An *in vitro* biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: Design, implementation, and evaluation

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Abstract

Purpose: Design an *in vitro* methodology for studying gastrointestinal transfer in the fasted state and implement the methodology *in vitro* by using a biorelevant gastrointestinal transfer system (BioGIT); evaluate the usefulness of BioGIT in predicting luminal concentrations of lipophilic weak bases in fasted upper small intestine.

Methods: The methodology was designed after modeling existing luminal data. Its implementation *in vitro* was based on a three compartment setup. Reproducibility of the transfer process was evaluated under conditions where solutions and/or suspensions were present in gastric and/or duodenal compartment and by using ranitidine, dipyridamole, ketoconazole, and posaconazole as model drugs. The transfer process as well as concentrations of dipyridamole, ketoconazole and posaconazole measured in the duodenal compartment were compared with data previously collected in the upper small intestine, after administration of identical preparations/dosage forms to fasted adults.

Results: Using BioGIT, the transfer process was performed reproducibly in all cases (RSD<12.9%); data with dipyridamole and ketoconazole were in line with luminal data in humans. Dipyridamole, ketoconazole and posaconazole concentrations in duodenal compartment were also in line with previously measured concentrations in the fasted upper small intestine of healthy adults.

Conclusions: BioGIT system could be useful for the evaluation of the impact of gastrointestinal transfer on concentrations in upper intestinal lumen during the first hour, after oral administration of dispersing/solution dosage forms of lipophilic weak bases.

45 **Keywords**

46 BioGIT, gastrointestinal transfer, biorelevant evaluation, luminal concentrations,

47 precipitation, weak bases

1. Introduction

Gastrointestinal transfer is a prerequisite for the initiation of drug absorption after oral administration. In cases where a poorly water soluble active pharmaceutical ingredient (API) is a weak base and/or it has been formulated as a bioenhanced (enabling) product the impact of gastrointestinal transfer increases, because precipitation of the API in the upper small intestine following transfer will reduce intraluminal concentrations. In these cases, maintenance of supersaturation in upper small intestine for as long as most of the dose is absorbed, would be advantageous for the rate and, possibly, the extent of absorption. This is an important issue during the development of drugs for oral administration, not only because weak bases comprise the majority of orally administered APIs (Paulekuhn et al. 2007), but, also, because aqueous solubility of new drugs and drug candidates is often very low and insufficient absorption frequently requires enabling formulations (e.g. Augustijns and Brewster, 2012).

Evaluation of the impact of gastrointestinal transfer on intraluminal drug concentrations and on drug absorption can be performed by using three approaches. The first involves *in silico* modeling of API presence in dissolved state in the upper gastrointestinal lumen by combining dissolution, nucleation, and particle growth kinetics, based on theories of particle dissolution and crystallization (Sugano et al. 2010). With this approach the average cumulative amount to be absorbed could be estimated (Sugano et al. 2010). The second approach is based on parameters controlling dissolution, supersaturation and precipitation which are estimated from *in vitro* experiments (Kostewicz et al. 2004, Carlert et al. 2010; Arnold et al. 2011). Most frequently, relevant parameters are introduced in physiologically based pharmacokinetic models and the average plasma profile is estimated (Shono et al. 2011; Berlin et al. 2014; Berlin et al. 2015). One issue of these approaches may be the

validation of methodologies used for estimating the key input parameters, because the biorelevance of *in vitro* data is evaluated indirectly by monitoring plasma concentrations, and, therefore, by assuming adequate description of all other processes which lead to drug arrival in and elimination from plasma. The third approach involves direct estimation of concentrations in the upper small intestine using biorelevant *in vitro* setups. To date, various *in vitro* methodologies have been proposed for simulating, gastrointestinal transfer and duodenal elimination in order to estimate duodenal drug concentrations (Blanquet et al. 2004; Gu et al. 2005; Polster et al. 2010; Psachoulis et al. 2012; Mitra and Fadda 2014; Matsui et al. 2015). Concentrations in the upper small intestine drive absorption and are, as such, useful for selecting the appropriate dosage form and/or the physical and chemical state of the API. Provided that adequate modeling approaches are available, relevant data may also be useful for evaluating the impact of concentrations in the upper small intestine on plasma levels. An advantage of this approach is that the simulations can be directly validated with luminal concentrations measured in humans.

The Psachoulis et al. (2012) methodology is a representative example of the third approach. It has been useful in forecasting luminal drug concentrations and precipitation in humans, after administration of solutions of dipyridamole and ketoconazole (Psachoulis et al. 2011). Data with two other weak bases under development were also in line with plasma data, after administration of both solutions and suspensions (Psachoulis et al. 2012). In addition, it has been shown to be more useful than other *in silico* or *in vitro* methodologies in simulating the gastrointestinal transfer of a hydrophilic non-absorbable marker, after administration in the fasted state (Hens, Brouwers et al. 2014). An extension of this methodology has recently been proposed for the study of weak bases, after oral administration of solid dosage forms, which enables studying the impact of intragastric dissolution on duodenal concentrations (Dimopoulou et al. 2015).

