HELLENIC REPUBLIC
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Optimized fecal material for simulating bacterial drug degradation in the lower intestine

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2nd Symposium
Drug disposition in the colon
20 January 2022

COLOTAN

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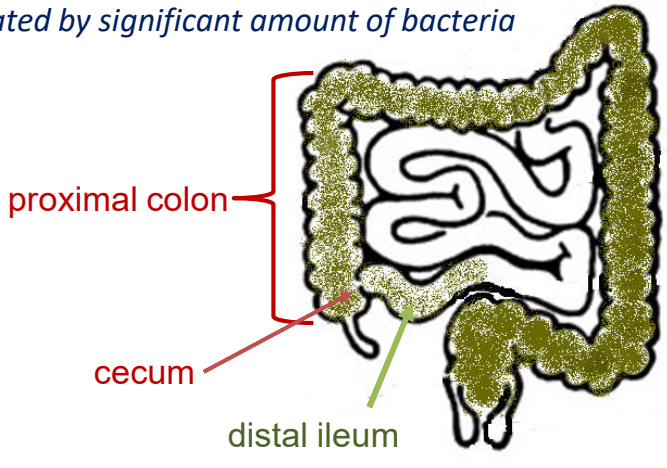
In this presentation…

… lower intestine refers to
- Proximal Colon
- cecum
- distal ileum

populated by significant amount of bacteria



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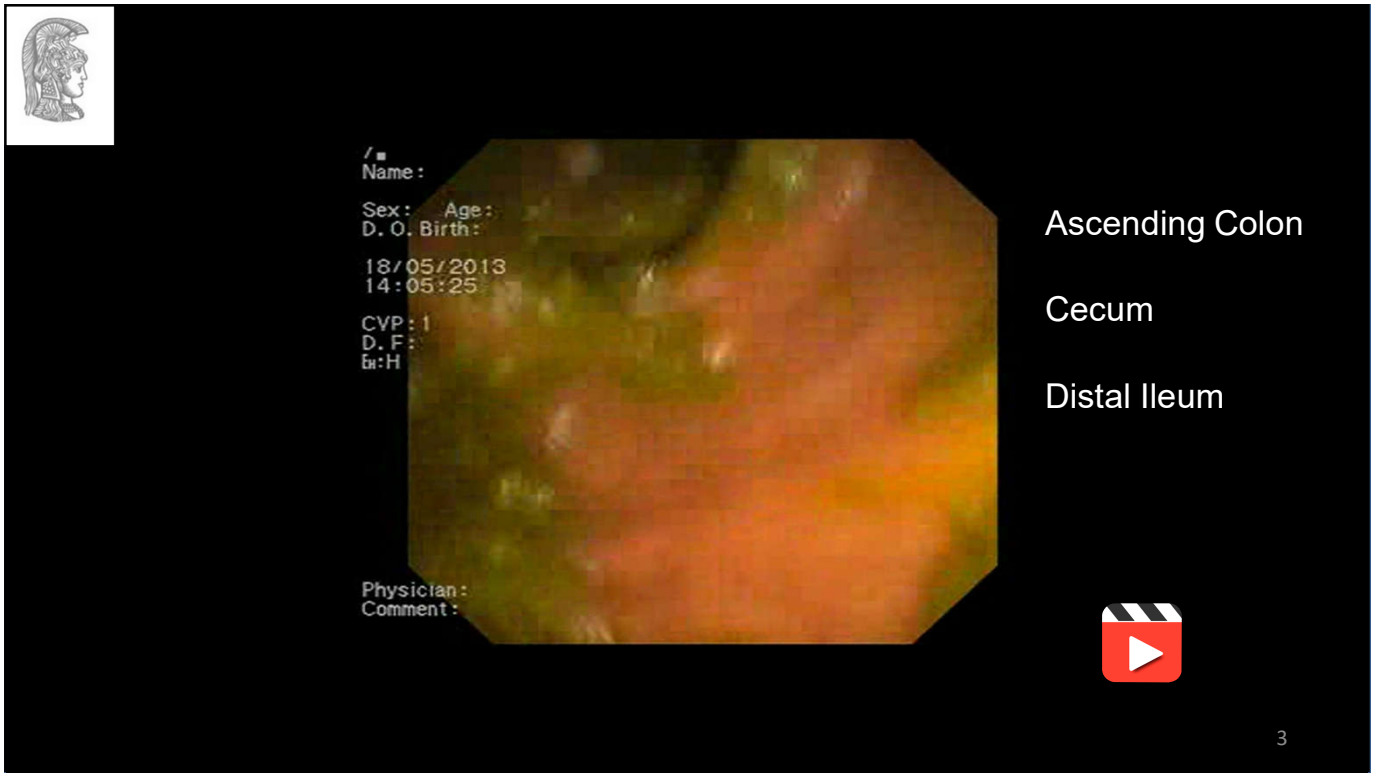

Bacterial degradation of orally administered therapeutic agents in the lower intestine: When is of interest?

