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**Evaluation of the impact of excipients and an albendazole salt on albendazole concentrations in upper small intestine using an *in vitro* biorelevant gastrointestinal transfer (BioGIT) system**

**Alexandros Kourentas<sup>1</sup>, Maria Vertzoni<sup>1</sup>, Ibrahim Khadra<sup>2</sup>, Mira Symillides<sup>1</sup>, Hugh Clark<sup>3</sup>, Gavin Halbert<sup>2</sup>, James Butler<sup>4</sup>, Christos Reppas<sup>1,\*</sup>**

<sup>1</sup>Faculty of Pharmacy, National and Kapodistrian University of Athens, Zografou, Greece

<sup>2</sup>Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, Scotland, UK

<sup>3</sup>Product Development, GlaxoSmithKline, Stevenage, UK

<sup>4</sup>Product Development, GlaxoSmithKline, Ware, UK

Running Title: Evaluation of albendazole concentrations in upper small intestine using BioGIT

\*To whom correspondence should be addressed:

Christos Reppas PhD

Faculty of Pharmacy, School of Health Sciences,

National and Kapodistrian University of Athens,

Panepistimiopolis, 157 84 Zografou, Greece

Tel. 00 30 210 727 4678 / Fax: 00 30 210 727 4027

[reppas@pharm.uoa.gr](mailto:reppas@pharm.uoa.gr)

## Abstract

An *in vitro* biorelevant transfer (BioGIT) system was assessed for its ability to mimic recently reported albendazole concentrations in human upper small intestine after administration of free base suspensions to fasted adults in absence and in presence of supersaturation promoting excipients (hydroxypropylmethylcellulose and lipidic self-emulsifying vehicles). The *in vitro* method was then used to evaluate the likely impact of using the sulfate salt on albendazole concentrations in upper small intestine. In addition, BioGIT data were compared with equilibrium solubility data of the salt and the free base in human aspirates and biorelevant media. The BioGIT system adequately mimicked the average albendazole gastrointestinal transfer process and *in vivo* concentrations in upper small intestine after administration of the free base suspensions to fasted adults. However, the degree of supersaturation seen initially in the duodenum was greater *in-vitro* than *in-vivo*. Albendazole sulfate resulted in minimal increase of albendazole concentrations in the duodenal compartment of the BioGIT, despite improved equilibrium solubility observed in human aspirates and biorelevant media, indicating that the use of a salt is unlikely to lead to any significant oral absorption advantage for albendazole.

## **Keywords**

BioGIT; gastrointestinal transfer; dissolution; supersaturation; poorly soluble weak base;

albendazole salts; lipid excipients; precipitation inhibitor; HPMC E5

## Introduction

New drug candidates often have low aqueous solubility to an extent that require administration in enabling formulations to promote supersaturation, in order to achieve luminal concentrations to get most of the dose absorbed (e.g. Brouwers et al. 2009). In case of a poorly soluble weak base, the impact of gastrointestinal transfer in the fasted state on absorption rates may be substantial, because, due to the abrupt increase in pH, precipitation in the upper small intestine is likely. The use of supersaturation promoting formulations may then be required.

Various methodologies have been proposed to evaluate to impact of gastrointestinal transfer on the performance of the dosage form and of the drug intraluminally (Kostewicz et al. 2014). Recently, an *in vitro* biorelevant gastrointestinal transfer (BioGIT) system was proposed 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 in the fasted state (Kourentas et al. 2016).

In the present investigation, the impact of excipients and of free base vs. salt on concentrations of lipophilic weak bases in upper small intestine was investigated with BioGIT by using albendazole free base [ABZ, pka 2.80 (Jung et al 1998), logP 3.46 (Rivera et al. 2007)] as a model lipophilic weak base. There were three specific objectives of the present study:

Firstly, to evaluate the usefulness of BioGIT in reproducing recently observed ABZ concentrations and supersaturation of contents of the upper small intestine of healthy

adults after administration of various ABZ free base suspensions in absence and in presence of supersaturation promoting excipients in the fasted state (Kourentas et al. submitted).