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101 The Psachoulis et al. (2012) *in vitro* methodology involves the use of a three compartment
102 *in vitro* setup, consisting of a gastric, a duodenal, and a reservoir compartment. This *in vitro*
103 system has been used as a non-continuous (stepwise) model consisting of 5 individual
104 experiments. Contents of the duodenal compartment are completely renewed every 15
105 minutes with fresh Level II biorelevant medium simulating the composition in upper small
106 intestine (Psachoulis et al. 2012; Markopoulos, Andreas et al. 2015). Upon each renewal,
107 the gastrointestinal flow rate is decreased to simulate first order gastric emptying kinetics
108 (Psachoulis et al. 2012; Dimopoulou et al. 2015). An overall duodenal concentration-time
109 profile is obtained by combining the concentrations measured at 7.5 minutes, after each
110 renewal. However, the stepwise mode of application does not comply with the continuous
111 gastrointestinal transfer process *in vivo*. In addition, the required equipment for its
112 application in practice is not commercially available.

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114 The objective of the present study was threefold.

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116 First, to design an *in vitro* model for simulating gastrointestinal transfer in the fasted state,
117 after modelling existing luminal data in humans (Psachoulis et al. 2011) that could be
118 applied in a continuous mode, by using a three compartment setup [Biorelevant Gastro-
119 Intestinal Transfer (BioGIT) system].

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121 The second objective was to implement the new transfer model *in vitro*. Implementation
122 was evaluated by testing the reproducibility of the transfer process and by testing whether
123 the process takes place according to theory. Three different situations were considered, i.e.
124 (i) when contents of both gastric and duodenal compartment are solutions (by using
125 ranitidine), (ii) when contents of gastric compartment are a solution but contents of

126 duodenal compartment may contain precipitated particles (by using dipyridamole and
127 ketoconazole), and (iii) when contents in both gastric and duodenal compartment are
128 suspensions (by using posaconazole).

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130 The third objective was to evaluate the usefulness of the proposed methodology in
131 forecasting the concentrations of weak bases in the upper small intestine (by using
132 dipyridamole, ketoconazole and posaconazole). Concentrations in the duodenal
133 compartment were compared with previously reported luminal concentrations in humans
134 (Psachoulis et al. 2011; Walravens et al. 2011).

2. Materials and Methods

2.1 Materials

Ranitidine was from Unipharma, S.A. (Athens, Greece), dipyridamole (DPD) was from Boehringer Ingelheim España, S.A. (Malgrat De Mar, Spain) and ketoconazole (KCZ) was from Janssen Pharmaceutical Ltd (Little Ireland, Cork, Republic of Ireland). Posaconazole (PSC) as well as the marketed product Noxafil® suspension (40 mg/mL) was from Merck Sharp & Dohme Ltd (Hertfordshire, UK). All APIs were of at least 99% purity. Acetonitrile, dichloromethane and methanol of HPLC grade were from Sigma Aldrich (St. Louis, U.S.A.). Egg phosphatidylcholine (Lipoid E PC® 99.1% pure, lot# 105019-1/14) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Sodium phosphate monobasic dihydrate, sodium hydroxide, sodium chloride, sodium oleate (88.9% w/w pure) and cholesterol (minimum 99%) were purchased from Sigma-Aldrich (St. Louis, U.S.A.), respectively. FaSSIF and FaSSIF-V2 powders were kindly donated by Biorelevant.com (Surrey, U.K.). Diethylamine was purchased from Riedel-de Haën® (Seelze, Germany).

2.2 Methods

2.2.1 Estimation of key parameters for evaluating drug transfer in the upper small intestine based on existing luminal data and design of the BioGIT system

A compartmental model was fitted to previously published intraluminal data (Psachoulas et al. 2011) that had been collected after single dose administrations of dipyridamole and ketoconazole solutions to fasted healthy adults. Two doses of dipyridamole (30 mg and 90 mg) and two doses of ketoconazole (100 mg and 300 mg) had been administered as 240 ml

aqueous solutions. Drug content had been measured in individual samples of intestinal contents aspirated at specific times post-dosing (Psachoulis et al. 2011). The following compartmental model was used to describe the drug transfer through the upper small intestine:

$$y = Dose \frac{1}{V_I} \frac{k_G}{k_G - k_I} (e^{-k_I t} - e^{-k_G t}) \quad (\text{Eq.1})$$

where y is the total drug amount per volume of intestinal contents, t is the time after administration, V_I is the volume of contents in upper small intestine, k_G is the first order gastric emptying rate constant, and k_I is the first order upper small intestine elimination rate constant. The elimination rate constant of the upper small intestine is the sum of intestinal transit rate constant and absorption rate constant. For simultaneous analysis of all luminal data, y was normalized with each drug's dose. Key parameter values, estimated after least square fitting of the compartmental model equation to the luminal data, were used to propose the experimental conditions of a three compartment *in vitro* setup. Specifically, the *in vitro* setup consists of the gastric compartment, the duodenal compartment and the reservoir compartment (Figure 1).

