

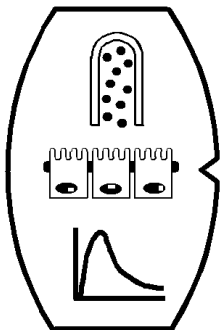


**GPEN 2006**  
The University of Kansas

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***Biorelevant media for in vitro permeability  
assessment of phosphate ester prodrugs:  
a case study with fosamprenavir***

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Laboratory for Pharmacotechnology and Biopharmacy

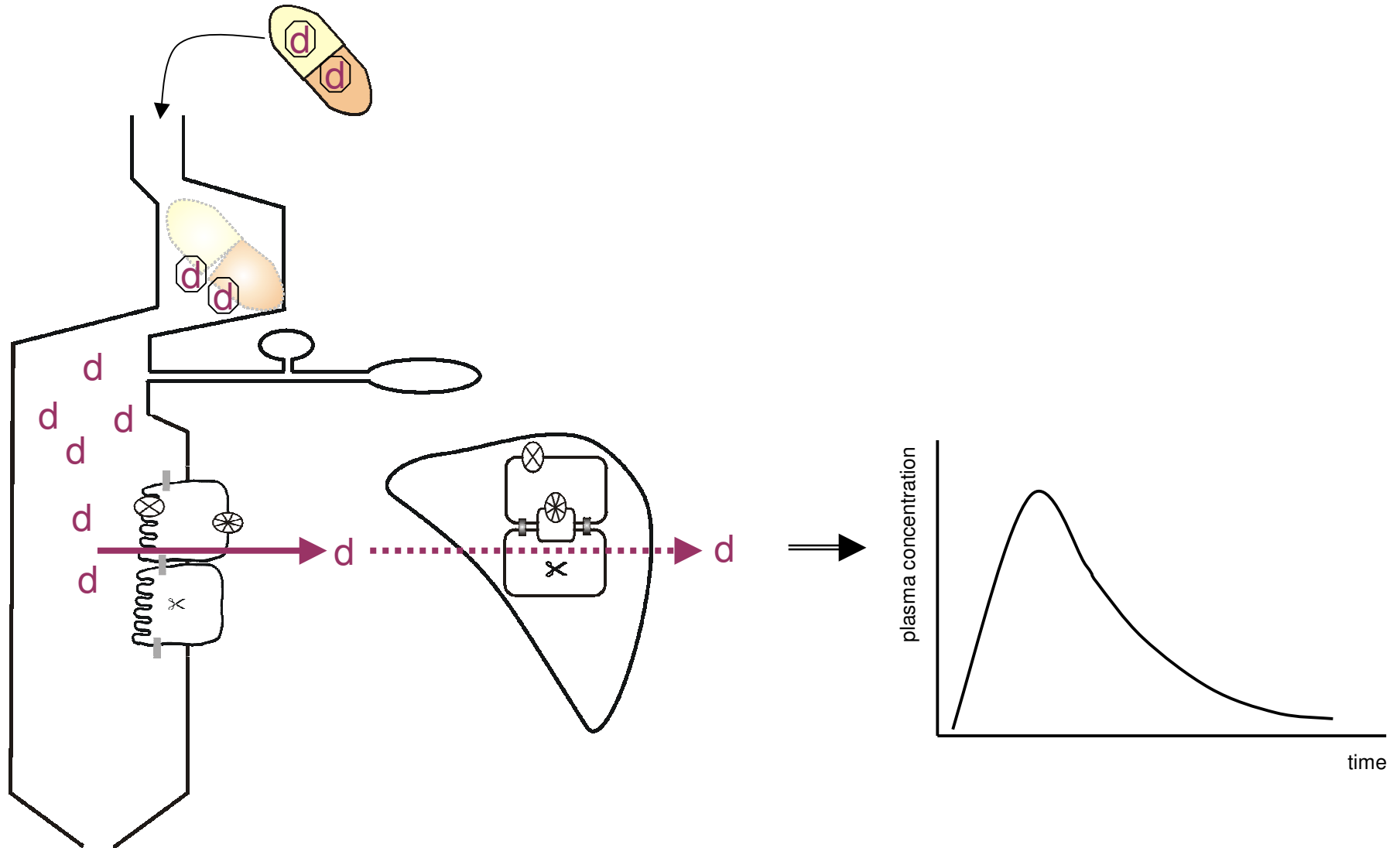
Katholieke Universiteit Leuven

GPEN, Kansas, October 25<sup>th</sup> 2006



# Introduction

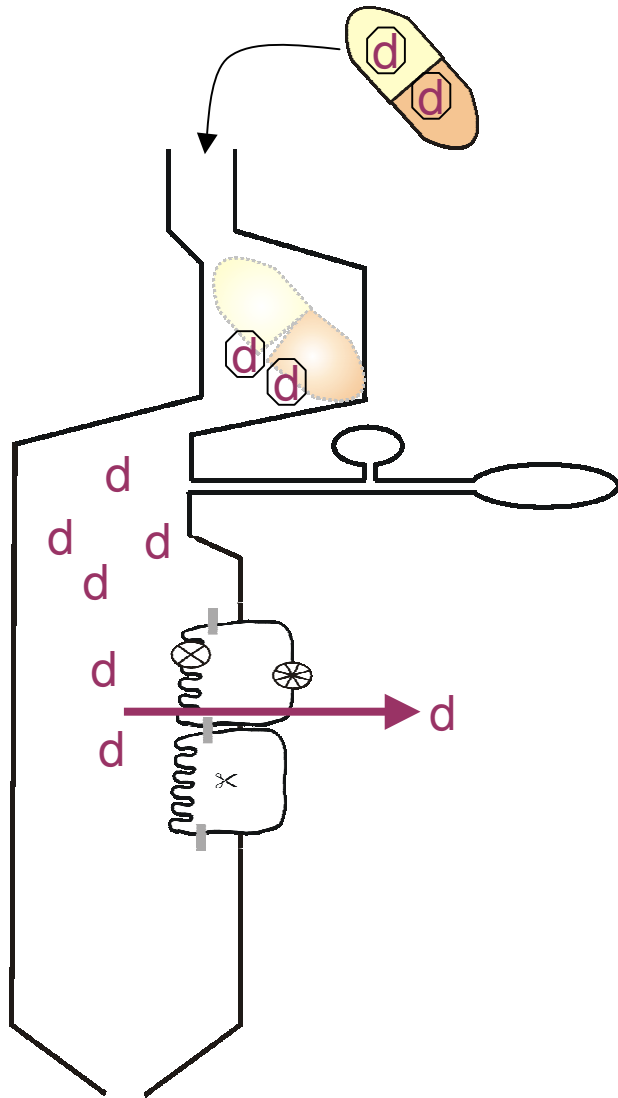
## *intraluminal drug & formulation behavior*



# Introduction

## *intraluminal drug & formulation behavior*

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Intraluminal conditions in function of time after oral drug intake?