Secondly, to evaluate the impact of free base vs. salt on ABZ concentrations and degrees of supersaturation of contents of the upper small intestine in absence and in presence of HPMC E5 using BioGIT.

Thirdly, to compare BioGIT data with equilibrium solubility data (in human aspirates and in biorelevant media) in order to evaluate the impact of free base vs. salt on ABZ behaviour in the upper gastrointestinal lumen.

Since only the free base of ABZ is commercially available, a salt had to be prepared for the needs of the present investigation. Although various salts of ABZ have been prepared and characterized in the past (Paulekuhn et al. 2013), the relatively low pKa makes ABZ salts sensitive to disproportionation (e.g. Stephenson et al. 2011). After considering various counter-ions, the sulfate salt of ABZ was selected to be used, based on its comparatively better crystallinity and stability characteristics.

## Materials and Methods

### Materials

Albendazole free base was from Sigma Aldrich (Saint Louis, U.S.A., 99% pure). Sodium phosphate monobasic, sodium hydroxide, sodium chloride, ammonium formate, and pepsin from porcine gastric mucosa (15.8% protein) were purchased from Sigma Aldrich (Saint Louis, U.S.A.). Acetonitrile and water (HPLC grade) as well as tetrahydrofuran and ethanol were also from Sigma Aldrich (Saint Louis, U.S.A.). n-butanol was purchased from Fluka (Neu-Ulm, Germany). SIF® Powder Original was kindly donated by Biorelevant.com (Surrey, U.K.). Hydrochloric acid was from Panreac Co. (Barcelona, Spain). Hydroxypropylmethylcellulose E5 was from JRS Pharma (Zacapu, Mexico). Miglyol 812N [Caprylic/Capric (C8–C10) triglycerides] was received from Sasol Germany. Cremophor RH 40 was from BASF (Ludwigshafen, Germany). Polysorbate 80 was from Sigma Aldrich (Saint Louis, U.S.A.).

### Preparation of various ABZ salts and selection of the sulfate salt

Initially, the solubility of ABZ free base was assessed in tetrahydrofuran, ethanol, water, and n-butanol at a range of temperatures (25–80 °C). In order to obtain albendazole hydrochloride, albendazole sulfate and albendazole mesylate, 10, 20, 30, 40 and 50 mg of ABZ free base were transferred into 2 ml HPLC vials followed by the addition of 1 ml of solvent (tetrahydrofuran, water, ethanol or n-butanol) and the addition of an equi-molar amount of acid (HCl, H<sub>2</sub>SO<sub>4</sub> or methanesulfonic acid). The vials were placed in a Crystal 16 apparatus (Avantium Crystallization System, Amsterdam, Netherlands), temperature was cycled with the maximum temperature set 10°C below the solvents boiling point and the salt was formed at the end of the experiment. Two scale-up experiments were conducted using

25 ml and 50 ml of solvent, to ensure the scale-up procedure was viable. Equi-molar amounts of ABZ free base and the acids (HCl, H<sub>2</sub>SO<sub>4</sub>, or methanesulfonic acid) were added to 100 ml glass beakers containing either 25 or 50 ml of solvent and the mixture heated to 10 degrees below the boiling point of the solvent. At the elevated temperature the ABZ salt was completely dissolved; it was then left at room temperature to cool down and the salt precipitated. Residual solvent was evaporated at room temperature and the material was further dried at 40 °C, over 24 hours, and weighed. The formation of ABZ salts (as hydrates, based on Karl-Fisher titration data) was confirmed using Nuclear Magnetic Resonance, Differential Scanning Calorimetry (30 – 300 °C in 10 min, Mettler Toledo DSC 822e), and Elemental Analysis for carbon, hydrogen and nitrogen (data on file). Based on X-ray diffraction data, albendazole hydrochloride was poorly crystalline (unlike with a previous report (Paulekuhn et al. 2013) whereas albendazole mesylate crystals and crystal stability characteristics varied with the solvent used for their isolation. In contrast, albendazole sulfate salt (ABZ sulfate) samples obtained with tetrahydrofuran and ethanol were concordant (some differences in peak intensities may suggest small differences in crystallinity). Also, based on the one-month stability data ABZ sulfate, peaks were concordant at two tested conditions (40 °C / 75% humidity and 50 °C / ambient humidity) with the initial analysis. Based on these data, ABZ sulfate obtained using tetrahydrofuran was selected to be used in the present investigation.