- Absorption from the region is required for achieving or maintaining therapeutic effect, systemic or local

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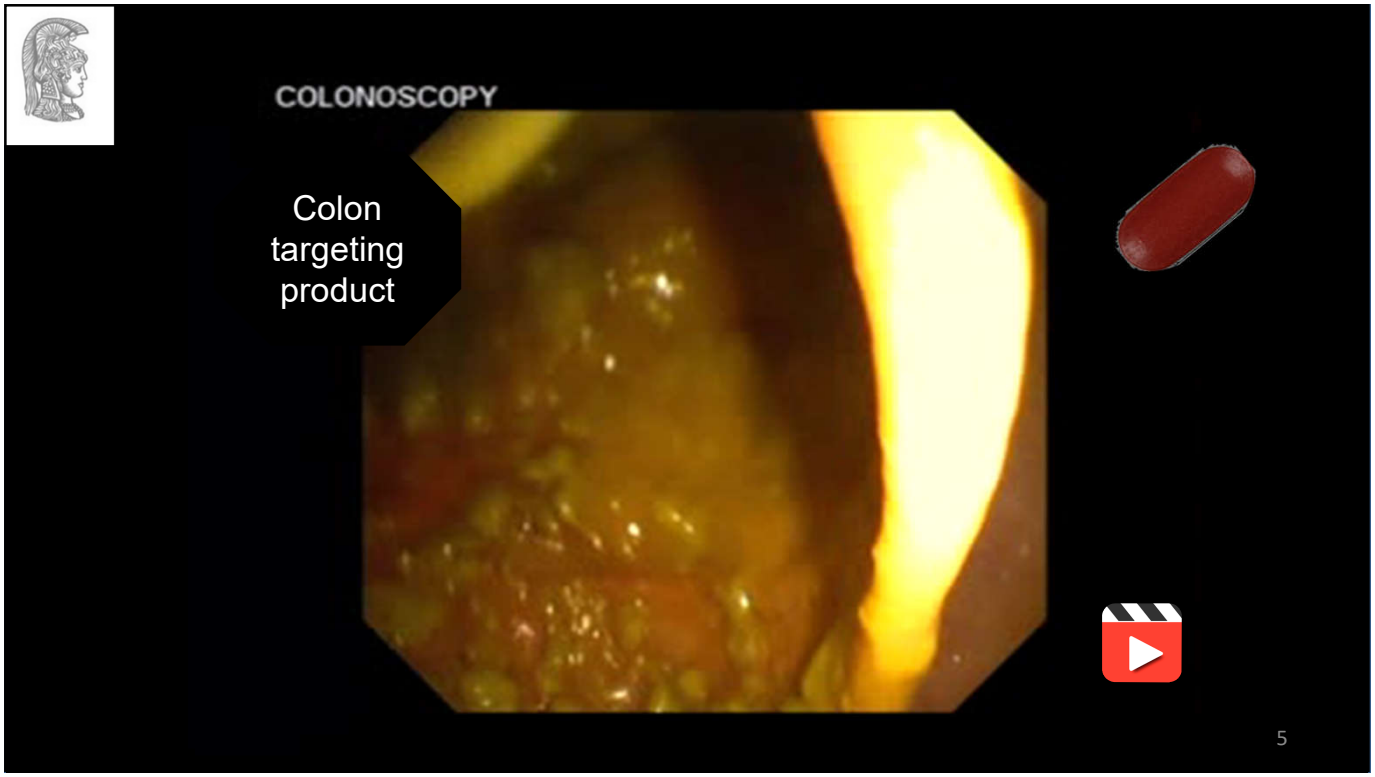

Bacterial degradation of orally administered therapeutic agents in the lower intestine: When is of interest?

- Absorption from the region is required for achieving or maintaining therapeutic effect, systemic or local
- A prodrug is administered and its conversion to the active form is expected to take place by the bacteria in the lumen of the lower intestine
- There is a risk of drug or drug-metabolite induced toxicity

  The combination of sorivudine (an antiviral agent) with 5-fluorouracil (an antitumor agent) caused >15 deaths and numerous cases of serious side effects in the 1990s:

  sorivudine bacterial metabolite inhibits 5-fluorouracil metabolism in liver resulting in its abnormal accumulation in the blood and toxicity of 5-fluorouracil

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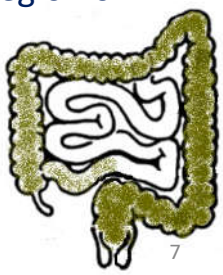
Karatza et al Int. J. Pharm. 2017

Outline

- How bacterial degradation of orally administered therapeutic agents in the lower intestine could be evaluated?
- Handling, storage conditions and reproducibility of fecal material
- Optimization of fecal material to reflect bacterial activity in the region of interest



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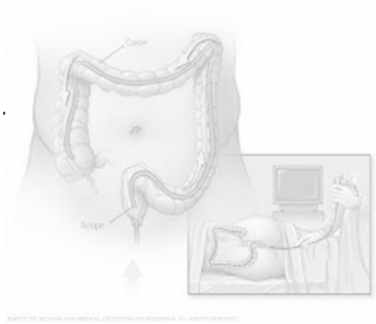
Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

Evaluation on drug degradation in the contents of the lower intestine

Measurement in humans by direct sampling from the lower intestine (colonoscopy) is associated with various difficulties

- medical supervision
- ethical issues
- sampling issues
  - tube's positioning, limited number of samples, volume availability

