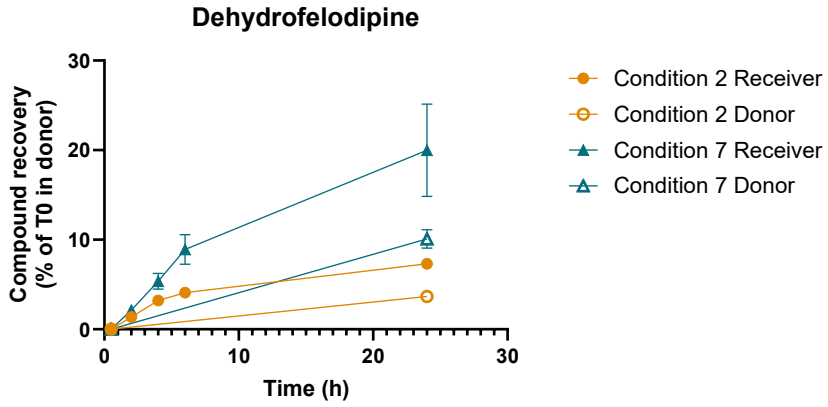
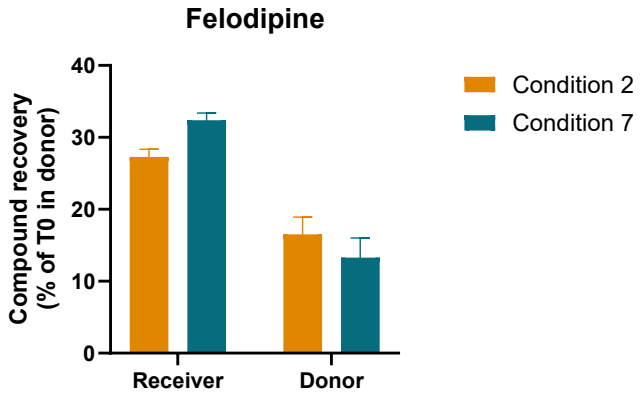
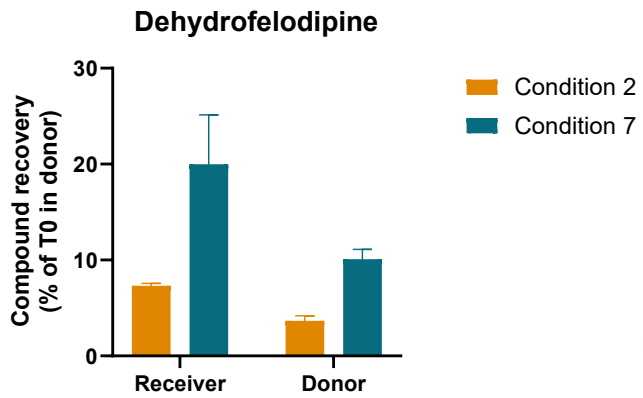
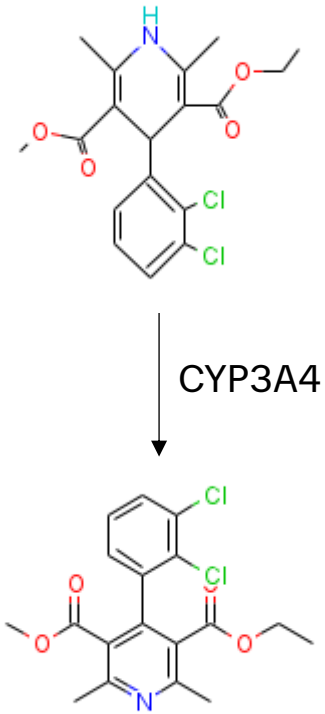
Human Duodenal Organoids as Monolayer: CYP3A4 Activities











Unpublished results

- CYP3A4 activities: Condition 7 > condition 2

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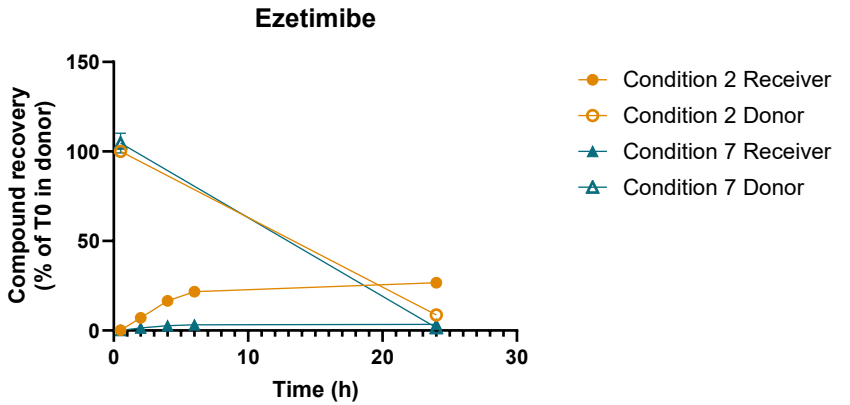
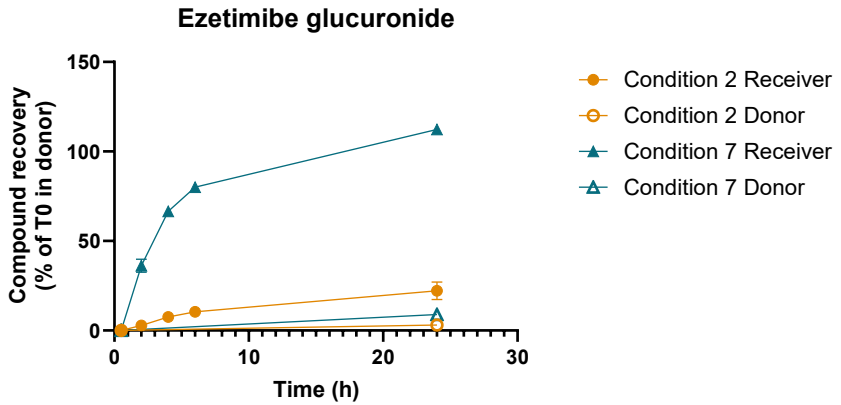
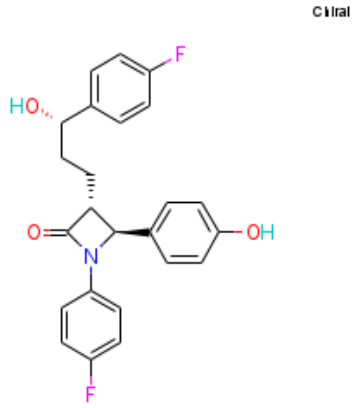
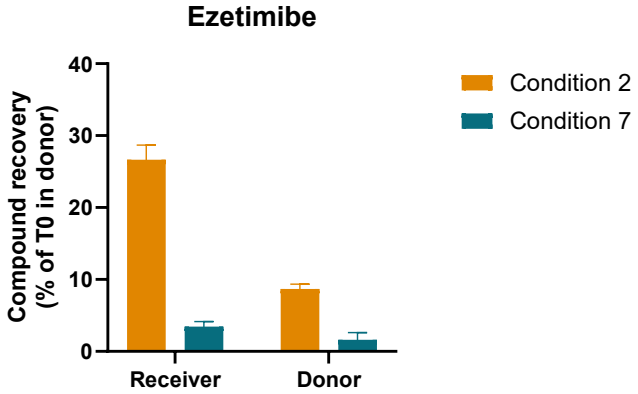
Human Duodenal organoids as Monolayer : UGT Activities