### Experiments with BioGIT

#### *Methodology*

BioGIT is an *in vitro* three compartment setup simulating the drug transfer from the stomach into the fasted upper small intestine (Kourentas et al. 2016). The initial volume of the gastric



compartment is 250 ml. The duodenal volume is maintained at 40 ml during the entire experiment. The mini - paddles in gastric and duodenal compartments rotate at 75 rpm. The emptying of contents of gastric compartment (on a volume basis) follows first-order kinetics with a half-life of 15 min. Experiments are performed using a three-channel peristaltic pump (Reglo ICC pump, part ISM 4308, Ismatec®) for 45 min, after the initiation of an experiment. Incoming flow rates are changed every 10 min and sampling is performed at midpoint (Kourentas et al. 2016). In this study, experiments were performed in triplicate at 37 °C.

#### *Contents of gastric compartment*

Two aqueous suspensions of ABZ free base were tested. For the first, 50 mg ABZ free base were mixed with 200 ml table water (Nera Kritis, Heraklion, Greece) under vigorous magnetic stirring for 5 min at room temperature. Preparation of the second suspension was achieved by dissolving 5 mg HPMC E5 in 200 ml table water, prior to mixing 50 mg ABZ free base with the aqueous HPMC E5 solution. Regardless of the presence of HPMC E5, the suspension was mixed with 50 ml concentrated Level III FaSSGF. The resulting 250 ml suspension in Level III FaSSGF (Markopoulos, Andreas et al. 2015), Susp or Susp-HPMC, was immediately added to the gastric compartment, and the transfer experiment was initiated.

Two lipid based suspensions of ABZ free base were tested, prepared from one Type IIIA and one Type IV lipid based formulation (Pouton, 2006). The appropriate quantities of excipients were weighed into a screw cap glass bottle of 50 ml capacity, i.e. 0.4 g Miglyol 812N, 0.2 g Cremophor RH 40 and 0.4 g Tween 80 for Type IIIA lipid based formulation and 0.2 g Cremophor RH40 and 0.4 g Tween 80 for Type IV lipid based formulation (Kourentas et al. submitted). An accurately weighed quantity of 50 mg ABZ free base was added into the glass bottle. The components were mixed using a magnetic stirrer for 1 h at room temperature.

The lipid based formulations of ABZ free base were stored at room temperature overnight (Kourentas et al. submitted). Each lipid based formulation was transferred to a screw cap glass bottle of 250 ml capacity by rinsing the 50 ml capacity glass bottle with small amounts of table water (total volume of table water used for rinsing was 200 ml). The 250 ml capacity bottle was then vigorously stirred using a magnetic stirrer for 5 min at room temperature (Kourentas et al. submitted), 50 ml of concentrated Level III FaSSGF were added into the bottle and mixed. The resulting 250 ml suspension in Level III FaSSGF, Susp-III A or Susp-IV, was immediately added to the gastric compartment, and the experiment initiated.