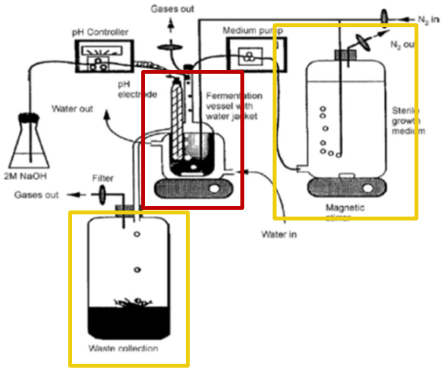


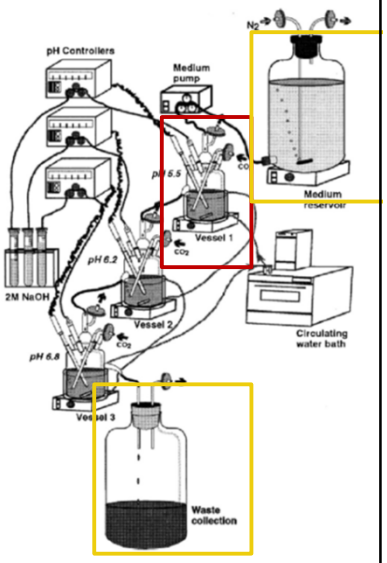
Lemmens et al J. Pharm. Sci. 2021

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

(Semi)continuous culture systems

- Long periods of incubation
- Part of the culture media is removed and replaced at intervals
- Addition of fermentable substrates (nutrient for bacteria) into the system





Sousa et al. Int. J. Pharm. 2008

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

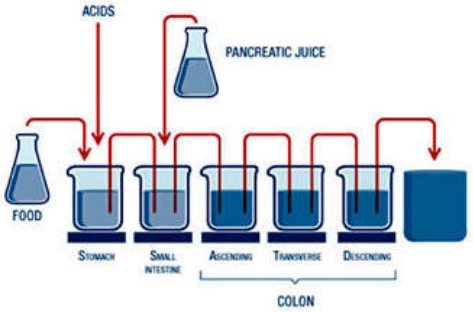
Simulator of the human intestinal microbial ecosystem (SHIME)

Five-stage reactor

The first two reactors are of the fill-and-draw principle to simulate different steps in food uptake and digestion.

The last three compartments simulate the large intestine. Upon inoculation with faecal microbiota, these reactors simulate the ascending, transverse and descending colon.

The model has been originally designed to study the metabolic fate of food over a period of several weeks.

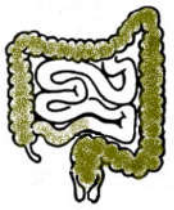


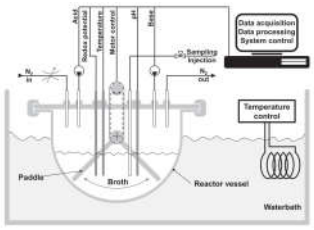
https://www.prodigest.eu

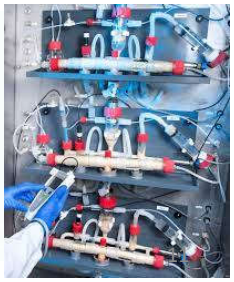
Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

MimiCol, Biostat®, TIM-2

	in vivo (Yang, 2008)	MimiCol	Biostat®	TIM-2
Volume	170 ± 40 mL ascending colon (Badley et al., 1993)	150 mL	2000 mL	160 mL
Motility	pendulum movements, segmentations and fast mass transfer	6 rpm	200 rpm stirring	Peristaltic mixing by indirect pressure application
pH	7.4 to 6.2	6.2 ± 0.25pH profile starting at pH 7.4	6.2 ± 0.1	5.8–7.0
Microbiota	10^{11} CFU/mL (Cummings and Macfarlane, 1991)	Standard microbiota derived from a faecal sample of a healthy human volunteer	Standard microbiota derived from a faecal sample of a healthy human volunteer	Standard fermenter microflora or faecal inoculum







Beeck et al. Int. J. Pharm. 2021; www.thetimcompany.com; www.sartorius.com

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

Static batch cultures

Caecal contents of animals (rats) or faeces (animal or human) are placed into a medium (saline or buffer solution)

- ✓ Suitable for short incubation periods
- ✓ Easy and flexible screening tool
- ✓ Estimation of bacterial degradation half-life of APIs (PBPK modelling)

Points for consideration

- Source: animals *vs.* humans
- Handling, storage conditions and reproducibility of bulk material
- Optimization of bacterial activity in the region of interest

The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine (literature data: ranges from 5 to 25%)

Sousa et al. Int. J. Pharm. 2008, Karatza et al. Int. J. Pharm. 2017

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

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	Species	Bacterial numbers (log/g)	Enzymes activities (mol/h per g caecal contents or faeces)			
			β-Glucosidase	β-Glucoronidase	Nitro reductase	Nitrate reductase
caecal contents	Rat	10.8 ± 0.8	34.3 ± 5.5	156.3 ± 28.8	4.0 ± 0.7	3.9 ± 1.0
	Mouse	10.2 ± 0.2	55.6 ± 22.0	42.9 ± 4.6	6.5 ± 0.7	1.8 ± 0.4
	Hamster	10.4 ± 0.1	30.1 ± 2.3	60.8 ± 14.8	3.9 ± 0.6	1.7 ± 0.2
	Guinea-pig	10.3 ± 0.1	8.4 ± 3.0	11.3 ± 1.5	0.4 ± 0.1	5.6 ± 1.8
faeces	Marmoset	10.8 ± 0.3	35.1 ± 8.0	11.7 ± 5.5	0.6 ± 0.2	1.9 ± 1.0
	Human	11.3 ± 0.2	49.5 ± 8.1	35.5 ± 19.9	1.0 ± 0.2	8.0 ± 2.3

- The rat, guinea-pig and marmoset differed significantly from the human in three of the four enzymes
- The mouse and hamster differed significantly from the human two out of four enzymes