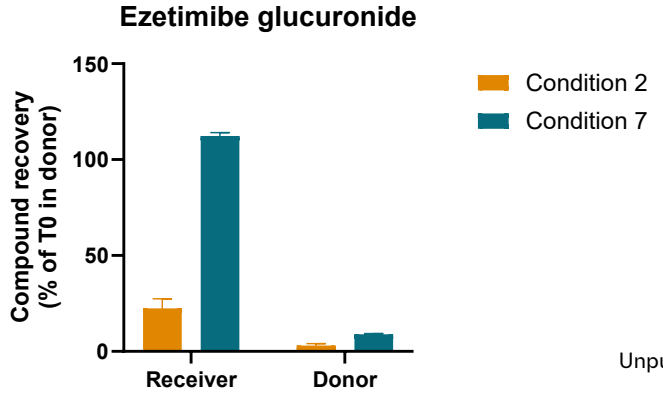




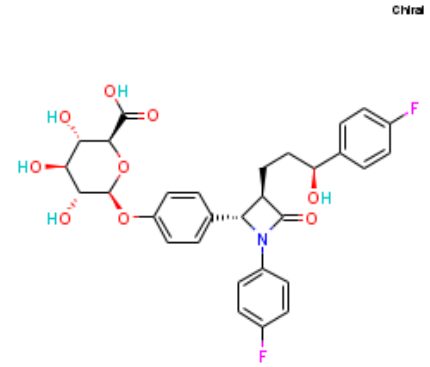


UGTs





Unpublished results



- UGT activities: Condition 7 >> condition 2

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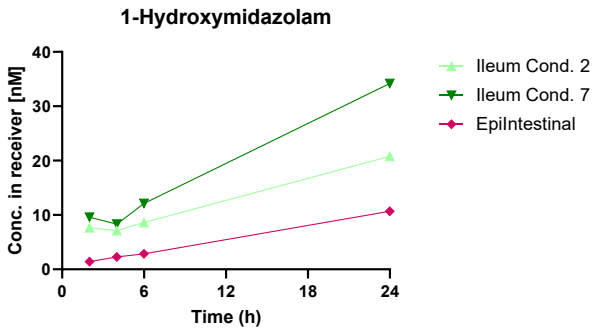
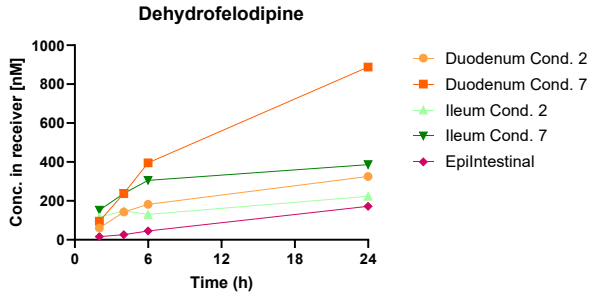
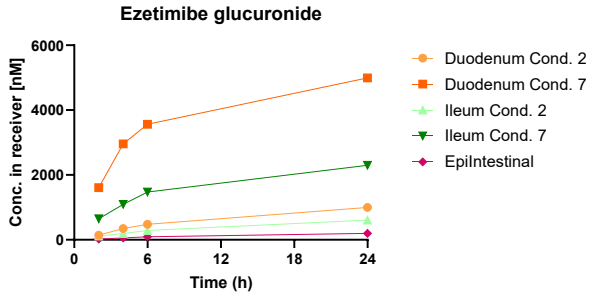
Comparison between In-house Models and EpiIntestinal Model



CYP3A



CYP3A



UGT

- Activities of major DMEs: Duodenal organoids > Ileal organoids > EpiIntestinal

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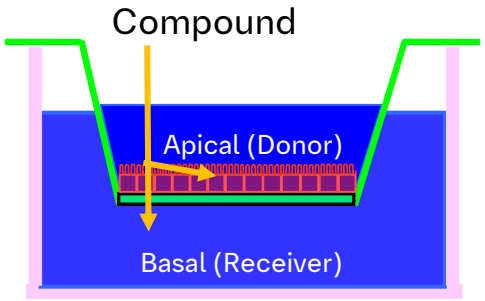
Differentiation of Intestinal Organoids to Epithelial Monolayer: Summary

- A differentiation protocol identified which results in tight epithelial monolayer and substantial activities of drug transporters and drug metabolizing enzymes
- Activities of CYP3A4 and UGTs: Duodenal organoids > ileal organoids > EpiIntestinal
- SULT activities: Ileal organoids are closer to physiological situation compared to EpiIntestinal

- Further experimental planning
  - Induction of drug metabolizing enzymes
  - Disease models
  - Gut-on-chip
  - How useful are the results for PBPK modeling

Prediction of intestinal first-pass availability with duodenum organoids

	Permeability coefficient (e-6 cm/s)		Fg (%) (Mean±SD)	Fa*Fg (%) (Human)
	Apparent[1]	Intrinsic[2]		
Midazolam	9.9[3]	14.5[3]	53.4 ± 21.8[4]	59
Felodipine	8.5[3]	12.1[3]	51.0 ± 24.6[4]	62



1: Calculation without considering compound loss due to metabolic clearance: $P_{apparent} = \frac{A_{Acceptor}}{C_{Donor} \times Surface_{filter} \times T}$