The mathematical description of total mass transfer through the *in vitro* system (Figure 1), assuming solution or perfectly mixed dispersion in gastric and duodenal compartment, is presented below:

The amount of drug emptying from the gastric compartment, A_G , per time interval dt is

$$\frac{dA_G}{dt} = F_1 \frac{Dose}{V_G} = F \frac{A_G}{V_G} = F \frac{Dose}{V_G} e^{-k_G t} \quad (\text{Eq.2})$$

where V_G is the initial volume of gastric contents, F_1 , is the fluid flow rate from the gastric compartment that is decreasing with time,

$$F_1 = F e^{-k_G t} \quad (\text{Eq.3})$$

and F is a constant fluid flow rate in and out of the duodenal compartment.

The volume of contents in the duodenal compartment, V_I , is kept constant as fluid without drug is transferred also from the reservoir compartment to the duodenal compartment, with flow rate F_2 , so that $F_1 + F_2 = F$. The value of F can be calculated from

$$F = V_G k_G = V_G \ln(2)/t_{1/2G}$$

where $t_{1/2G}$ is the gastric emptying half-life. The amount of drug change per time interval in the duodenal compartment, dA_I/dt , is estimated as follows:

$$\frac{dA_I}{dt} = F_1 \frac{Dose}{V_G} - \frac{F}{V_I} A_I \quad (\text{Eq.4})$$

After substituting Eq.3 in Eq.4, integrating and dividing by V_I , the following equation results:

$$\frac{A_I}{V_I} = Dose \frac{1}{V_I} \frac{k_G}{k_G - F/V_I} (e^{-(F/V_I)t} - e^{-k_G t}) \quad (\text{Eq.5})$$

The ratio F/V_I corresponds to k_I , the first order elimination rate constant of the upper small intestine. Equation 5 is the *in vitro* transfer model equation.

2.2.2 Implementation of the theoretical methodology in *in vitro* experiments using the BioGIT system

For practical reasons, the following approximation of equation 5 can be considered:

During a small observation period (e.g. 10 min), flow rate F_1 is kept constant with a value, F_{1i} , corresponding to the midpoint of the observation period i , calculated from Eq.3. During this time period i , the rate of change of drug amount in the duodenal compartment, resulting from the i drug input, may be expressed as

$$\frac{dA_{1i}}{dt} = F_{1i} \frac{Dose}{V_G} - \frac{F}{V_I} A_{1i} \quad (\text{Eq.6})$$

which, after integration, results in

$$\frac{A_{li}}{V_l} = \frac{F_{li}}{F} \frac{Dose}{V_G} (1 - e^{-\frac{F}{V_l} t}) \quad (\text{Eq.7})$$

where t is the time after initiation of the i drug input. Using a similar reasoning, the amount of drug change per time in the duodenal compartment resulting from the i drug input can be calculated for each consecutive observation time period. The overall drug transfer can be estimated using the superposition principle, i.e. by taking also into account the amount of drug remaining in the duodenal compartment at the end of each observation period. The best approximation of the drug amount is then obtained at the midpoint of each observation period.

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2.2.3 Evaluation of the transfer process and concentrations in the upper small intestine using the BioGIT system

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The degree of similarity of data estimated using equation 5 (*in vitro* transfer model equation) with the data expected after implementing the theoretical methodology in *in vitro* experiments (Expected values for the transfer process) and with actual *in vitro* data (Experimental values in the duodenal compartment, referring to total drug, solid and in solution, in the duodenal compartment) was evaluated using solutions of ranitidine, dipyridamole, and ketoconazole as well as a suspension of posaconazole in the gastric compartment.

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In vitro data were collected with a DT6 dissolution system (Erweka, Heusenstamm, Germany) by using a mini vessel with 500 ml capacity (Erweka, Heusenstamm, Germany) for the gastric compartment and a mini vessel with 100 ml capacity (Distek, New Brunswick NJ, USA) for the duodenal compartment (Figure 1). The Distek mini vessel and mini paddle were placed in the Erweka dissolution apparatus by using appropriate adjusters. In all cases, the

temperature was set at 37 °C and the paddle rotation at 75 rpm, both in the gastric and duodenal compartment. Transfer was performed via a three channel peristaltic pump (Reglo ICC pump, part ISM 4308, Ismatec®). The exact positions of the rotating paddle and inlet and outlet tubes in the duodenal compartment were selected, after preliminary experiments, so that adequate mixing and reproducible transfer is ensured.