Sousa et al. Int. J. Pharm. 2008

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Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

Static batch cultures

Caecal contents of animals (rats) or faeces (animal or human) are placed into a medium (saline or buffer solution)

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Sousa et al. Int. J. Pharm. 2008, Karatza et al. Int. J. Pharm. 2017

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Handling and storage of human fecal material

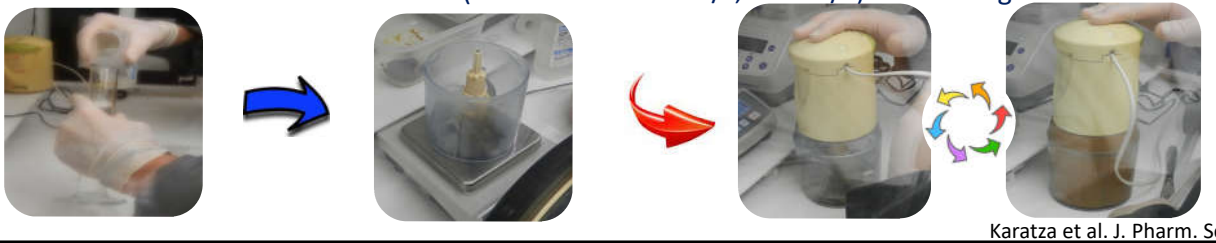
1. Freshly voided stools are collected in pre-weighted containers

All works are performed in the workstation
10 % H_2, 10% CO_2, 80% N_2, 37 °C, 70% RH

2. Stools are transferred in an anaerobic workstation

3. Stools are weighted

4. Stools are diluted with normal saline (feces:saline 1:3.8 w/v, 25% w/v) and homogenized in a mixer



Karatza et al. J. Pharm. Sci. 2016

Handling and storage of human fecal material

5. Material is sieved through a 0.35mm metal mesh to remove any heterogeneous fibrous material

6. Sieved homogenate is poured in sealed (screw cap) glass vials (to avoid multiple thawing freezing cycles)

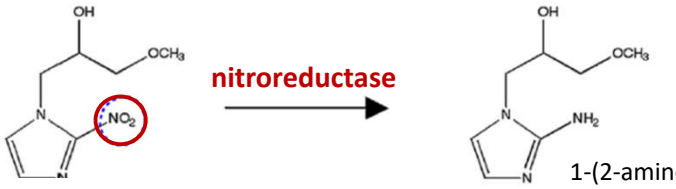
7. pH of fecal material is measured

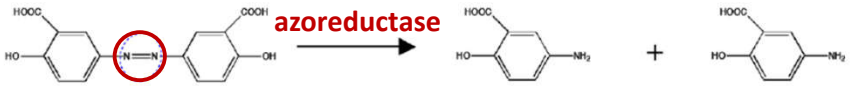
8. Sealed vials are stored at -70°C

Karatza et al. J. Pharm. Sci. 2016

Handling, storage and reproducibility of human fecal material

Enzyme activity is assessed indirectly by evaluating the degradation characteristics of 2 substrates of **nitroreductase** and **azoreductase**.





Metronidazole and olsalazine are chemically stable
in the range of pH values that were achieved in human fecal material prepared and tested (5 < pH < 7)

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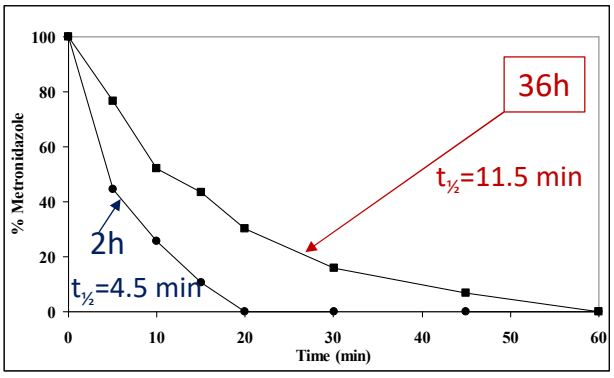
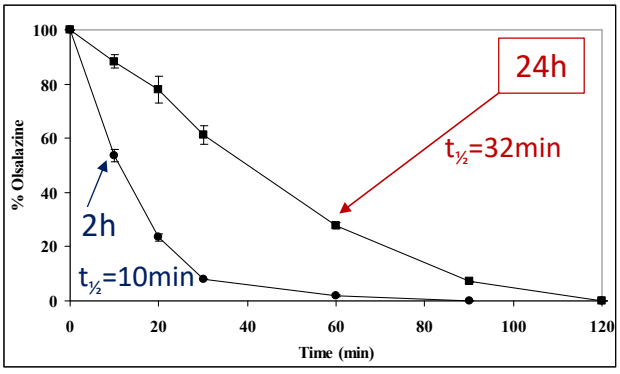
Handling human fecal material

Optimization of incubation period of frozen fecal material on bacterial activity





Bacterial degradation experiments in previously frozen (- 70 °C) human fecal material should be performed **only after incubating the frozen material for about 2 h** in the anaerobic workstation