pH

bile salts

phospholipids

drug concentration

excipient concentration

influence of food

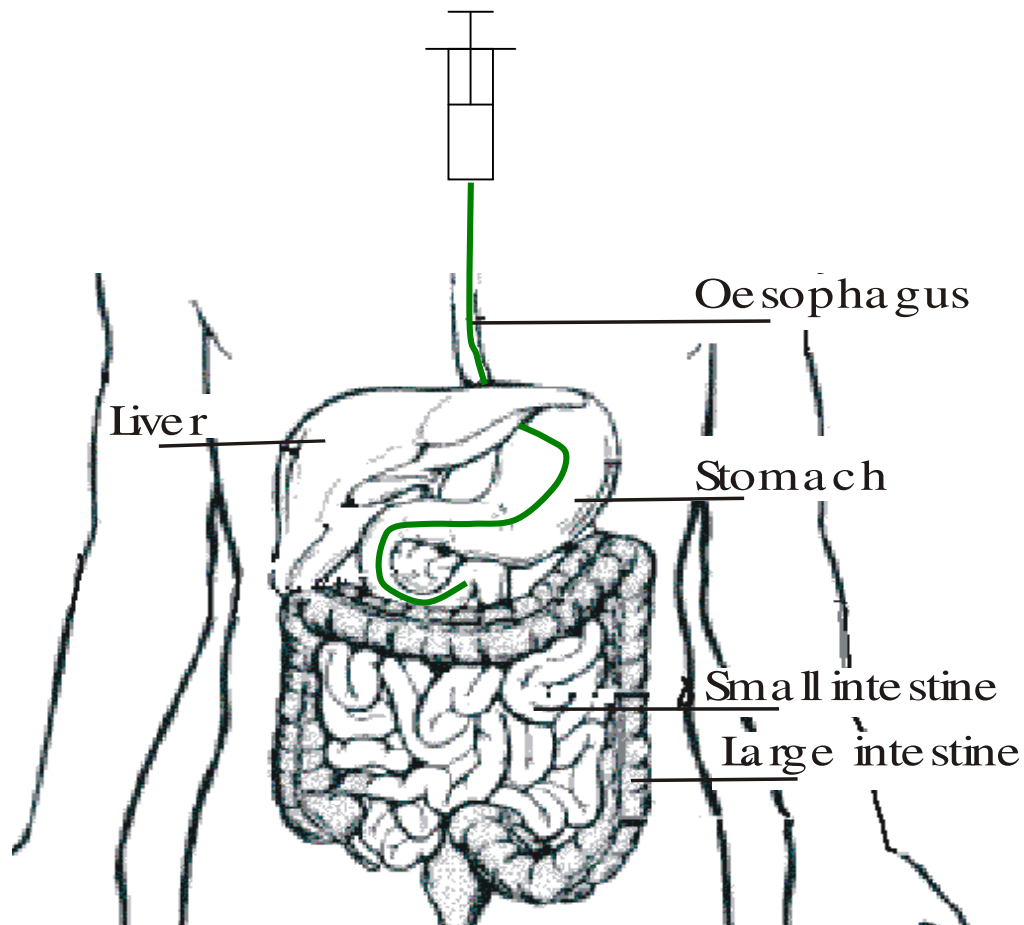
...

# Introduction

## *intraluminal drug & formulation behavior*

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### Sampling of human gastro-intestinal fluids



- healthy volunteers
- double lumen catheter(s)
- blank fluid
- after intake of oral dosage form
- fasted vs fed

# Introduction

## *intraluminal drug & formulation behavior*

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### Sampling of human gastro-intestinal fluids

- characterization: pH, bile salts, phospholipids, drug, excipient...

⇒ intraluminal conditions (after oral drug intake)

descriptive

relation to pharmacokinetics

working mechanisms of formulations

...

- integration in in vitro studies (dissolution / solubility / stability / permeability)

⇒ influence of real intraluminal conditions on drug absorption

⇒ biorelevance of model systems: aqueous buffers vs intraluminal conditions

# Introduction

## *amprenavir / fosamprenavir*

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Amprenavir:

HIV protease inhibitor

poorly water-soluble (0.08 mM in H<sub>2</sub>O, pH 7, 37 °C)

substrate of the efflux carrier P-gp

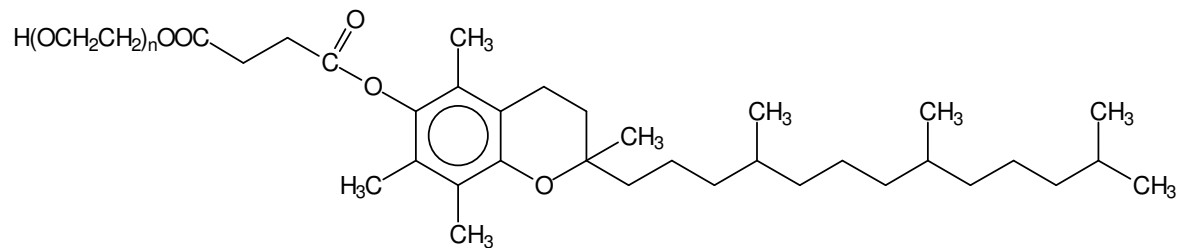
Standard formulation (Agenerase<sup>®</sup>):

**high pill burden!**

soft gelatin capsules

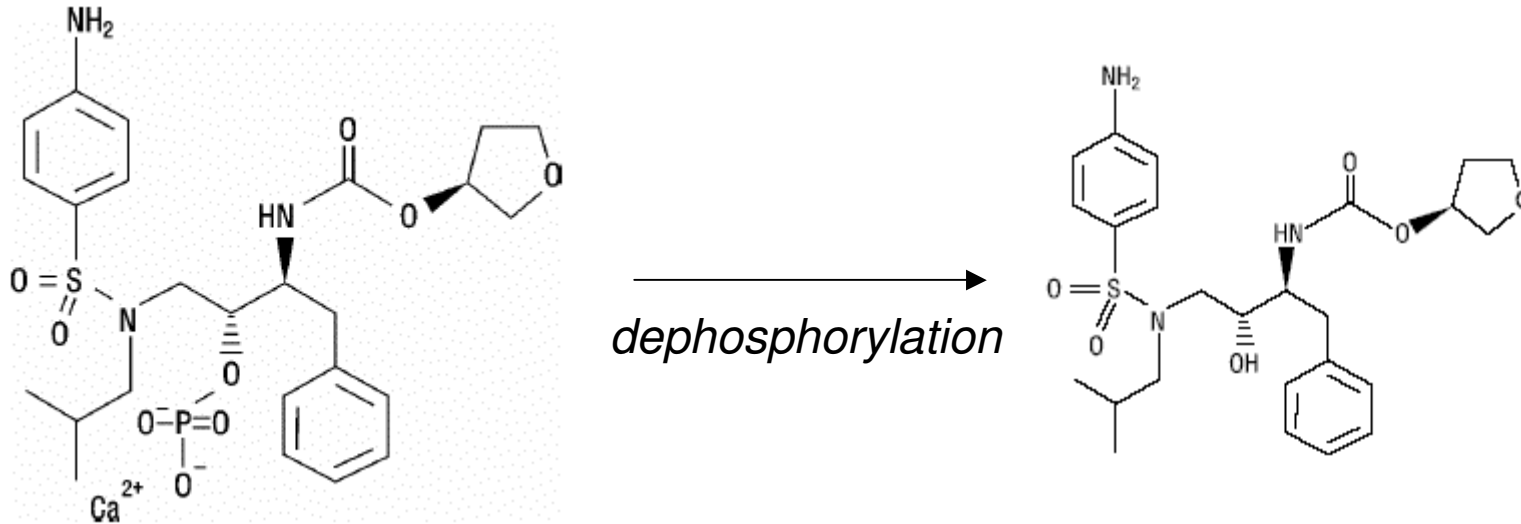
amprenavir 150 mg (single dose: 8 capsules, amprenavir 1200 mg)

solubilizing excipient TPGS



# Introduction

## *amprenavir / fosamprenavir*



Fosamprenavir  
water-soluble

→ Telzir<sup>®</sup>:  
2 tablets (1400 mg APV)

Amprenavir  
poorly water-soluble

→ Agenerase<sup>®</sup>:  
8 capsules (1200 mg APV)

# Introduction

## *amprenavir / fosamprenavir*

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Prodrug with increased solubility compared to parent drug

→ enhanced intestinal absorption of parent drug!

⇒ What happens in the gastro-intestinal tract?

in vivo study

in vitro study





# Purpose

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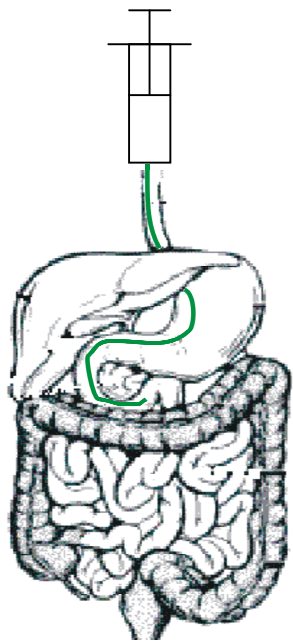
To characterize the in vitro behavior of fosamprenavir in the Caco-2 model system using different media:

- transport medium (aqueous buffer)
- human intestinal fluids
- “biorelevant” media: FaSSIF (+ taurocholate/phospholipids)