Experiments with ranitidine were performed at dose levels of 30 mg and 300 mg in the gastric compartment using aqueous solutions. Distilled water was initially placed in the duodenal and the reservoir compartments.

Experiments with dipyridamole and ketoconazole were performed at two dose levels (30 mg and 90 mg for dipyridamole and 100 mg and 300 mg for ketoconazole) by using Level III Fasted state simulating gastric fluid (FaSSGF, Vertzoni et al. 2005; Markopoulos, Andreas et al. 2015) in the gastric compartment. Level II biorelevant media (Markopoulos, Andreas et al. 2015) simulating the environment in upper small intestine, FaSSIF (Galia et al. 1998) and FaSSIF-V2plus (Psachoulias et al. 2012), were initially placed in the duodenal compartment. In experiments where FaSSIF was employed in the duodenal compartment, a series of phosphate buffer solutions containing sodium chloride, bile salt and lecithin was placed in the reservoir compartment so that the composition of simulated duodenal contents (pH, buffer capacity, osmolality, bile salt and lecithin concentration) remains unaltered during the experiment. When FaSSIF-V2plus was employed in the duodenal compartment, the series of phosphate buffer solutions additionally contained cholesterol and sodium oleate to maintain also cholesterol and oleate concentration in the duodenal compartment unaltered during the experiment.

Experiments with posaconazole were performed at the 400 mg dose level (10 ml Noxafil® suspension, 40 mg/ml) using FaSSGF and FaSSIF in the gastric and in the duodenal compartment, respectively.

In experiments with dipyridamole, ketoconazole, and posaconazole, each sample from the duodenal compartment was divided in two parts. In the first part, total amount of API (solid and in solution) per volume (expressed in µg/ml) was measured. The second part was filtered through 0.45-µm regenerated cellulose filters (17 mm, Thermo Scientific; Germany) for assaying drug concentration. Adequacy of filtration versus centrifugation to separate dissolved from undissolved material and API adsorption to the filter were determined with preliminary experiments.

2.2.4 Analytical methods

All four drugs were assayed with HPLC-UV methods that were based in previously published methods (Kokoletsi et al. 2005; Vertzoni et al. 2006; Vertzoni et al. 2007; Cendejas et al. 2012). The chromatographic conditions are presented in Table 1.

2.2.5 Data analysis

Data collected with dipyridamole and ketoconazole solutions were treated so that the precipitated fraction was estimated in each sample using the following equation

$$\pi = 1 - \frac{\text{Concentration}}{\text{Total amount per volume}} \quad (\text{Eq.8})$$

The precipitated fraction during the 55 minutes, after initiation of an experiment, was estimated by averaging the precipitated fractions estimated at each sampling time point (n=3) and calculating the grand mean of these averages.

282 Total drug amount per volume and drug concentrations of dipyridamole and ketoconazole in
283 the duodenal compartment were compared with previously published luminal data from
284 Psachoulis et al. (2011). Due to the lack of luminal total drug amount per volume data, only
285 concentrations of posaconazole in the duodenal compartment were compared with
286 previously published luminal data from Walravens et al. (2011).

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288 In this manuscript, previously published luminal data are presented as individual and mean
289 data points (graphically) and/or as box-whisker plots depicting the median value, the 10th,
290 25th, 50th, 75th, and 90th percentiles, and the individual outlying data points.

3. Results and discussion

3.1 Key parameter values for gastrointestinal transfer kinetics estimated from existing luminal data

Figure 2 shows the normalized total dipyridamole and ketoconazole amount per volume of intestinal contents [individual data ($n=336$) and mean values at each sampling time point from Psachoulis et al. (2011)] and the best fitted line to the individual data according to equation 1. Parameter values estimated using dipyridamole data ($n=165$), ketoconazole data ($n=171$), and both dipyridamole and ketoconazole data ($n=336$) are presented in Table 2. Based on Table 2, a half-life of gastric emptying of about 19 min is estimated (value of $k_G \approx 0.037 \text{ min}^{-1}$) which is within the range of previously estimated values using magnetic resonance imaging (Koziolek et al. 2014; Mudie et al. 2014; Steingoetter et al. 2006). Further, duodenal volume is estimated to be between 25 and 30 ml and duodenal elimination half-life is about 2 min ($k_r \approx 0.37 \text{ min}^{-1}$) (Table 2). These values are in line with recently published estimations for the total volume of water in the entire fasted small intestine following the administration of a glass of water (mean values ranged from 43 ml to 94 ml, Mudie et al. 2014).