2: Calculation taking into account of compound loss due to metabolic clearance (Tran et al. 2004): $P_{intrinsic} = -\frac{V_{Acceptor} \times V_{Dnor}}{(V_{Acceptor} + V_{Donor}) \times Surface_{filter} \times T} \times \ln\left\{1 - \frac{C_{Acceptor}}{C_{average}}\right\}$

3: Values from one representative experiment

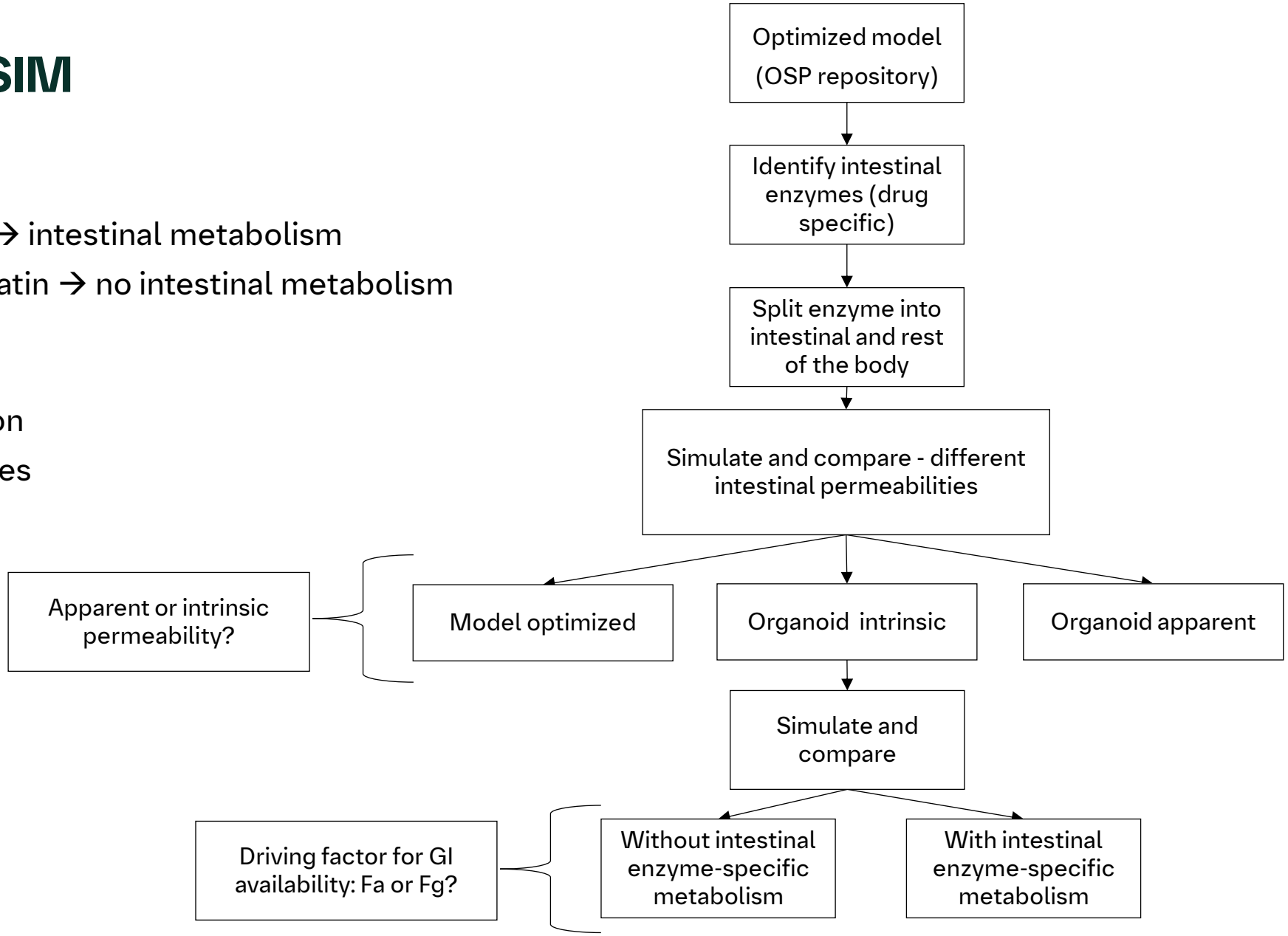
4: Calculated from 3 independent experiments with 2 – 3 replicates/experiment: $F_g = P_{app}/P_{intrinsic}$

Simulation with PK-SIM

- Four drugs were tested
  - Felodipine and Midazolam → intestinal metabolism
  - Cyclosporine and Rosuvastatin → no intestinal metabolism (i.e. negative control)