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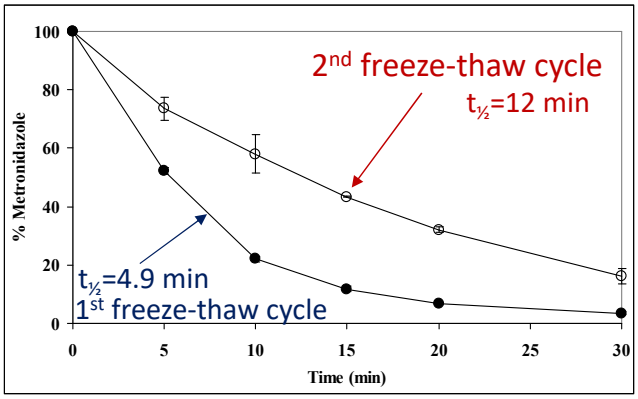
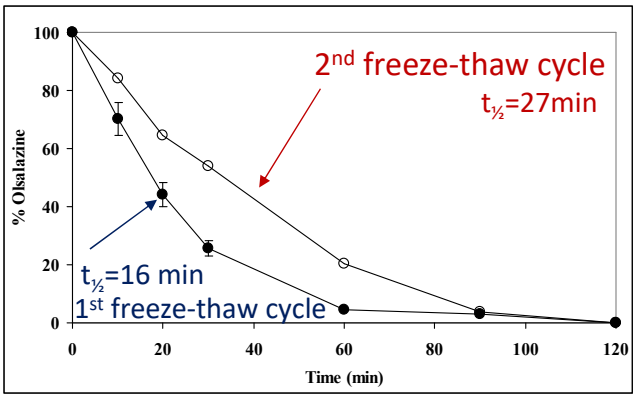
Handling human fecal material

Impact of second freeze-thaw cycle of fecal material on bacterial activity





Bacterial degradation experiments in previously frozen (- 70 °C) human fecal material should be performed **only after the first thawing** of the material

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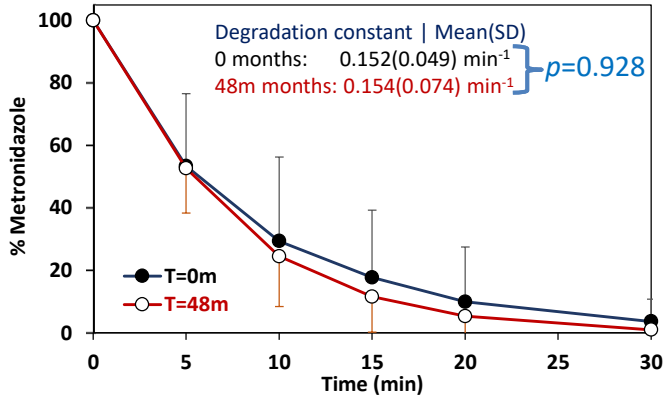
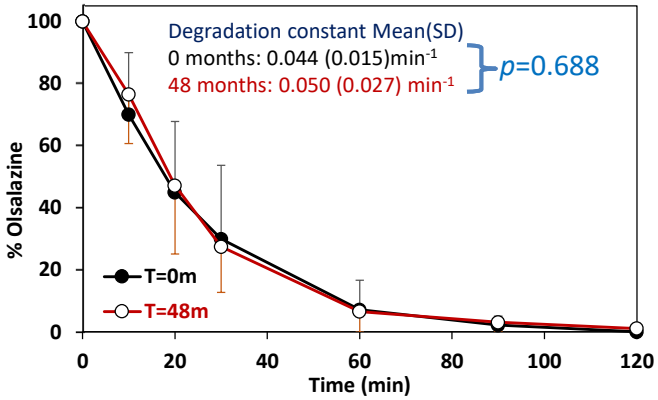
Storage of human fecal material

Impact of storage period of frozen fecal material on bacterial activity





During **48 months** of storage at **- 70°C** bacterial activity remained unaltered

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Reproducibility of human fecal material

Evaluation of fecal material collected in two different occasions

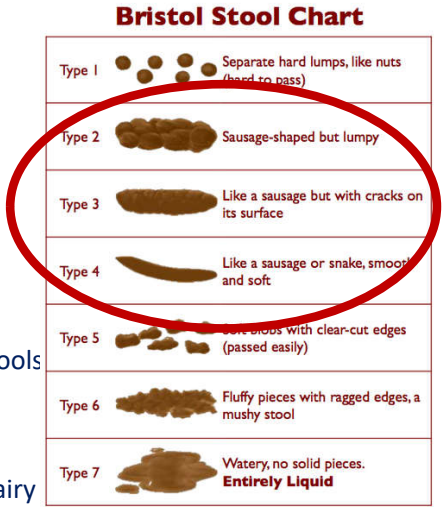
Healthy adults (18-60 y)

Inclusion Criteria

- Within 20% of ideal body weight as determined by Metropolitan Life Tables.
- Willingness to abstain from smoking and drinking alcohol for at least 3 days before the day of stool collection
- No treatment with antibiotics during the last 6 months before stool collection.
- No use of laxatives or cathartics for the last 7 days.
- Regular intestinal habits and stools consistency of type 2, 3, or 4, according to Bristol stool scale for at least a month before the day of stools collection.

Exclusion Criteria

- Exclusion of a specific food category from his/her diet (e.g., meat or dairy habits.