# Methods

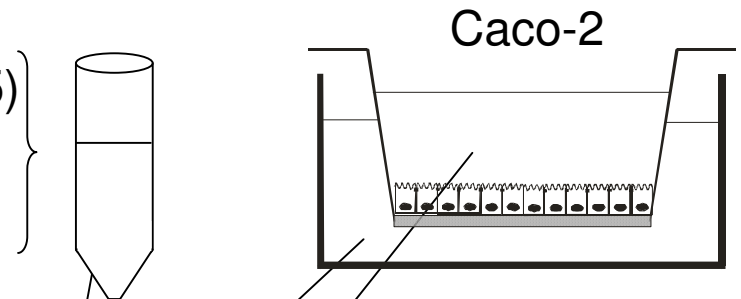
## Stability of fosamprenavir?

transport medium (MES-buffered HBSS, pH 6.5)  
“biorelevant” media  
human intestinal fluids (HIF)



### Sampling of HIF

- 3 volunteers
- duodenum
- fasted state
- in function of time
- samples pooled per volunteer
- pH / inorganic phosphate



### Sampling in function of time

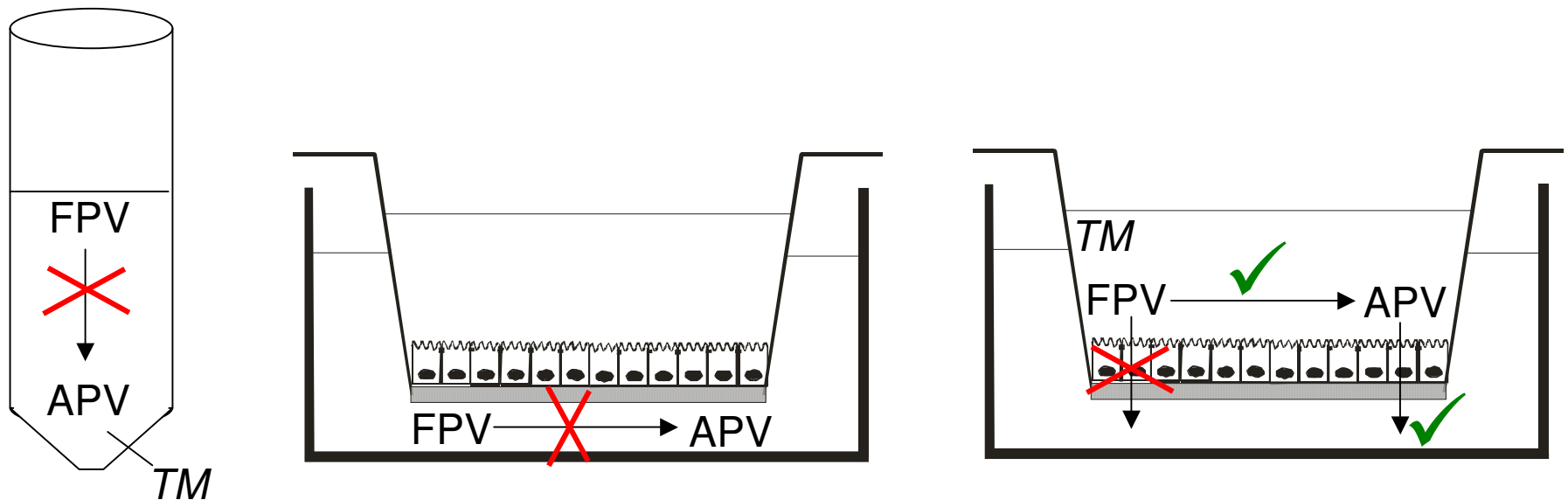
- (filtration)
- analysis of amprenavir and fosamprenavir (HPLC + fluorescence detection)

# Results

## *fosamprenavir in transport medium / Caco-2*

Stability of fosamprenavir upon incubation in transport medium?

Transport medium: MES-buffered HBSS pH 6.5

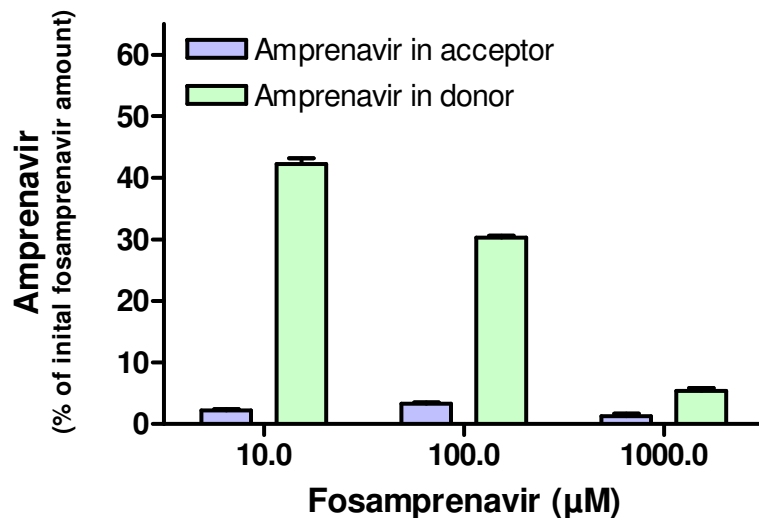


# Results

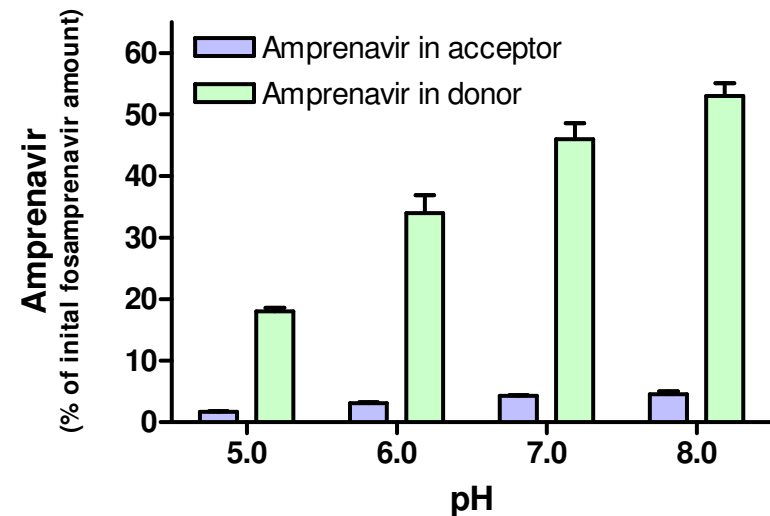
## *fosamprenavir in transport medium / Caco-2*

Incubation at the apical side of Caco-2 monolayers (60 min, 37°C)

Concentration-dependency (pH 6.5)



pH-dependency (FPV 10 µM)