Assuming that resting gastric volume in the fasted state is 10 ml (Psachoulis et al. 2011; Vertzoni et al. 2012), volume and composition of contents in the gastric compartment when using ABZ free base suspensions reflected the expected composition of gastric contents after administration of the following suspensions to fasted adults (Kourentas et al. submitted):

- Suspension of ABZ free base in 240 ml table water (Susp)
- Suspension of ABZ free base in 240 ml table water in which HPMC E5 had been pre-dissolved (Susp-HPMC)
- Suspension of a Type III A lipid based formulation of ABZ free base in 240 ml table water (Susp-III A)
- Suspension of a Type IV lipid based formulation of ABZ free base in 240 ml table water (Susp-IV)

Two aqueous suspensions of ABZ sulfate were then tested. For the first, 50 mg ABZ equivalent were mixed with 200 ml table water under vigorous magnetic stirring for 5 min at room temperature. Preparation of the second suspension was achieved by dissolving 5 mg HPMC E5 in 200 ml table water, prior to mixing 50 mg ABZ equivalent with the aqueous HPMC E5 solution. Regardless of the presence of HPMC E5, the suspension was mixed with

50 ml concentrated Level III FaSSGF. The resulting 250 ml suspension in Level III FaSSGF (Markopoulos, Andreas et al. 2015), S-Susp or S-Susp-HPMC, was immediately added to the gastric compartment, and the transfer experiment was initiated.

The composition of contents in the gastric compartment were designed to reflect intragastric conditions using the same assumptions made for the ABZ free base suspensions and mimicking administration of the following ABZ sulfate suspensions to fasted adults:

-Suspension of ABZ sulfate in 240 ml table water (S-Susp)

-Suspension of ABZ sulfate in 240 ml table water containing HPMC E5 (S-Susp-HPMC)

In all experiments (with ABZ free base and with ABZ sulfate), concentrated Level III FaSSGF had pH 0.9 whereas concentrations of sodium chloride, pepsin, sodium taurocholate and phosphatidylcholine were 5-fold higher than in Level III FaSSGF pH 1.6 (Markopoulos, Andreas et al. 2015).

#### *Contents of duodenal compartment at time zero and contents of reservoir compartment*

Level II FaSSIF (Markopoulos, Andreas et al. 2015) was employed in the duodenal compartment in order to simulate the fasted state conditions in upper small intestine. A series of phosphate buffer solutions containing sodium chloride, bile salt and lecithin were employed in the reservoir compartment so that the composition of contents in the duodenal compartment (pH, buffer capacity, osmolality, bile salt and lecithin concentration) remains unaltered during an experiment (Kourentas et al. 2016).

#### *Sample treatment*

Upon collection, each sample from the duodenal compartment was divided into two parts:

i) The first part was immediately filtered through regenerated cellulose filters (Titan 3, 17

mm, 0.45  $\mu$ m, SUN SRI, Rockwood, U.S.A.) and the filtrate was divided in two portions:

- The first, after immediate dilution with mobile phase (so that precipitation during subsequent handling is avoided), was used for measuring ABZ concentration.
- The second portion was incubated (37 °C, 75 oscillations/min, model Unitronic OR, j.p. Selecta, s.a., Barcelona, Spain) in presence of excess of ABZ free base or ABZ sulfate solid material for 16 hours and, after filtration through a regenerated cellulose 0.45  $\mu$ m filter, equilibrium solubility was determined. The duration of incubation for achieving equilibration had been evaluated with preliminary experiments.

Adsorption of ABZ on to the regenerated cellulose filter had been evaluated and found to be negligible.

ii) The second part was used for measuring total ABZ amount (solid and dissolved drug) per volume, after appropriate dilution with mobile phase.

*Equilibrium solubility of ABZ free base and ABZ sulfate in biorelevant media and in humans aspirates*