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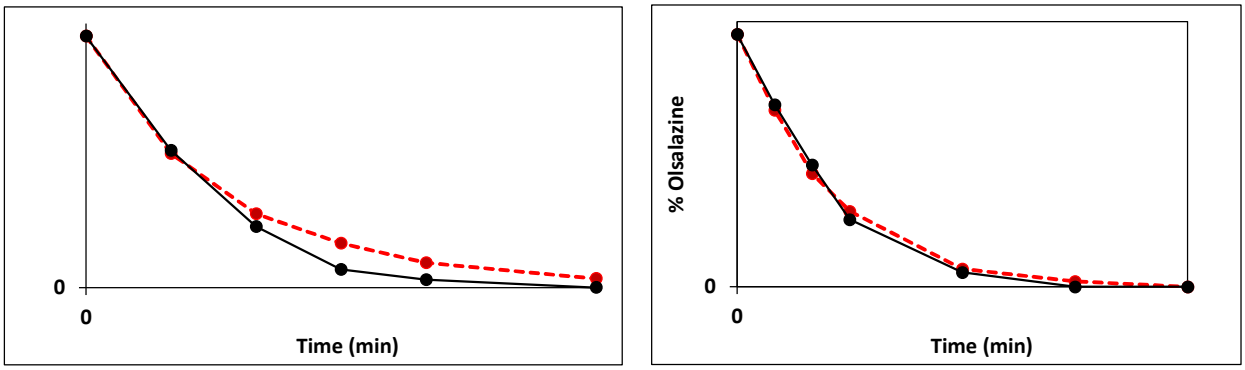
Reproducibility of human fecal material

Evaluation of fecal material collected in two different occasions



No significant difference was observed

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Optimization of the dilution of feces with normal saline

The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine
Literature data: dilution ranges from 5 to 25% w/v

Lack of justification of level of dilution of stools and the questionable clinical relevance of collected data may lead to

- rejection of potentially useful therapeutic agents (**false negative decision**) or
- selection of problematic compounds for further development (**false positive decision**)

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Optimization of the dilution of feces with normal saline

The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine
Literature data: dilution ranges from 5 to 25% w/v

For the optimization of dilution of the fecal material with normal saline to simulate **ileal bacteria**, the degradation profiles of model compounds were compared with their degradation profiles in the contents of the **distal ileum**
For the optimization of dilution of the fecal material with normal saline to simulate **colonic bacteria**, the degradation profiles of model compounds were compared with their degradation profiles in the contents of the **proximal colon**

Two issues

- Sampling from the lower intestine
- Population and Dosing conditions

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Sampling from lower intestine...

… requires preparation of the **distal colon** without disturbing the physiological state of **distal ileum/cecum/proximal colon** at sampling time

One-time enema of 250ml of water appears sufficient to cleanse the left hemicolon
KULeuven |Lemmens et al. 2021

50h and 44h prior to colonoscopy 10mg bisacodyl [bis(p-acetoxyphenyl)-2-pyridylmethane] (Dulcolax®, mild laxative) is orally administered to prepare primarily the descending and sigmoid colon
NKUA | Diakidou et al. 2009

Bisacodyl for distal colon preparation?

After oral administration **bisacodyl is rapidly converted to the active metabolite** bis(p-hydroxyphenyl)-2-pyridylmethane (**BHPM**) and its action is initiated by activating protein kinase C releasing prostaglandin E2 and, thereby, **inducing net fluid secretion** (in vitro & human data)

Effects on the intraluminal physiology have been shown to be reversible (rat and human data).

Mucus secretion occurs **only** at **BHPM** concentrations of at least **1 μg/ml**
Sodium and fluid secretion occurs at intracolonic BHPM concentrations of **≥5 μg/ml** (rat data)

With the NKUA protocol for colon preparation, after overnight fasting and 5h after a glass of water,
BHPM at proximal colon is **~ 0.5 μg/ml**
Also, stools consistency and osmolality of contents at the proximal colon are used as supporting evidence

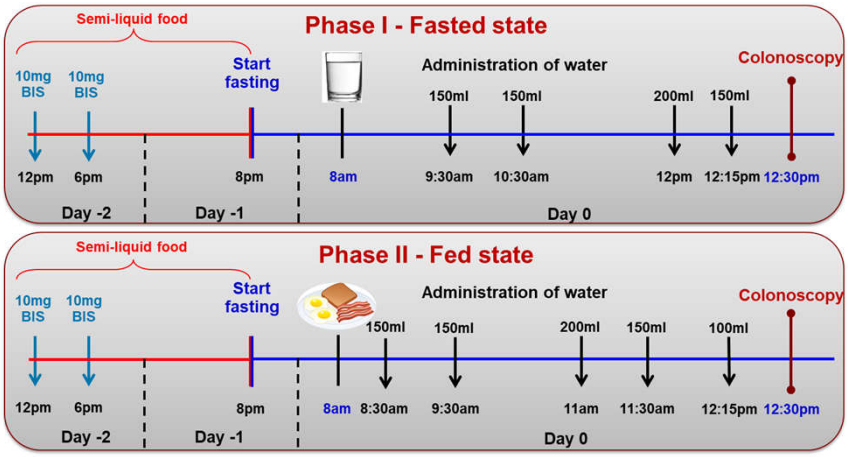
A useful methodology, especially when considering sensitive populations

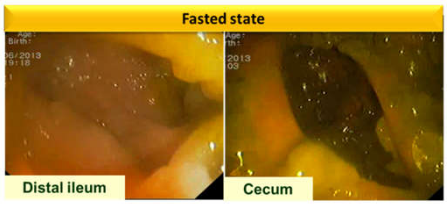
Diakidou et al. Pharm. Res 2009

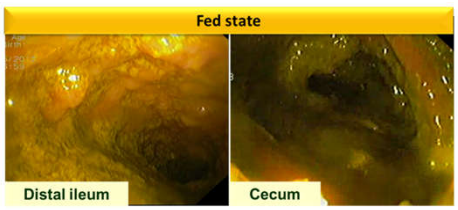
Collection of contents of distal ileum, cecum, proximal colon