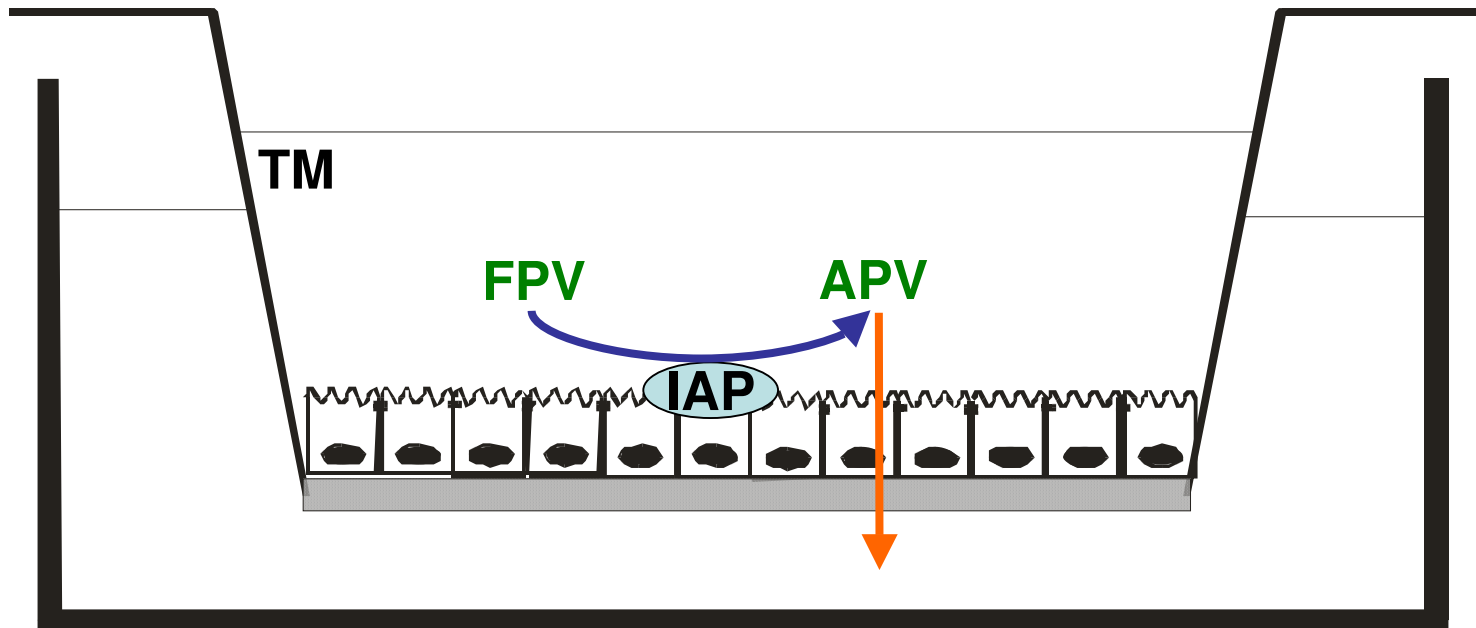


⇒ Conversion to amprenavir: concentration-dependent  
pH-dependent

⇒ Ca. 8% of the amprenavir formed is transported across the cell monolayer

# Results

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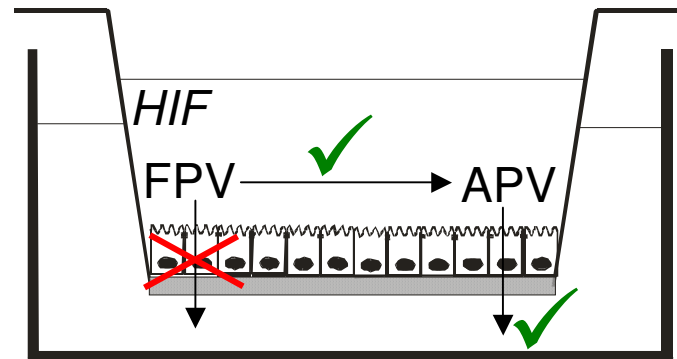
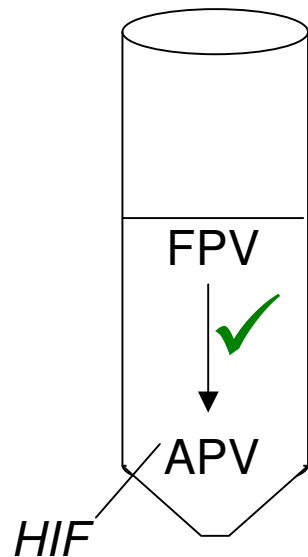
IAP: intestinal alkaline phosphatase

# Results

## *fosamprenavir in HIF / Caco-2*

Stability of fosamprenavir upon incubation in HIF?

HIF (human intestinal fluid): 3 volunteers, fasted, duodenum

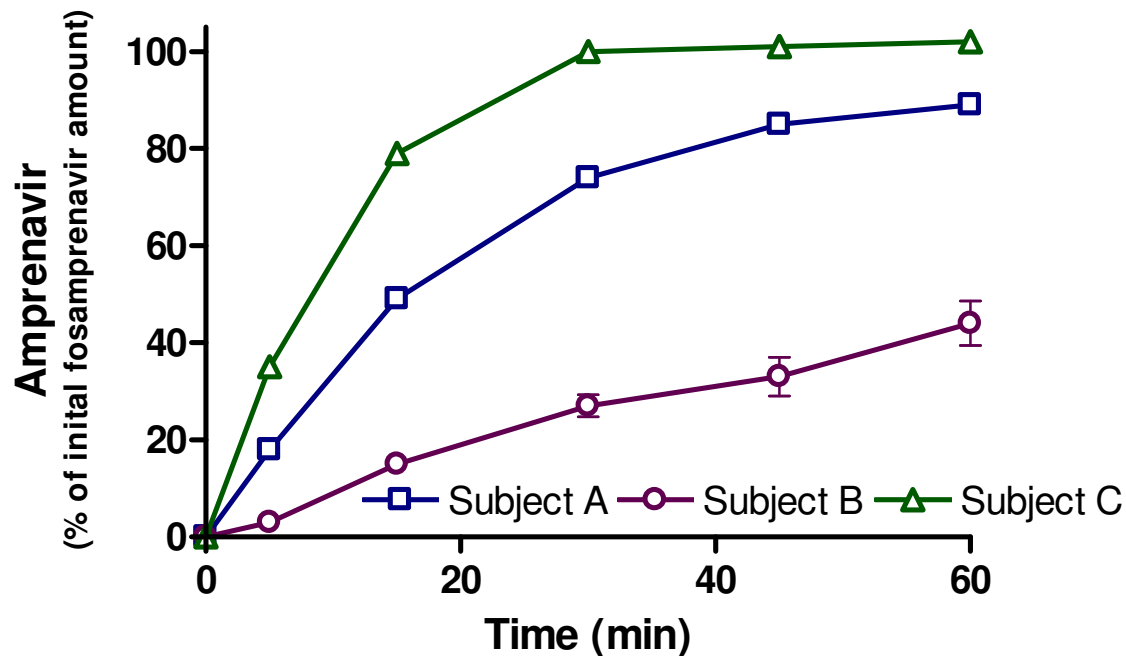


# Results

## *fosamprenavir in HIF*

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Stability of fosamprenavir (10  $\mu$ M) upon incubation in HIF (37°C)



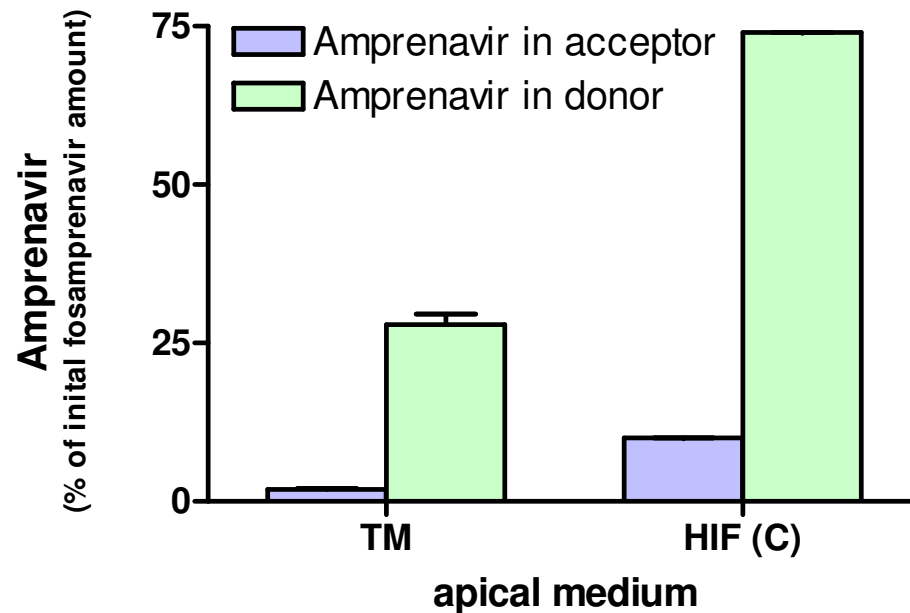
⇒ Phosphatase activity in HIF, depending on subject.