3.2 Implementation of the luminal conditions in the BioGIT system

The exact parameter values used in the *in vitro* transfer model equation (equation 5) were a compromise between data in Table 2 and practicalities, i.e. half-life of gastric emptying was set at 15 min, and volume of duodenal contents was set at 40 ml. The use of 40 ml as volume of duodenal contents was a compromise between the estimated volumes (Table 2) and technical issues; during an experiment adequate mixing is required to ensure

reproducible sampling from a potentially heterogeneous medium, due to precipitation and/or incoming solids from the gastric compartment. This involved also appropriate positioning of the three ports via which incoming and outgoing fluids are operating. The exact positions of the inlet and outlet tubes in the duodenal compartment were 10 cm and 9 cm, respectively, from the top of the Distek mini vessel. To simulate the dosing conditions in *in vivo* studies, the initial volume of contents in the gastric compartment was set at 250 ml. The selection of the volumes in the gastric (250 ml) and duodenal (40 ml) compartments necessitates the use of an Erweka and a Distek mini vessel, respectively, and corresponding paddles. The exact positions of the rotating paddles in the Erweka and the Distek vessels were 1.1 cm and 0.5 cm from the bottom of the corresponding mini vessel. To avoid substantial deviations from theory as well as for practical reasons, incoming flow rates were changed every 10 min and sampling was performed at midpoint. Table 3 shows the combination of flow rates that were used in the *in vitro* experiments. Exact compositions of phosphate buffers added in the duodenal compartment, to maintain the composition of FaSSIF and FaSSIF-V2plus unaltered, are presented in Table 4 and in Table 5, respectively.

3.3 Simulation of the gastrointestinal transfer process using the BioGIT system

3.3.1 Solution of a highly soluble drug in gastric compartment: Ranitidine

Theoretical values (according to the transfer model, equation 5), expected values (due to the approximation of the process) and experimental data in the duodenal compartment during the transfer of ranitidine solutions are presented in Figure 3. Transfer of ranitidine solutions was achieved reproducibly at all time points and experimental data are in line with the expected values (Table 6). It is worth mentioning that equation 5 has also been shown to be

useful in reproducing gastrointestinal mass transfer of another highly soluble API,
paramomycin, in fasted healthy adults (Hens, Brouwers et al. 2014).

3.3.2 Solutions of two lipophilic weak bases in gastric compartment: Dipyridamole and Ketoconazole

In vitro data with the low dose of dipyridamole indicate that the transfer of total amount (dissolved and solid) in and out of the duodenal compartment is achieved reproducibly and occurs according to theoretically expected values (Figure 4A). Similarly, *in vitro* data with the high dose of dipyridamole indicate that the transfer is reproducible and in line with the expected values both in FaSSIF and in FaSSIF-V2plus (Table 6, Figure 4B). At both dose levels, *in vitro* estimated mass transfer adequately simulates the observed mass transfer *in vivo* (Figures 4A and 4B).

For the low dose of ketoconazole, transfer was reproducible and occurred according to theoretically expected values (Table 6, Figure 5A). Data with the high dose of ketoconazole indicate that the transfer was also achieved reproducibly and relevant data are in line with the expected values both in FaSSIF and in FaSSIF-V2plus (Table 6, Figure 5B). As with dipyridamole, at both dose levels, *in vitro* estimated mass transfer adequately simulates the observed mass transfer *in vivo* (Figures 5A and 5B).

3.3.3 Suspension of a lipophilic weak base in gastric compartment: Posaconazole

Transfer of posaconazole using the BioGIT system was reproducible and occurred according to theoretically expected values (Table 6, Figure 6A). It is interesting to note that the level of bias is higher at early time points (Figure 6A). However, the overall mass transfer process is

close to theoretically expected values, when solid particles are present both in gastric and duodenal compartments.

3.4 In vitro estimation of concentrations of dipyridamole, ketoconazole and posaconazole in upper small intestine using the BioGIT system

3.4.1 Solutions of two lipophilic weak bases in gastric compartment: Dipyridamole and Ketoconazole

For the low dose of dipyridamole (30 mg) estimated concentrations were in line with luminal data (Figure 4C). The mean precipitated fraction in FaSSIF during the first 55 min, post initiation of the *in vitro* experiment, was estimated to be 0.03, i.e. identical to the mean precipitated fraction in the upper small intestine of adults during the same time period post administration (Psachoulis et al. 2011). At the high dose level of dipyridamole (90 mg), the concentrations in the duodenal compartment slightly underestimated luminal concentrations (Figure 4D) whereas estimated precipitated fractions overestimated luminal values reported previously (Psachoulis et al. 2011). Mean precipitated fractions in FaSSIF, FaSSIF-V2plus and *in vivo* were 0.19, 0.17 and 0.04, respectively. The overestimation of precipitation was most pronounced at early time points; at 5 and 15 min, values for π estimated in FaSSIF or FaSSIF-V2plus ranged from 0.21 to 0.49 whereas the corresponding values at 5 and 10 min *in vivo* amounted to 0.03-0.06 (Psachoulis et al. 2011). The difference may be related to the fact that in the relevant *in vivo* study, the duodenal environment 5 min and 10 min after drug administration deviated from that expected for the average general population [e.g. pH in 7 out of 12 subjects in that Phase was 2.3-5.8, (Psachoulis et al. 2011)].