Population Healthy Adults

Dosing conditions Oral drug absorption studies







Vertzoni et al. Int. J. Pharm. 2011; Karatza et al. Int. J. Pharm. 2017

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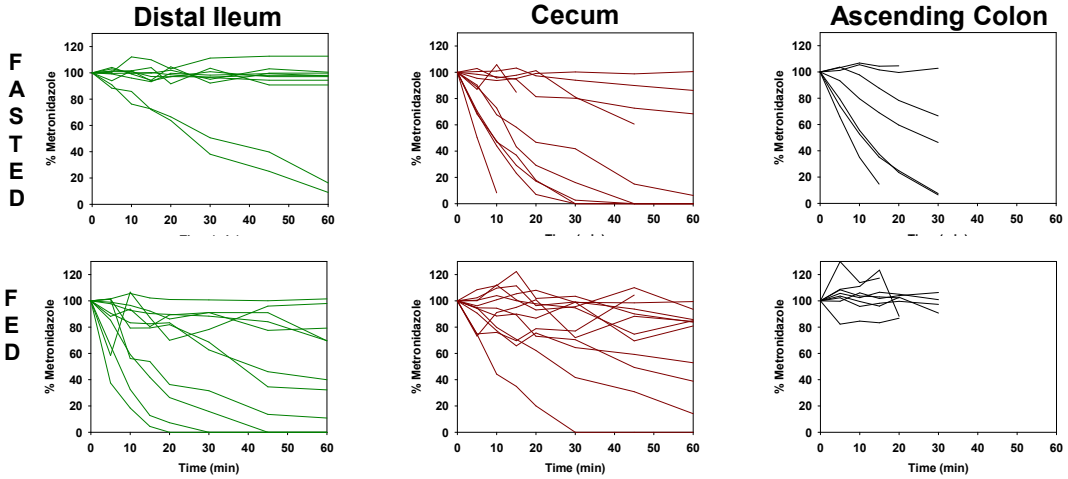
Bacterial degradation of metronidazole in the lower intestine



- In the fed state, degradation, if any, does not increase much from distal ileum to proximal colon
  - Bacterial degradation in cecum and ascending colon is more substantial in the fasted state

Vertzoni et al. Int. J. Pharm. 2011; Karatza et al. Int. J. Pharm. 2017

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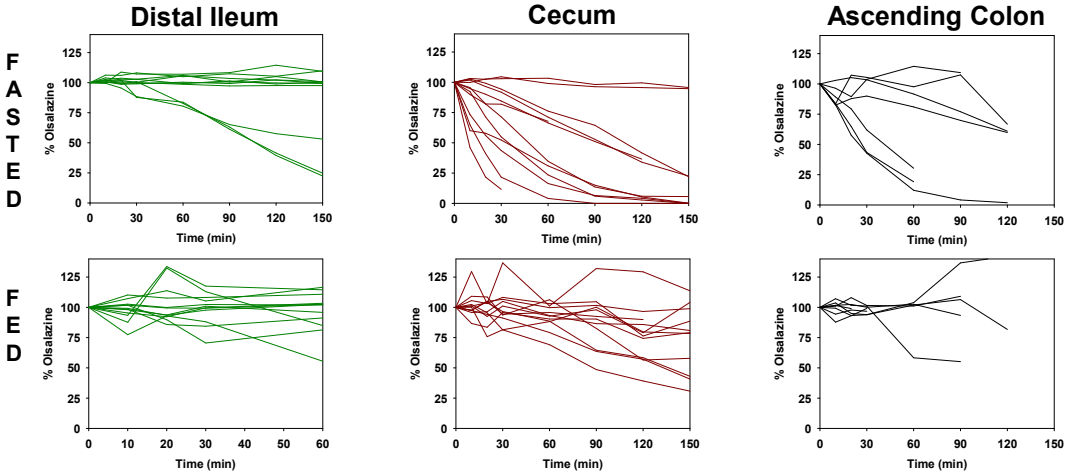
Bacterial degradation of olsalazine in the lower intestine



- In the fed state, degradation, if any, does not increase much from distal ileum to proximal colon
- Bacterial degradation in cecum and ascending colon is more substantial in the fasted state
- High inter-subject variability complicates the development of screening tools

Vertzoni et al. Int. J. Pharm. 2011; Karatza et al. Int. J. Pharm. 2017

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Optimization of the dilution of feces with normal saline

Clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults *ex vivo* using adult **fecal material** was evaluated

- Based on
  - P_{eff} human ileal and colonic permeability values of BCS highly permeable APIs
  - Residence times in the regions
- Assuming that
  - degradation is clinically important when ≥20% reduction in absorption from distal ileum or proximal colon occurs
  - degradation and absorption occurs according to 1st order kinetics

30min in distal ileum and **95min in proximal colon** seem to be reasonable **point estimates** of maximum bacterial degradation half-lives in order bacterial degradation to be clinically important

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Optimization of the dilution of feces with normal saline