# Results

## *fosamprenavir in HIF / Caco-2*

Incubation at the apical side of Caco-2 monolayers

(fosamprenavir 10  $\mu$ M, 60 min, 37°C)



⇒ Phosphatase activity of Caco + HIF



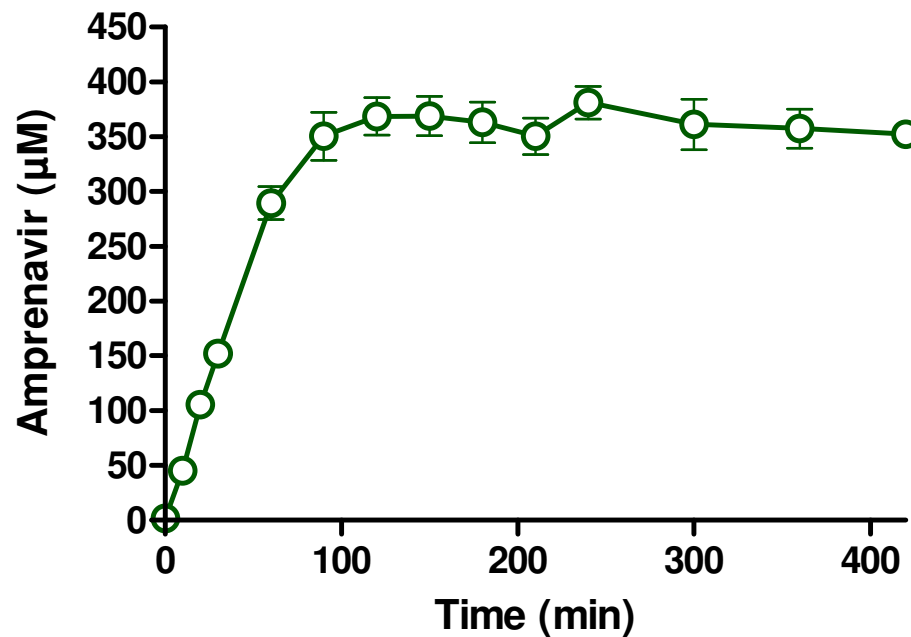
# Results

## *supersaturation in HIF*

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High dose of fosamprenavir → amprenavir??

incubation of fosamprenavir 500  $\mu\text{M}$  in HIF



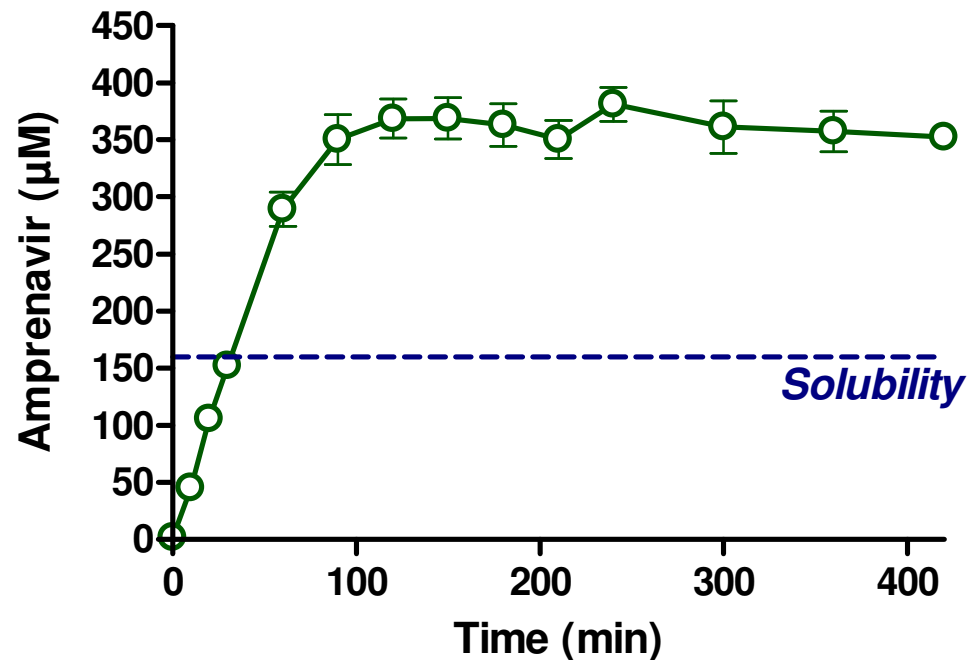
# Results

## *supersaturation in HIF*

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High dose of fosamprenavir → amprenavir??

incubation of fosamprenavir 500  $\mu\text{M}$  in HIF



⇒ create and maintain supersaturation of amprenavir in HIF

# Results

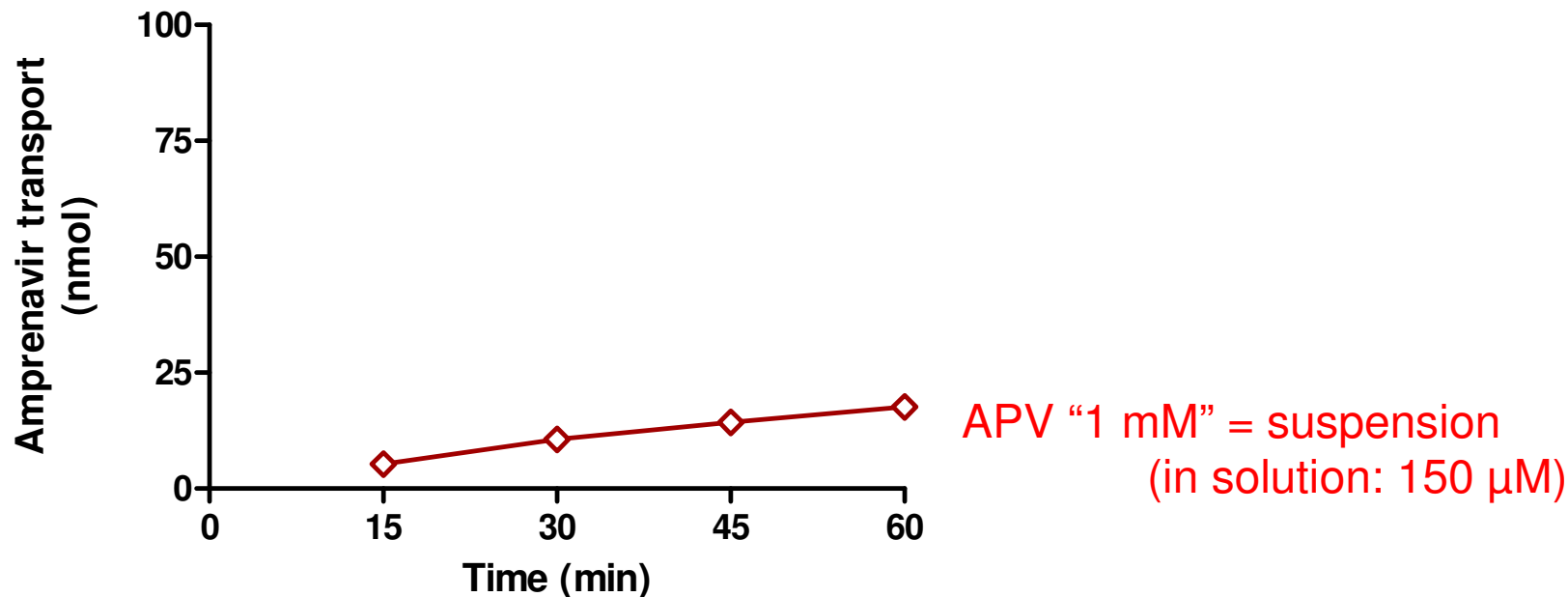
## *supersaturation and flux*

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Supersaturation  $\Rightarrow C_0 \uparrow \Rightarrow$  flux?

incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers

$\rightarrow$  transport of amprenavir in function of time?