- Selected dosage form: solution
  - No effect of tablet properties

- Software: PK-SIM v11



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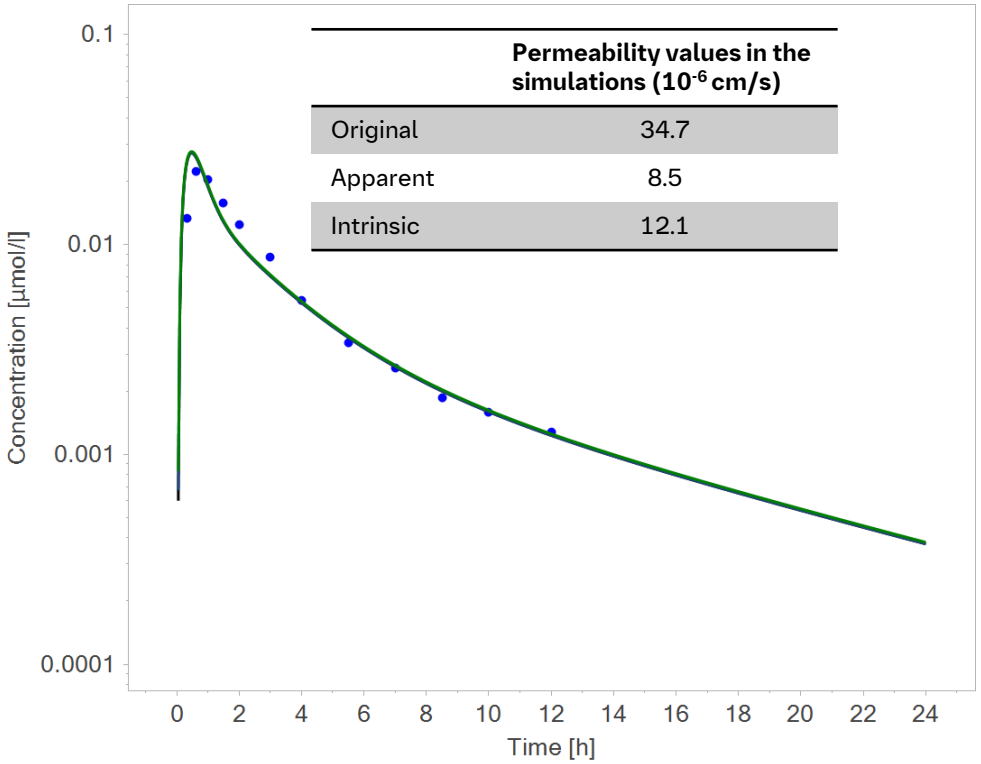
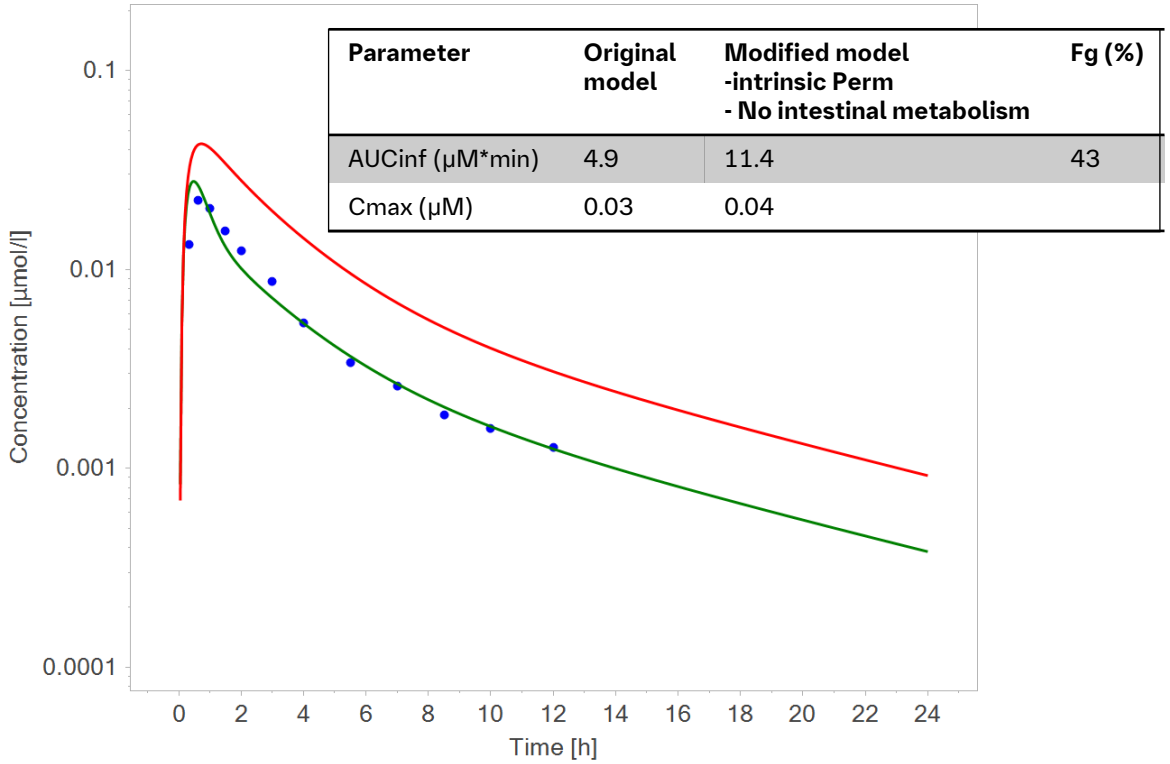
Felodipine: PBPK modelling based on results from intestinal organoids



	Permeability values in the simulations (10^{-6} cm/s)
Original	34.7
Apparent	8.5
Intrinsic	12.1

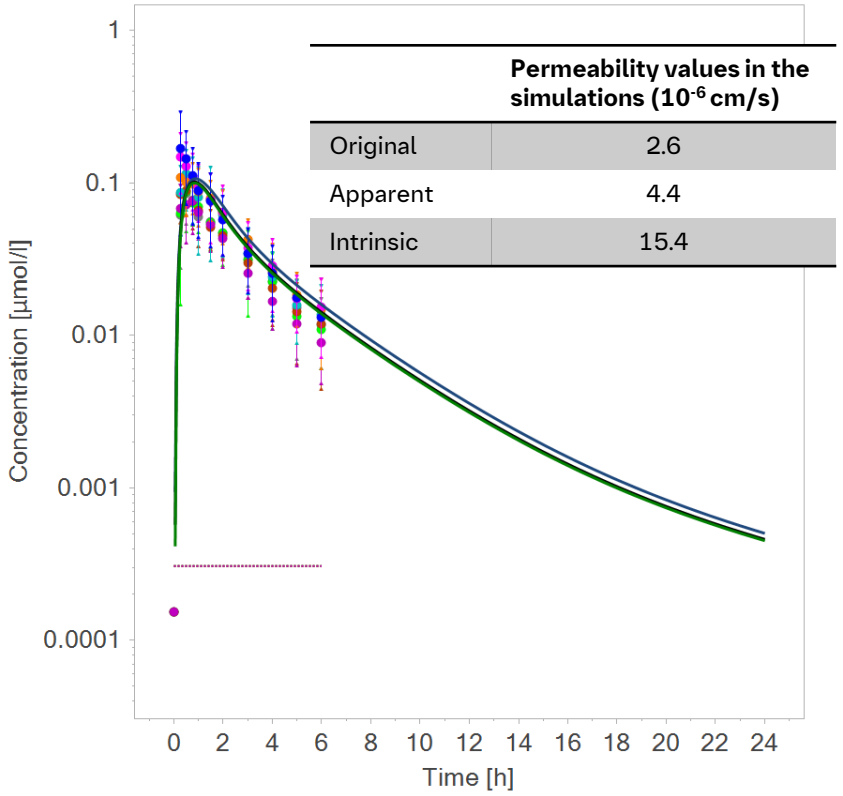


Parameter	Original model	Modified model -intrinsic Perm - No intestinal metabolism	Fg (%)
AUCinf (µM*min)	4.9	11.4	43
Cmax (µM)	0.03	0.04	