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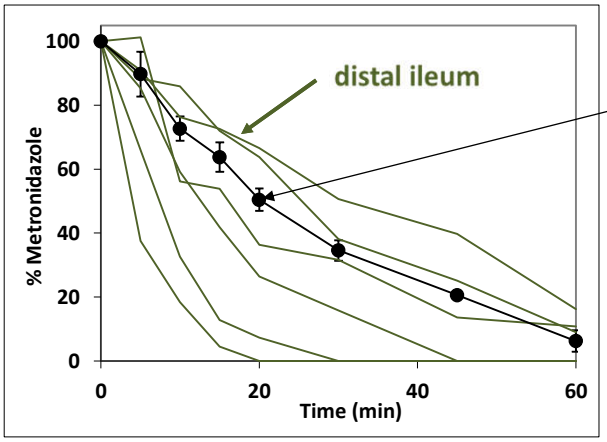
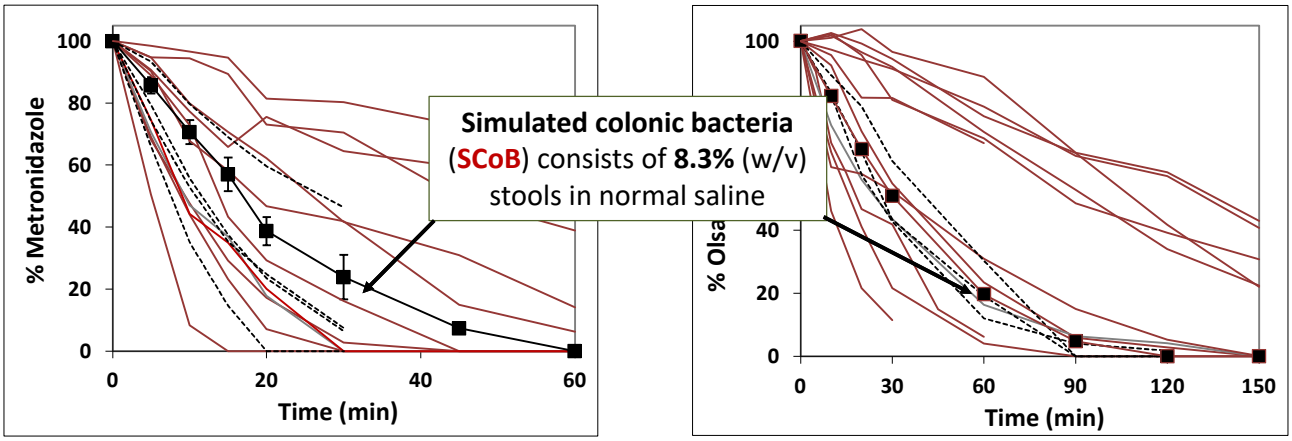


Simulated ileal bacteria (**SIB**) consists of **5.5%** (w/v) stools in normal saline

Karatza et al. Int. J. Pharm. 2017; Vertzoni et al Eur J Pharm Sci 2018

Optimization of the dilution of feces with normal saline

95min in proximal colon seems to be reasonable **point estimate** of maximum bacterial degradation half-lives in order bacterial degradation to be clinically important



Simulated colonic bacteria (**SCoB**) consists of **8.3%** (w/v) stools in normal saline

Vertzoni et al. Int. J. Pharm. 2011; Karatza et al. Int. J. Pharm. 2017; Vertzoni et al Eur J Pharm Sci 2018

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults using adult fecal material

Mean (SD) values for the bacterial degradation half-life (min) of model compounds tested in simulated intestinal bacteria (SIB) and in simulated colonic bacteria (SCoB)

	Simulated Ileal Bacteria (SIB)	Simulated Colonic Bacteria (SCoB)
Nitrendipine	**26.9 ± 3.4**	**16.7 ± 1.6**
Nimodipine	73.2 ± 7.8	**48.7 ± 2.7**
Sulfasalazine	**13.42 ± 0.45**	**7.897 ± 0.090**

$t_{1/2,SIB}$<30min

$t_{1/2,SCoB}$<95min

Data in line with available literature data in humans

Vertzoni et al Eur J Pharm Sci 2018

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults using adult fecal material

Is **nitroreductase** or **azoreductase** mediated degradation in the lower intestine likely to be clinically important for an orally administered therapeutic agent?

$t_{1/2, SCoB}$ < 95min

$t_{1/2, SCoB}$ > 95min

Proximal colon: Yes

Proximal colon: No
Distal ileum: No

$(t_{1/2, SIB})$<30min

$(t_{1/2, SIB})$>30min

Distal ileum: Yes

Distal ileum: No

Currently, investigating the usefulness of SCoB in simulating sulfoxide reduction

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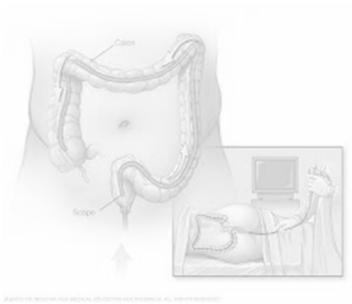
Key question: Can data be extrapolated to other populations?

To date,
we have started considering this question for older people

Older people
individuals older than 65 years old without any systemic diseases



Preliminary data

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Concluding Remarks

Data in SCoB are useful for evaluating whether bacterial azoreductase or nitroreductase activity in the proximal colon or in the distal ileum is likely to be clinically important for an orally administered therapeutic agent, provided that specific procedures are followed for

- collecting feces
  - preparing and storing fecal materials
    - evaluating the degradation kinetics.

The usefulness of this approach worth further investigation in case of

- therapeutic agents which are degraded by enzymes other than nitro or azo-reductases
  - formulations targeting the lower intestine
    - other populations (e.g. geriatric patients, paediatric populations, …).

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Thank you for your attention!

I would be happy to answer questions!

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