It is interesting to note that differences in the composition between FaSSIF-V2plus and FaSSIF did not have a significant impact on concentrations in the duodenal compartment (Figure 4C and 4D). Also, gastrointestinal transfer *in vitro* at the high dose level occurred according to theory (Figure 4B), despite the presence of precipitates formed in the duodenal compartment (Figure 4D).

The behavior of dipyridamole in the upper small intestine in the fasted state has previously been evaluated by others using conceptually similar *in vitro* setups (Gu et al. 2005; Mitra and Fadda 2014; Matsui et al. 2015). In contrast with the present study, in all previous attempts *in vitro* experiments were performed at 25, 50 and 100 mg levels using primarily powder or commercially available immediate release tablets (Gu et al. 2005; Mitra and Fadda 2014; Matsui et al. 2015). At the 50 mg dose level Gu et al. (2005) predicted up to 10% precipitation, i.e. higher than in the present study and in a previous *in vivo* study in humans where solutions in stomach had been used (Psachoulis et al. 2011). At the 100 mg dose level Mitra and Fadda (2014) measured concentrations in the duodenal compartment which were similar to those measured in humans, after a 90 mg dose administered as solution; when they used 25 mg dose, they overpredicted luminal concentrations previously measured, after intake of a 30 mg dose as solution. Matsui et al. (2015) overestimated *in vivo* precipitation; at the 50 mg dose level they estimated mean precipitated fraction of about 0.50, i.e. higher than the mean precipitated fraction measured *in vivo* (0.04, Psachoulis et al. 2011) or *in vitro* in the present investigation at the 90 mg dose level (0.19 in FaSSIF and 0.17 in FaSSIF-V2plus) when using solutions. Authors attributed the overestimation to the presence of solid particles, due to incomplete dissolution of the dose in the gastric compartment (Matsui et al. 2015). Although this may be a reasonable argument, other authors have shown that the presence of solids has minimal impact on the precipitation of dipyridamole, if any (Mitra and Fadda 2014).

For both dose levels of ketoconazole, estimated concentrations were in line with *in vivo* data (Figure 5C and 5D). For the low dose of ketoconazole, the mean precipitated fraction in FaSSIF was 0.03, i.e. close to the *in vivo* value of 0.06 (Psachoulis et al. 2011). For the high dose level of ketoconazole, mean precipitated fractions were 0.14 in FaSSIF and 0.10 in FaSSIF-V2plus, i.e. in line with intraluminal value of 0.14 (Psachoulis et al. 2011). As with the high dose of dipyridamole, it is worth noting that differences in the composition between FaSSIF-V2plus and FaSSIF did not affect ketoconazole concentrations in the duodenal compartment substantially. Also, the transfer process occurred according to theory (Figure 5A and 5B), despite the presence of precipitates formed in the duodenal compartment.

3.4.2 Suspension of a lipophilic weak base in gastric compartment: Posaconazole

Posaconazole concentrations in the upper small intestine of humans were highly variable (Figure 6B). However, using the BioGIT system, concentrations measured in the duodenal compartment were in line with median luminal data (Walravens et al. 2011) (Figure 6B). Since a suspension of posaconazole with incomplete dissolution in stomach or in gastric compartment was used, precipitated fractions in duodenum/duodenal compartment could not be estimated.

Concluding remarks

In the present investigation we designed, implemented *in vitro*, and evaluated a novel biorelevant system (BioGIT) for estimating drug concentrations in the upper small intestine in the fasted state. Data with three lipophilic weak bases suggest that BioGIT can be applied reproducibly and can provide *in vivo* relevant data, i.e. good estimates for the gastrointestinal mass transfer and for drug concentrations in the upper small intestine of healthy adults in the fasted state.

Compared with the previously proposed *in vitro* setup by Psachoulis et al. (2012), BioGIT system has the following advantages:

- The *in vitro* conditions are based on volume of duodenal contents and input/output duodenal rate estimated, after modelling previously collected luminal data.
- BioGIT system complies with the continuous gastrointestinal transfer process *in vivo*.
- Required equipment is commercially available.
- Concentrations seem to be much less affected by small variations in composition of the Level II biorelevant medium (Markopoulos, Andreas et al. 2015) used in the duodenal compartment.

BioGIT system can provide information on the impact of gastrointestinal transfer on concentrations in upper intestinal lumen during the first hour, after oral administration of dispersing/solution dosage forms of lipophilic weak bases. It could also be useful in providing information on the precipitated fraction in the upper intestinal lumen.