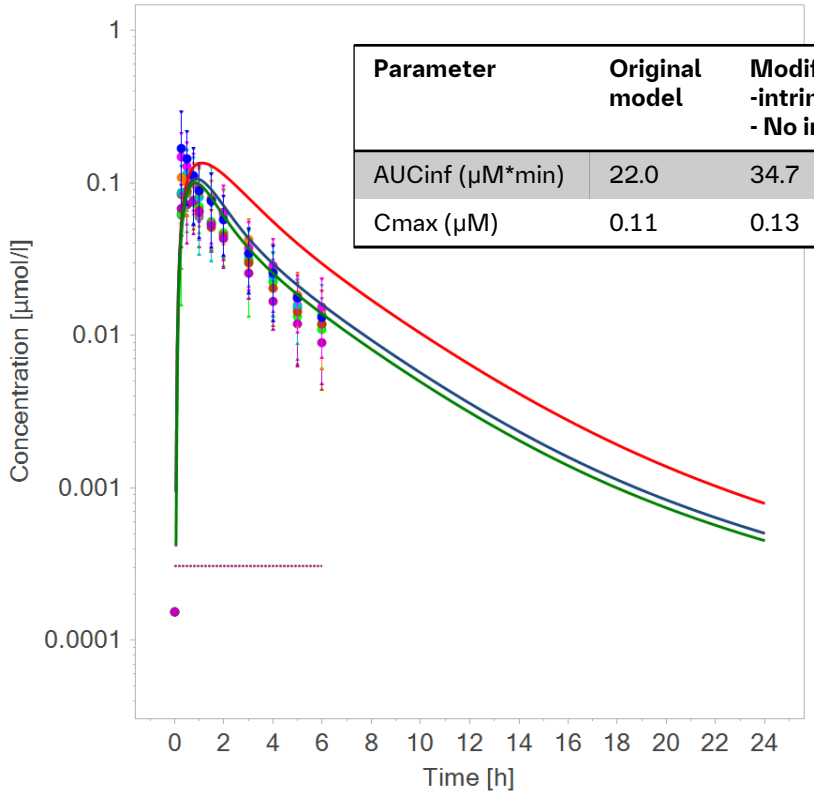
Original simulation
Organoid apparent permeability
Organoid intrinsic permeability
Organoid intrinsic permeability w/o intestinal metabolism

- Permeability is not rate limiting during the absorption of felodipine
- Metabolism in the gut wall is the limiting factor of GI firstpass availability

Midazolam: PBPK modelling based on results from intestinal organoids



	Permeability values in the simulations (10^{-6} cm/s)
Original	2.6
Apparent	4.4
Intrinsic	15.4



Parameter	Original model	Modified model -intrinsic Perm - No intestinal metabolism	Fg (%)
AUCinf (µM*min)	22.0	34.7	63
Cmax (µM)	0.11	0.13	

Original simulation
Organoid apparent permeability
Organoid intrinsic permeability
Organoid intrinsic permeability w/o intestinal metabolism

- Permeability is not rate limiting during the absorption of midazolam
- Metabolism in the gut wall is the limiting factor of GI firstpass availability

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Prediction of intestinal first-pass availability with duodenum organoids

	Permeability coefficient (e-6 cm/s)		Fg (%) (Mean±SD)	Fa*Fg (%) (Human)	Fg (%) (PBPK)
	Apparent[1]	Intrinsic[2]			
Midazolam	9.9[3]	14.5[3]	53.4 ± 21.8[4]	59	63
Felodipine	8.5[3]	12.1[3]	51.0 ± 24.6[4]	62	43

1: Calculation without considering compound loss due to metabolic clearance: $P_{apparent} = \frac{A_{Acceptor}}{C_{Donor} \times Surface_{filter} \times T}$

2: Calculation taking into account of compound loss due to metabolic clearance (Tran et al. 2004): $P_{intrinsic} = -\frac{V_{Acceptor} \times V_{Dnor}}{(V_{Acceptor} + V_{Donor}) \times Surface_{filter} \times T} \times \ln\left\{1 - \frac{C_{Acceptor}}{C_{average}}\right\}$

3: Values from one representative experiment

4: Calculated from 3 independent experiments with 2 – 3 replicates/experiment: $F_g = P_{app}/P_{intrinsic}$

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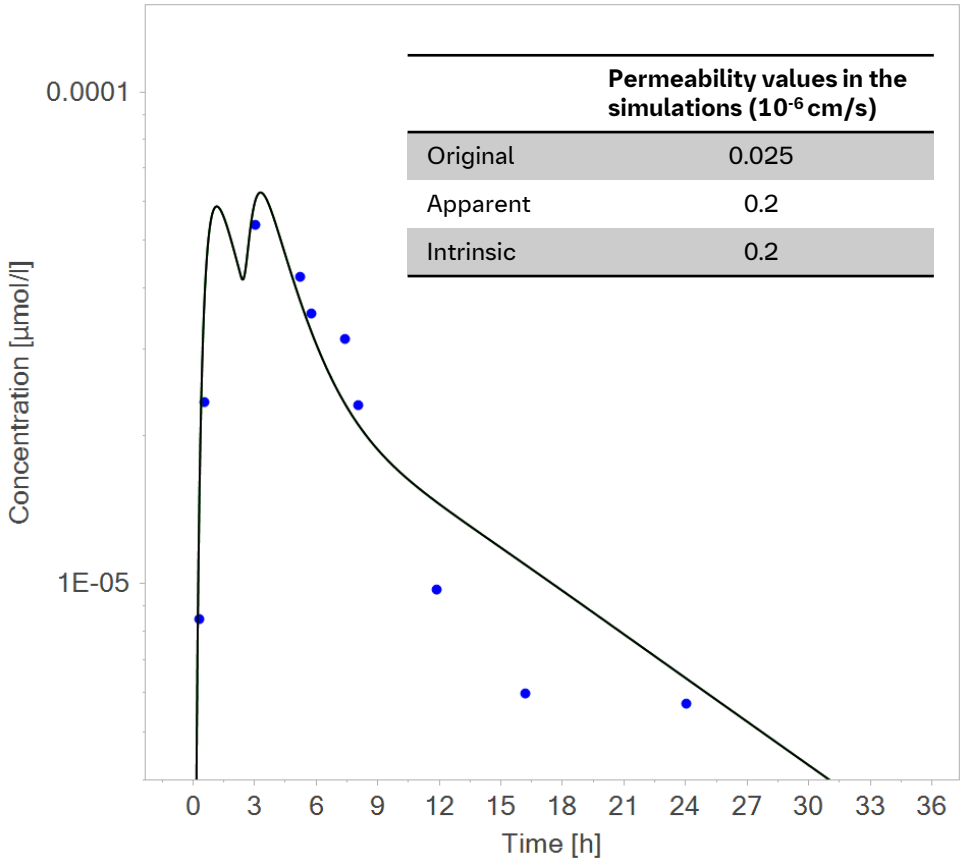
Rosuvastatin: PBPK modelling based on results from intestinal organoids