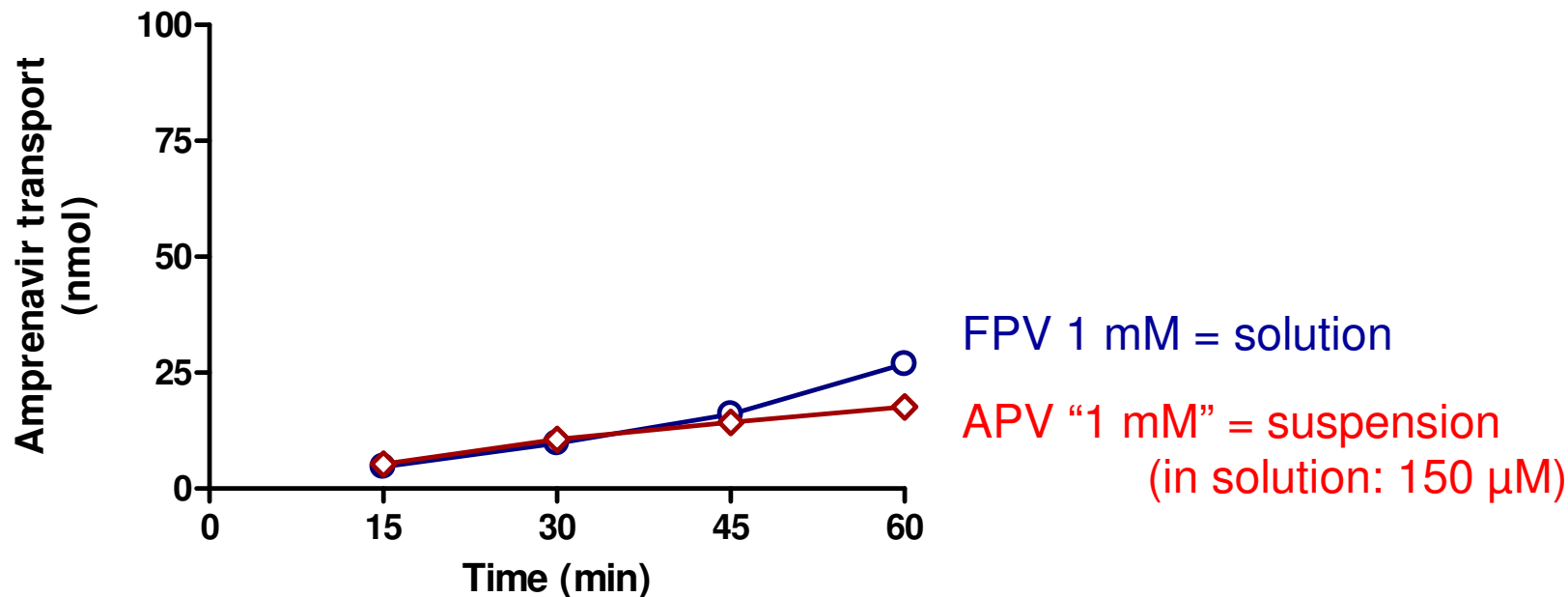
# Results

## *supersaturation and flux*

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incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers  
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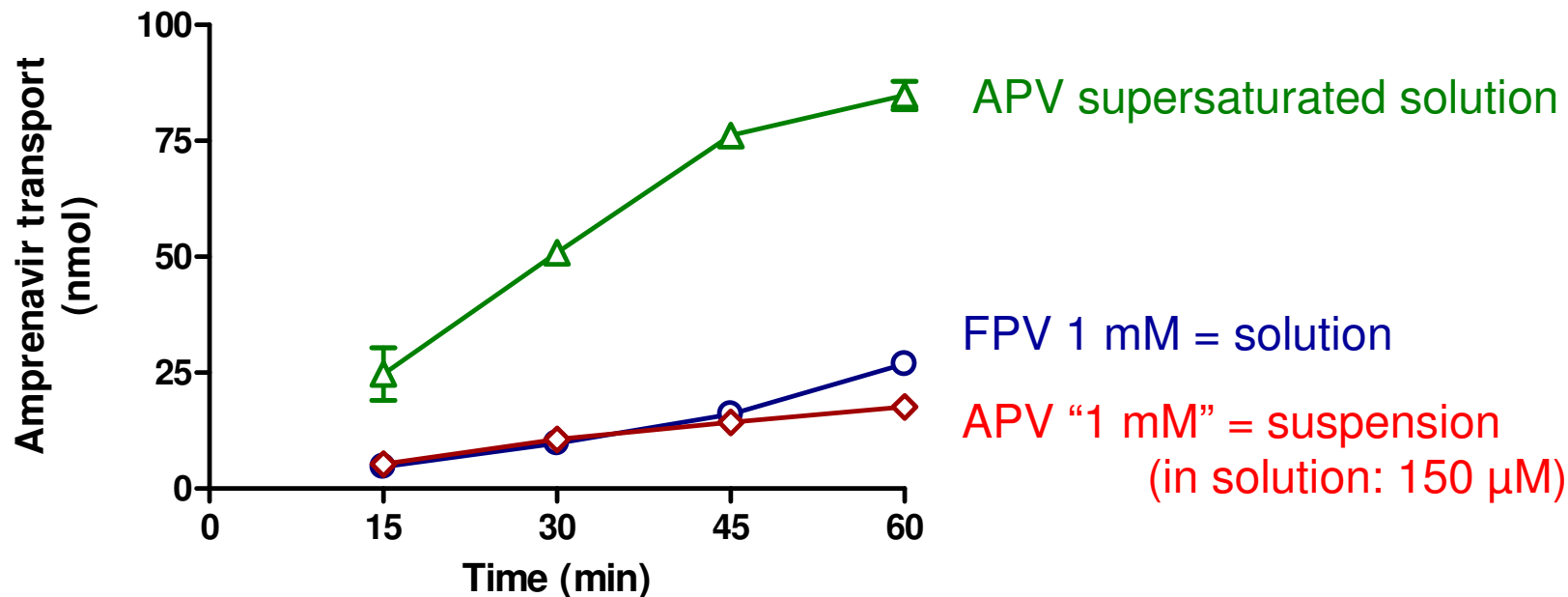
# Results

## *supersaturation and flux*

Supersaturation  $\Rightarrow C_0 \uparrow \Rightarrow$  flux?

incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers

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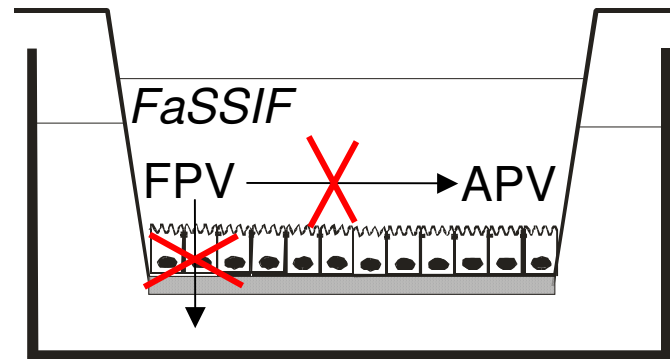
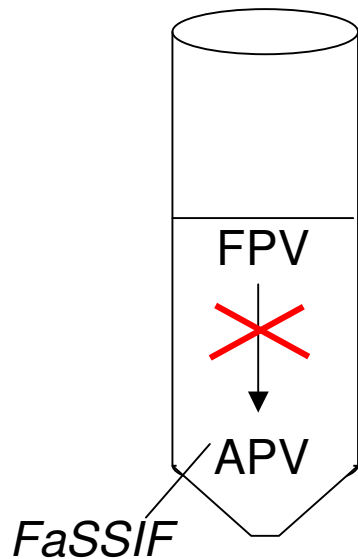
# Results

## *fosamprenavir in FaSSIF / Caco-2*

Stability of fosamprenavir upon incubation in FaSSIF?

FaSSIF (Fasted State Simulated Intestinal Fluid): phosphate buffer pH 6.5

poorly water-soluble drugs ← { taurocholate 3 mM  
lecithin 0.75 mM

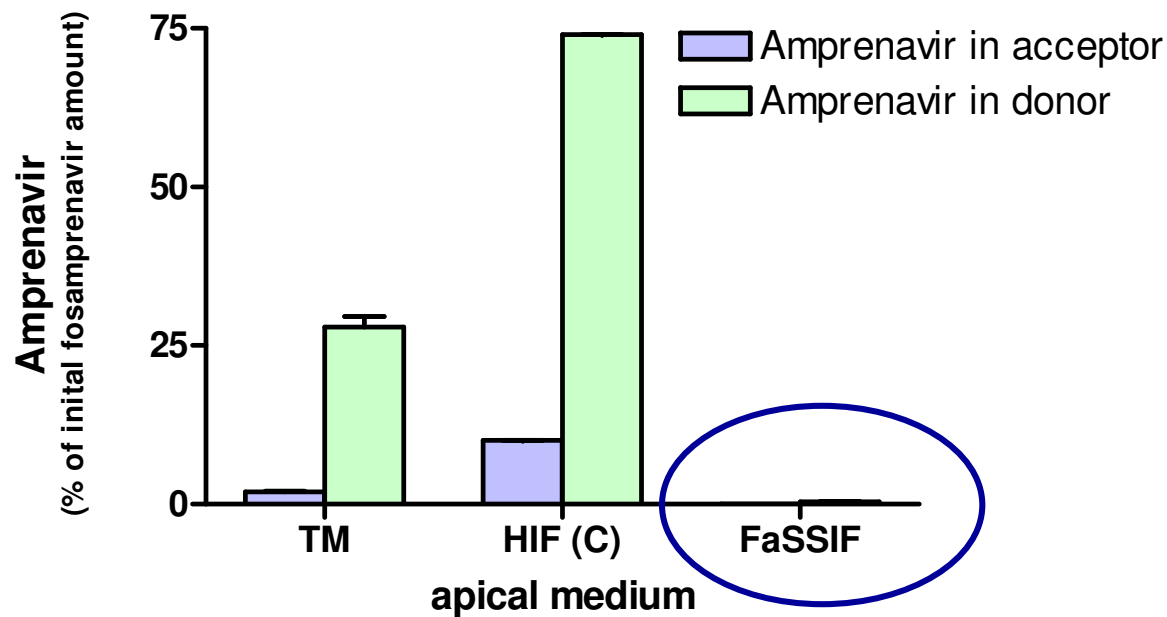


# Results

## *fosamprenavir in FaSSIF / Caco-2*

Incubation at the apical side of Caco-2 monolayers

(fosamprenavir 10  $\mu$ M, 60 min, 37°C)



⇒ Almost no dephosphorylation of fosamprenavir using FaSSIF as medium!