More data are needed on the importance of level of simulation of gastrointestinal composition when using the BioGIT system and on the usefulness of BioGIT system in

464 predicting concentrations in upper small intestine, after administration of solid dosage forms
465 and/or enabling drug products.

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 553 intraluminal and plasma drug concentrations. Clin. Pharmacokinet. 50, 725-734.

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555

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557 Conference 2013, Athens, Greece and Annual meeting and Exposition AAPS 2014, San Diego,

558 USA). This work was performed within the OrBiTo project which is funded by the Innovative

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560 Table 1: The chromatographic conditions used for the analysis of drugs in the present study.

Chromatographic Conditions	Ranitidine	Dipyridamole	Ketoconazole	Posaconazole
Pre-column	BDS C18 (10x4.6 mm, 5 µm)	BDS C18 (10x4.6 mm, 5 µm)	BDS C18 (10x4.6 mm, 5 µm)	BDS C18 (10x4.6 mm, 5 µm)
Column	Fortis C18 (150×3mm, 3µm)	Fortis C18 (150×3mm, 3µm)	BDS RP-C ₁₈ (250×4.6mm, 5µm)	Fortis C18 (150×3mm, 3µm)
Mobile Phase v/v/v	ammonium formate 10 mM (pH 4.7):acetonitrile 90:10	acetonitrile:water:diethylamine 50:50:0.1	methanol:water:diethylamine 74:26:0.1	acetonitrile:water 70:30
Flow Rate (ml/min)	0.5	0.5	1	0.5
Detection wavelength (nm)	230	280	240	262
Detection limit (ng/ml)	50	50	60	60
Injection volume (µl)	20	20	20	20
Reference	Kokoletsi et al. 2005	Vertzoni et al. 2007	Vertzoni et al. 2006	Cendejas et al. 2012

561 Table 2: Parameter values (s.e.) after fitting the theoretical model (Eq. 1) to individual
 562 normalized total drug amounts per volume of intestinal contents*.

	DPD	KCZ	DPD and KCZ
	$n=165$	$n=171$	$n=336$
	$R=0.648, p<0.0001$	$R=0.717, p<0.0001$	$R=0.670, p<0.0001$
$k_G \text{ (min}^{-1}\text{)}$	0.0423 (0.0077)	0.0328 (0.0043)	0.0371 (0.0041)
$k_I \text{ (min}^{-1}\text{)}$	0.34 (0.13)	0.40 (0.13)	0.372 (0.094)
$V_I \text{ (ml)}$	30 (13)	25.0 (8.8)	27.8 (7.7)

563 * R is the regression coefficient, p is the probability of committing type I error for the
 564 regression based on the ANOVA table, and n is the number of observations.

565 Table 3: Incoming flow rates to the duodenal compartment from the gastric compartment
 566 (F_1) and the reservoir compartment (F_2) applied in the *in vitro* experiments.*

Time Interval (min)	F_1 (ml/min)	F_2 (ml/min)
0-10	9.3	2.3
10-20	5.9	5.7
20-30	3.7	7.9
30-40	2.3	9.3
40-50	1.4	10.2
50-60	0.9	10.7

567 *The sum of incoming flow rates equals the outgoing flow rate F (11.6 ml/min).

568 Table 4: Composition of phosphate buffer solutions in the reservoir compartment in order to maintain the composition of FaSSIF in the duodenal
 569 compartment unaltered during an experiment.

Time interval (min)	pH*	Sodium phosphate monobasic dihydrate (mM)	Sodium Chloride (mM)	Sodium taurocholate (mM)**	Phosphatidyl- choline (mM)**
0-10	7.2	257.94	184	15.13	3.78
10-20	7.2	85.98	129	6.11	1.53
20-30	7.2	37.26	139	4.41	1.10
30-40	7.2	22.93	137	3.74	0.94
40-50	6.8	34.39	117	3.41	0.85
50-60	6.7	28.66	120	3.25	0.81

570 *The pH is adjusted with addition of appropriate volume of NaOH 1 M

571 **Sodium taurocholate and lecithin were included as FaSSIF powder.

572 Table 5: Composition of phosphate buffer solutions in the reservoir compartment in order to maintain the composition of FaSSIF-V2plus in duodenal
573 compartment unaltered during an experiment.

Time interval (min)	pH*	Sodium phosphate monobasic dihydrate (mM)	Sodium chloride (mM)	Sodium taurocholate (mM)	Phosphatidyl- choline (mM)**	Sodium oleate (mM)**	Cholesterol (mM)
0-10	7.2	257.94	0	15.13	1.01	2.52	1.01
10-20	7.2	85.98	30	6.11	0.41	1.02	0.41
20-30	7.2	37.26	67	4.41	0.29	0.73	0.29
30-40	7.2	22.93	76	3.74	0.25	0.62	0.25
40-50	6.8	34.39	61	3.41	0.23	0.57	0.23
50-60	6.7	28.66	67	3.25	0.22	0.54	0.22

574 *The pH is adjusted with addition of appropriate volume of NaOH 1 M.