	Permeability values in the simulations (10^{-6} cm/s)
Original	0.025
Apparent	0.2
Intrinsic	0.2

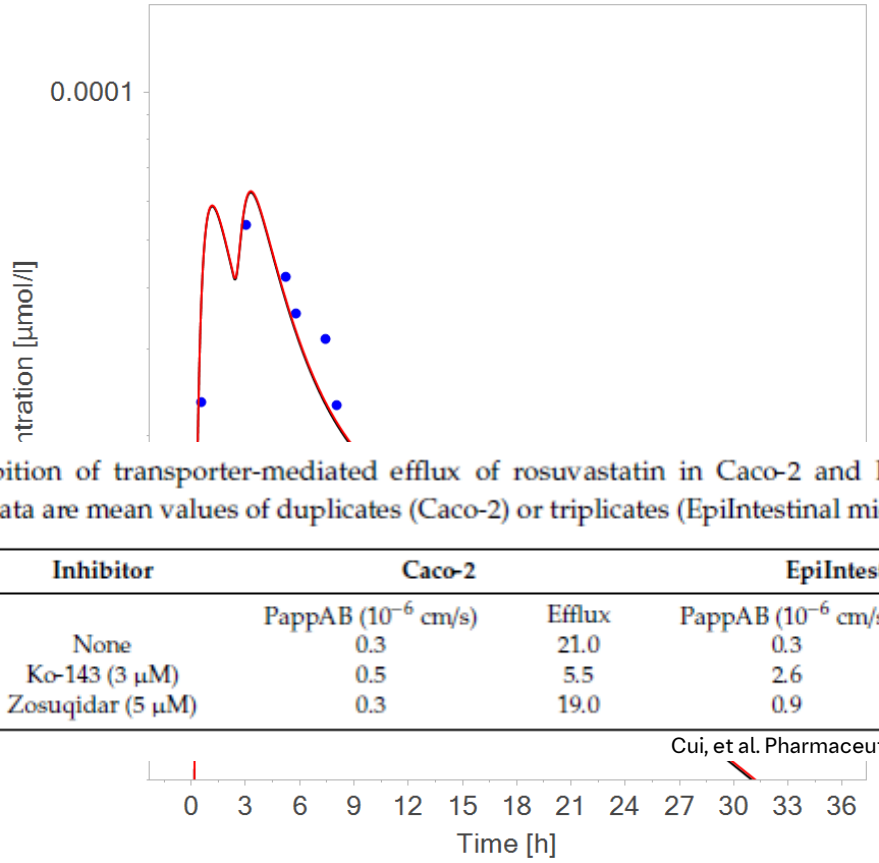


Table 3. Inhibition of transporter-mediated efflux of rosuvastatin in Caco-2 and EpiIntestinal microtissues. Data are mean values of duplicates (Caco-2) or triplicates (EpiIntestinal microtissues).

Substrate	Inhibitor	Caco-2		EpiIntestinal	
		PappAB (10^{-6} cm/s)	Efflux	PappAB (10^{-6} cm/s)	Efflux
Rosuvastatin	None	0.3	21.0	0.3	100.0
	Ko-143 (3 µM)	0.5	5.5	2.6	3.1
	Zosuqidar (5 µM)	0.3	19.0	0.9	25

Cui, et al. Pharmaceutics, 12:405 (2020)

Original simulation
Organoid apparent permeability
Organoid intrinsic permeability
Organoid intrinsic permeability w/o intestinal metabolism

- Metabolism in the gut wall does not affect GI firstpass availability
- Transporter (BCRP) is the rate-limiting factor for the absorption