→ not biorelevant!

# Results

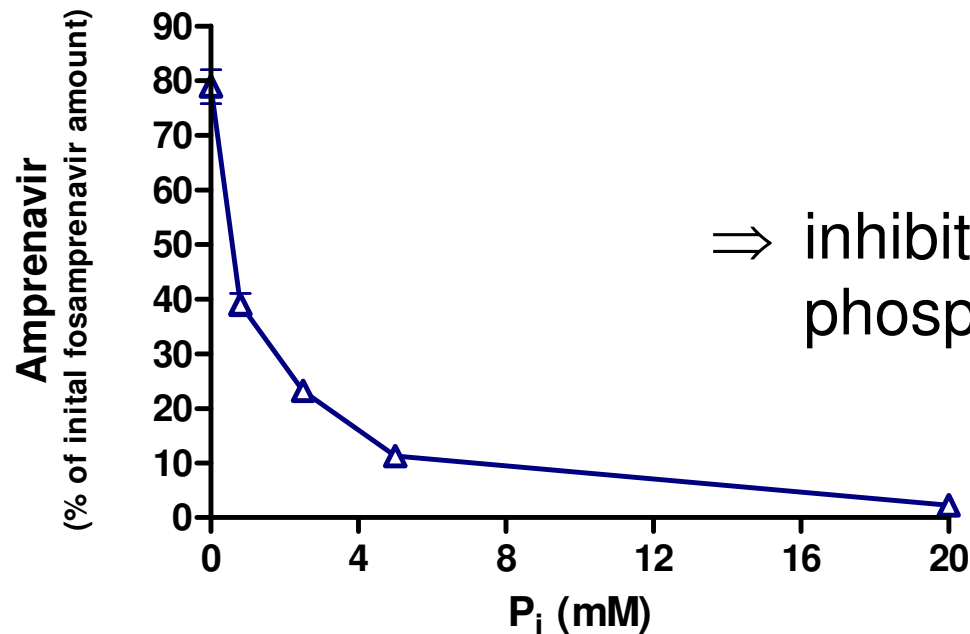
## *inorganic phosphate*

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Incubation at the apical side of Caco-2 monolayers

(fosamprenavir 10  $\mu$ M, 60 min, 37°C)

TM with different concentrations of inorganic phosphate ( $P_i$ )



⇒ inhibition of intestinal alkaline phosphatase by inorganic phosphate

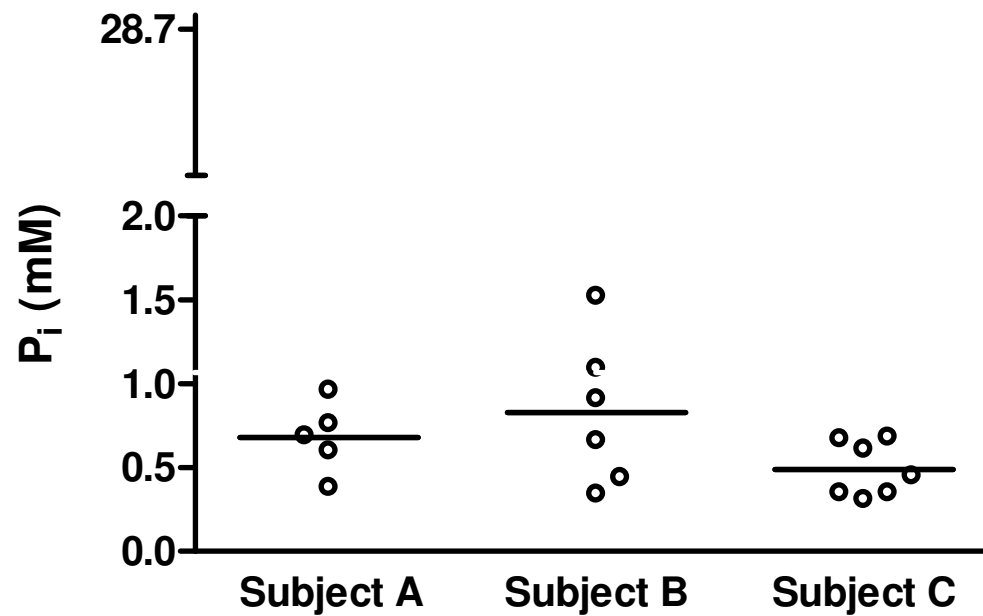


# Results

## *inorganic phosphate*

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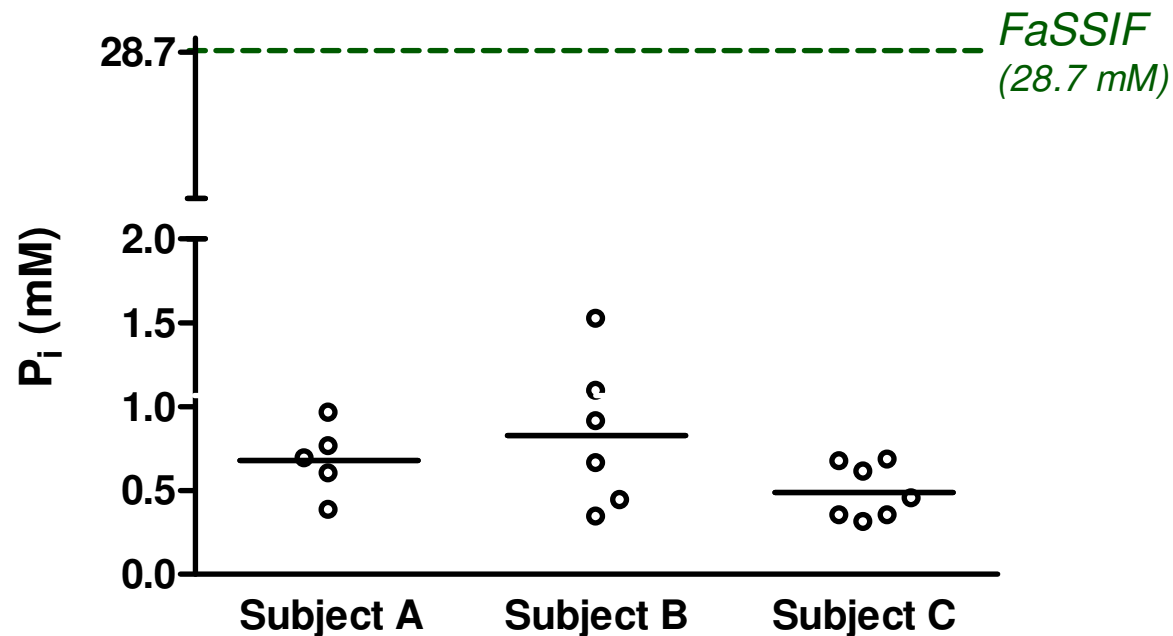
Intraluminal phosphate concentrations?



# Results

## *inorganic phosphate*

Intraluminal phosphate concentrations?