575 ** Sodium taurocholate and lecithin were included as FaSSIF-V2 powder.

Table 6: Maximum Bias from the expected values of total drug amount per volume in duodenal compartment and maximum Relative Standard Deviation (RSD) for the transfer experiments performed in the present investigation.*

Gastric compartment	Duodenal compartment		
	Medium	Total drug amount per volume	
		Maximum %Bias	Maximum %RSD
Composition at the beginning of the experiment			
30 mg RNT solution in water	Water	5.66	1.10
300 mg RNT solution in water	Water	3.61	4.18
30 mg DPD solution in FaSSGF	FaSSIF	8.56	8.87
90 mg DPD solution in FaSSGF	FaSSIF	15.36	7.61
90 mg DPD solution in FaSSGF	FaSSIF-V2plus	10.15	4.50
100 mg KCZ solution in FaSSGF	FaSSIF	12.26	3.44
300 mg KCZ solution in FaSSGF	FaSSIF	24.75	12.94
300 mg KCZ solution in FaSSGF	FaSSIF-V2plus	12.48	3.89
400 mg PSC suspension in FaSSGF	FaSSIF	20.31	11.39

*Deionized water was used in all cases; abbreviations are explained in the experimental section.

Figure 1: Schematic representation of the BioGIT system. F_1 and F_2 are the incoming flow rates and F is the outgoing flow rate; $F=F_1+F_2$.

Figure 2: Normalized total dipyridamole and ketoconazole amount per volume of intestinal contents vs. time after administration to healthy adults. The Figure depicts individual data (open circles, $n=336$) and mean data (grey circles) estimated from Psachoulas et al. (2011) and best fitted line to individual data, according to equation 1 (continuous line).

Figure 3: Ranitidine concentrations in the duodenal compartment vs. time after the initiation of the transfer from the gastric compartment. Key: Continuous line, values estimated using the transfer model equation (equation 5); grey stars, expected values due to the approximation of the process; filled squares, mean $\pm$ SD ($n=3$) experimental data.

Figure 4: Total amount of dipyridamole per volume (A and B) and concentration of dipyridamole (C and D) in the duodenal compartment vs. time after initiation of transfer from the gastric compartment. Key: Continuous line, values estimated using the transfer model equation (equation 5); grey stars, expected values due to the approximation of the process; diamonds, mean $\pm$ SD ($n=3$) experimental data using FaSSIF-V2plus in duodenal compartment; squares, mean $\pm$ SD ($n=3$) experimental data using FaSSIF in duodenal compartment. Grey filled symbols indicate total dipyridamole amount per volume and white filled symbols indicate dipyridamole concentrations in duodenal compartment. For comparative purposes, total amount of dipyridamole per volume (grey box-whisker plots) and concentration of dipyridamole (white box-whisker plots) in the contents of upper small intestine ($n=12$, Psachoulas et al. 2011) are also presented.

Figure 5: Total amount of ketoconazole per volume (A and B) and concentration of ketoconazole (C and D) in the duodenal compartment vs. time after initiation of transfer from the gastric compartment. Key: Continuous line, values estimated using the transfer model equation (equation 5); grey stars, expected values due to the approximation of the process; diamonds, mean $\pm$ SD (n=3) experimental data using FaSSIF-V2plus in duodenal compartment; squares, mean $\pm$ SD (n=3) experimental data using FaSSIF in duodenal compartment. Grey filled symbols indicate total ketoconazole amount per volume (ml) and white filled symbols indicate ketoconazole concentrations in duodenal compartment. For comparative purposes, total amount of ketoconazole per volume (grey box-whisker plots) and concentration of ketoconazole (white box-whisker plots) in the contents of upper small intestine (n=12, Psachoulis et al. 2011) are also presented.

Figure 6: Total amount of posaconazole per volume (A) and concentration of posaconazole (B) in the duodenal compartment vs. time after initiation of transfer from the gastric compartment. Key: Continuous line, values estimated using the transfer model equation (equation 5); grey stars, expected values due to the approximation of the process; squares, mean $\pm$ SD (n=3) experimental data using FaSSIF in duodenal compartment. Grey filled symbols indicate total posaconazole amount per volume and white filled symbols indicate posaconazole concentrations in duodenal compartment. For comparative purposes, concentration of posaconazole (white box-whisker plots) in the contents of upper small intestine (n=5, Walravens et al. 2011) are also presented.

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630 Figure 2

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634 Figure 4

635 Figure 5

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