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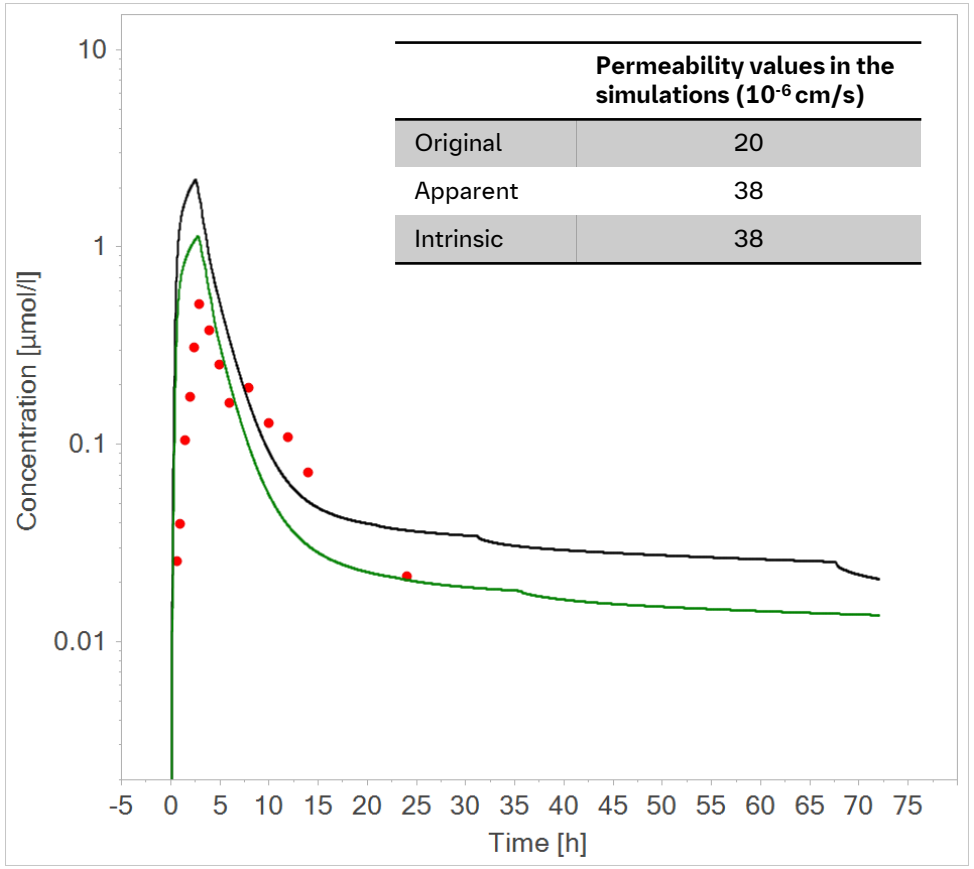
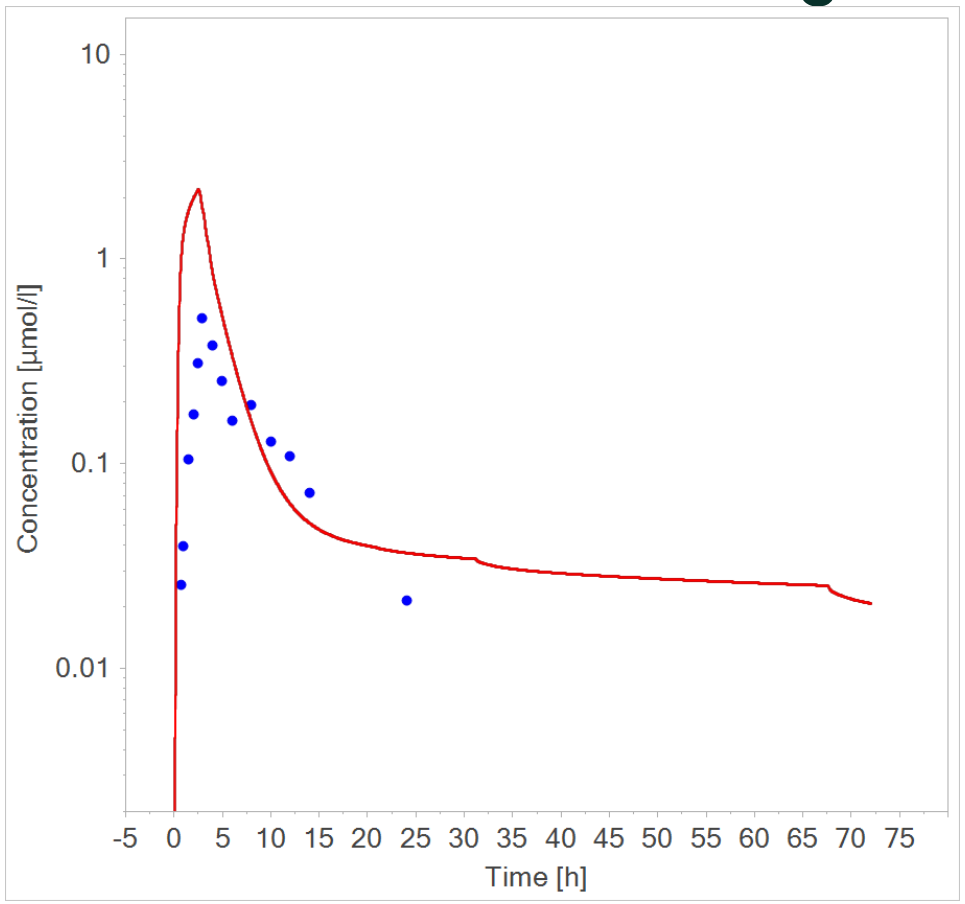
Cyclosporine-A: PBPK modelling based on results from intestinal organoids



	Permeability values in the simulations (10^{-6} cm/s)
Original	20
Apparent	38
Intrinsic	38



Original simulation
Organoid apparent permeability
Organoid intrinsic permeability
Organoid intrinsic permeability w/o intestinal metabolism

- Metabolism in the gut wall does not affect GI firstpass availability
- Low solubility of cyclosporine A is the main driver of the difference in simulation

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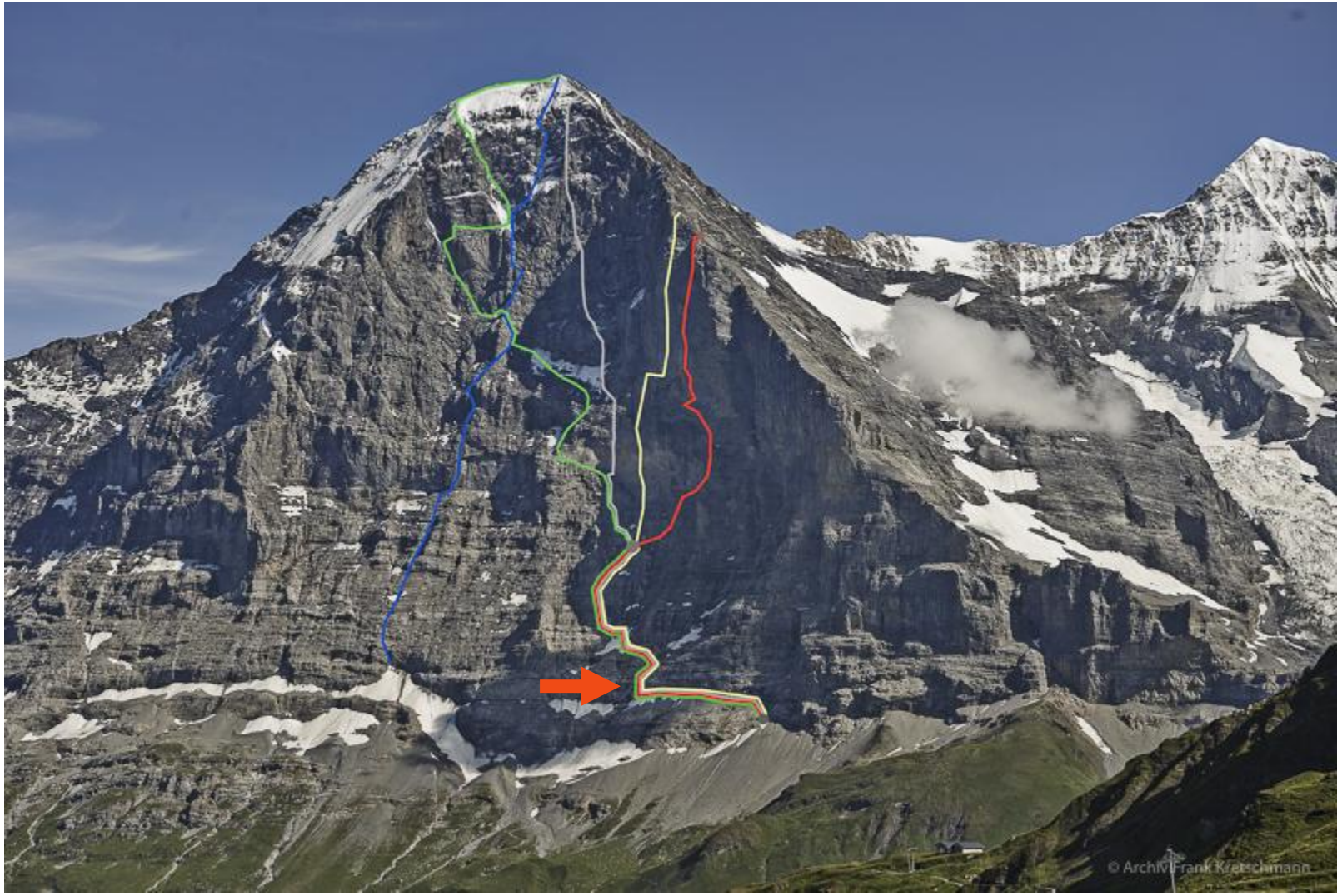
Lessons learned