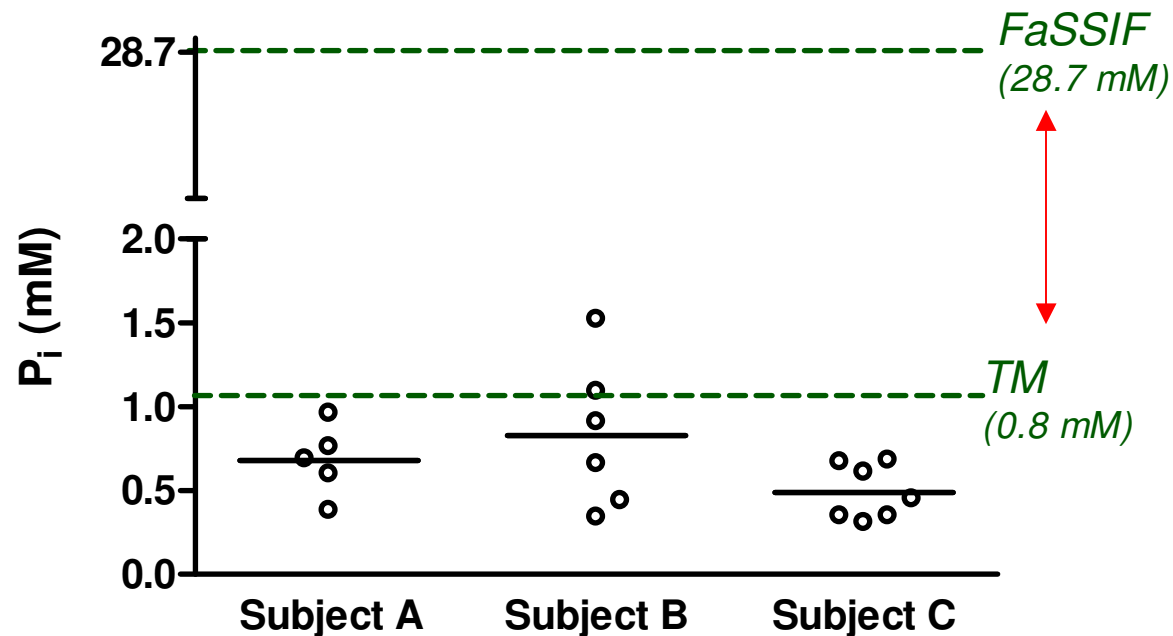


⇒ Phosphate-buffered FaSSIF is not compatible with phosphate ester prodrugs.

# Results

## *inorganic phosphate*

Intraluminal phosphate concentrations?



⇒ Phosphate-buffered FaSSIF is not compatible with phosphate ester prodrugs.

# Results

## *alternative for FaSSIF*

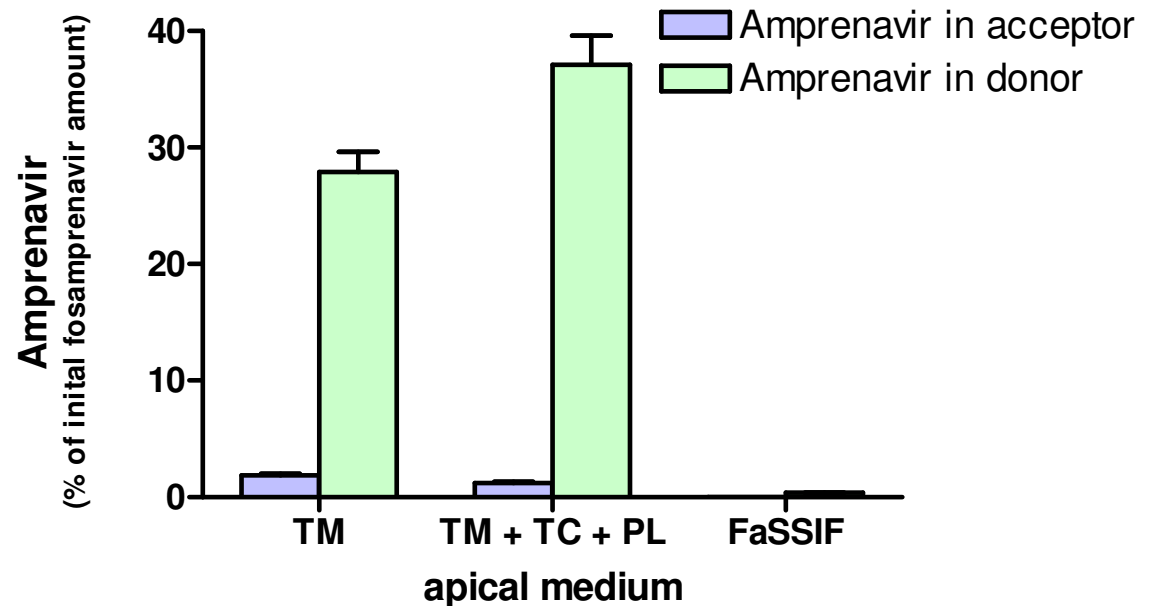
Incubation at the apical side of Caco-2 monolayers

(fosamprenavir 10  $\mu$ M, 60 min, 37°C)

TM (MES-buffered HBSS)

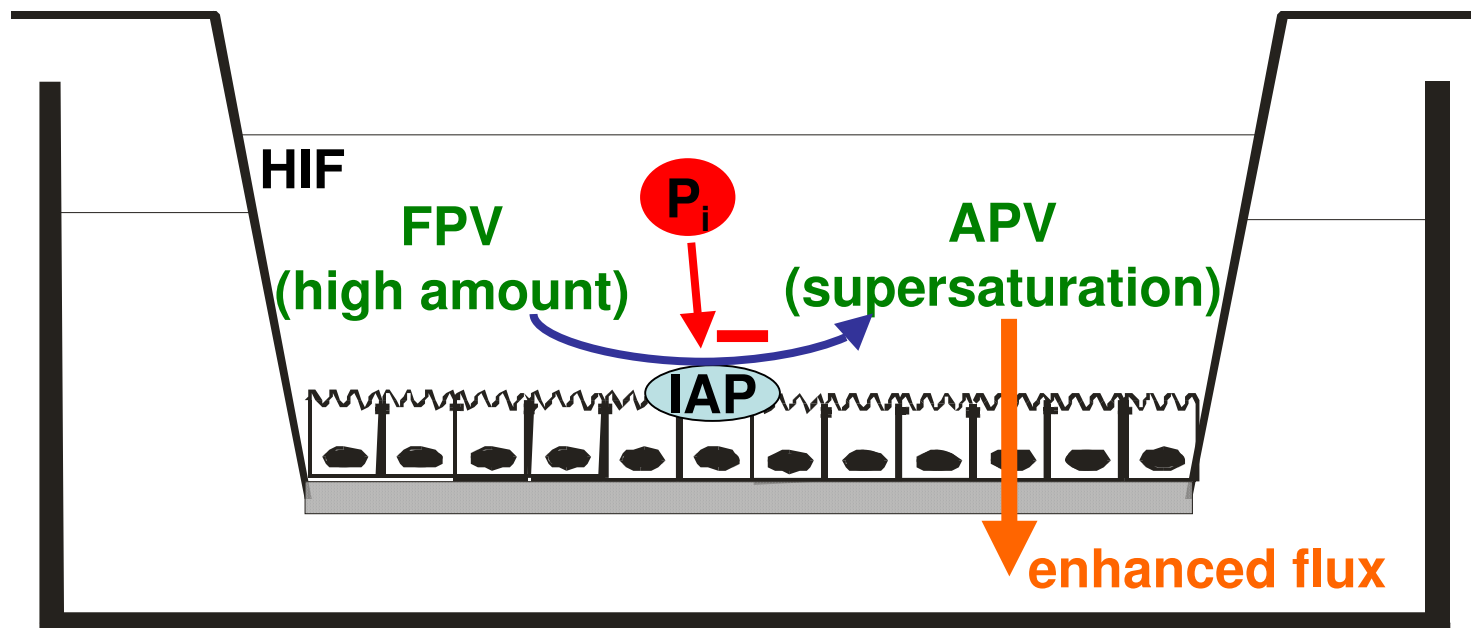
+ taurocholate (TC) 3 mM

+ phospholipids (PL) 0.75 mM



# Conclusion

- ✓ Illustration of intraluminal supersaturation of a poorly water-soluble drug from its soluble prodrug in real intestinal media.
- ✓ Dephosphorylation of fosamprenavir is inhibited by inorganic phosphate → biorelevant media!
- ✓ Ongoing: in vivo intraluminal behavior of fosamprenavir fasted vs fed



# Acknowledgements

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