- Experimental results from MPS are often of composite nature:
  - Apparent permeability coefficient contains information on permeability, transporter, and metabolic enzyme activities

- PBPK modeling requires defined parameters for each separate step

- Results from MPS need to be further processed for the use in PBPK modeling
  - Intrinsic permeability and transporter activities: Similar processing as for Caco-2 model. Deconvolution of metabolic activities necessary
  - Firstpass GI metabolism: Fg estimate from intestinal organoid model can be used to estimate the total metabolic clearance activities in GI wall in PBPK modeling, especially if the contribution of single enzymes not elucidated

Elimination of animal experiments for ADME testing: Where are we?



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Human Intestinal Organoids, OSP 2025, Paris

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Backup

Comparison between In-house Models and EpiIntestinal Model: SULTs

Substrate	EpiIntestinal		Human Intestinal Mucosa (HIM)	
	Ezetimibe	Raloxifene	Ezetimibe	Raloxifene
Parent (% of parent drug at T0)	8.4	2.4	40.3	33.2
Glucuronides (% of parent drug at T0)	39.1	2.2	58.6	18.7
Sulfates (% of parent drug at T0)	n.d.	14.1	n.d.	2.0

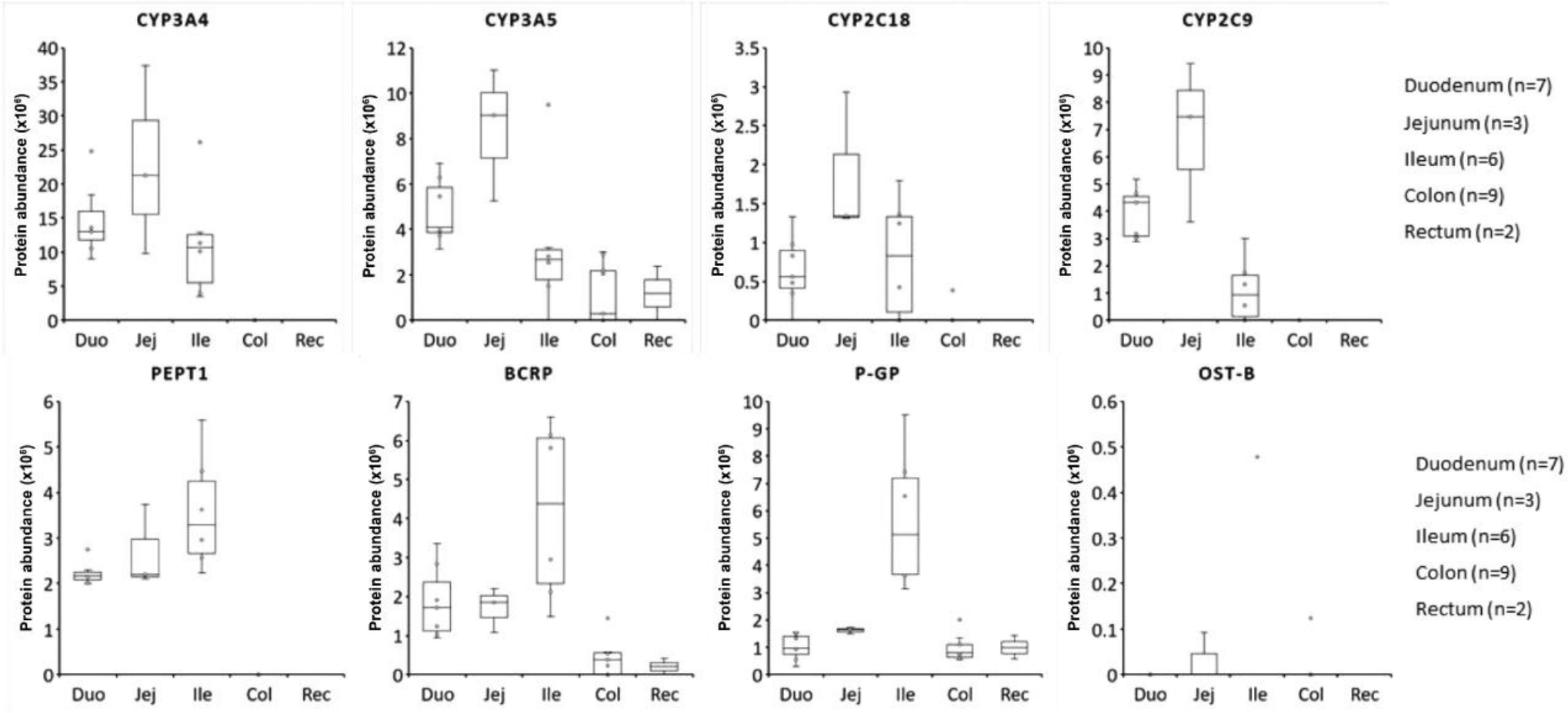
n.d.: Not detectable.

Cui, et al. Pharmaceutics, 12:405 (2020)

Human ileal Organoids
Raloxifene
44
14
0.01

- Activities of SULTs: Human ileal organoids resemble human intestinal mucosa compared to EpiIntestinal
- Human: Glucuronides are the only metabolites of raloxifene found in human plasma

Regional Proteomics in Human Intestine



Regional transcriptomics and proteomics of pharmacokinetics-related genes in human intestine, Murata M., et al., Mol Pharmaceutics 2023, 20:2876

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