The solubility of ABZ free base and ABZ sulfate were measured in Level III FaSSGF, in Level II FaSSIF (Markopoulos, Andreas et al. 2015) and in human aspirates collected from the stomach and from the upper small intestine in the fasted state. Human aspirates had been collected from healthy fasted adults (Petrakis et al. 2014) and pooled after one freeze-thaw cycle. The pH after pooling was 1.6 for the gastric pooled sample (human gastric fluid, HGF) and 7.4 for intestinal pooled sample (human intestinal fluid, HIF). An excess amount of ABZ

free base or ABZ sulfate solid material was added to glass vials containing the appropriate medium. The vials were incubated at 37 °C (75 oscillations/min) until equilibrium was reached. Equilibration times in human aspirates and in biorelevant media were evaluated with preliminary experiments at 4h, 16h and 24h, after initiation of the incubation. For each medium, concentrations were measured to be identical at all sampling times. For practical reasons solubility values were measured at 16h in all media. At equilibrium, samples from biorelevant media were filtered through 0.45 µm regenerated cellulose filters whereas HGF and HIF samples were centrifuged (11800 g, 37 °C, 10 min). Filtrates and supernatants were diluted with equal volume of mobile phase and samples were subjected to HPLC analysis. All measurements were performed in triplicate. The adequacy of filtration vs. centrifugation to separate dissolved from undissolved material had been evaluated with preliminary experiments (Kourentas et al. submitted).

#### ABZ assay

ABZ assay was performed on a Spectra HPLC system consisting of a P1000 pump, an AS1000 autosampler, a UV2000 detector, and an SN4000 controller which was programmed by the Chromquest® software (version 2.51, Thermoquest Inc., USA). Analysis of drug content involved the use of a Fortis C18 column (3 µm, 150×3 mm) equipped with a BDS C18 (5 µm, 10×4.6 mm) pre-column. The mobile phase consisted of ammonium formate (50 mM): acetonitrile 50:50 v/v, the flow rate was 0.5 ml/min, the wavelength was set at 292 nm and the injection volume was 20 µl. In all cases, results are expressed as ABZ free base.

Quantification limit was 10 ng/ml.

#### Data Analysis

Raw data are presented as Box-Whisker plots showing the median value, the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles, and the individual outlying data points.

Supersaturation in duodenal compartment was evaluated by estimating the Degree of Supersaturation, DS:

$$DS = \frac{\text{Concentration}}{\text{Equilibrium Solubility}} \quad (\text{Eq. 1})$$

DS>1 indicates supersaturation, DS=1 indicates saturation, and DS<1 indicates unsaturated solution.

One-sample t-test (Sigmaplot 11.0, Systat Software, Inc., San Jose, California, USA) was applied for evaluating whether a sample mean DS value comes from a population with mean of 1. Differences between solubility in human fluids and biorelevant media and between ABZ free base and ABZ sulfate were evaluated with unpaired t-test (Sigmaplot 11.0, Systat Software, Inc., San Jose, California, USA). Maximum type I error had been set at 0.05, in all cases.

## Results and Discussion

### BioGIT data vs. data in the upper small intestine after administration of ABZ free base suspensions

The total ABZ amount per volume in the duodenal compartment matched the average luminal data during the first 50 min of gastric emptying of Susp, Susp-HPMC, Susp-III A and Susp-IV (Figure 1). The BioGIT data are consistent with the lower total ABZ amount per volume in upper small intestine observed after administration of Susp and Susp-HPMC than after administration of Susp-III A or Susp-IV to fasted adults (diamonds and boxes in Figures 1a and 1b vs. diamonds and boxes in Figures 1c and 1d). As reported recently (Kourentas et al. submitted), total ABZ amount per volume in upper small intestine, after administration of Susp-III A and Susp-IV are in line with first-order gastric emptying kinetics and half-life of 15 minutes (Figure 1, continuous lines). Lower total ABZ amount per volume, after Susp or Susp-HPMC could be attributed to the slower emptying of Susp and Susp-HPMC, due to floating and/or adhesion of aggregates on to the walls of the gastric compartment (visually observed in this study) and in stomach (Kourentas et al. submitted).

When using Susp or Susp-HPMC, ABZ concentrations in the duodenal compartment early, i.e. about 5 minutes, after initiation of the experiment were somewhat higher than the average concentrations in the upper small intestine (Figures 2a and 2b). This may be attributed to the pH of contents in the gastric compartment (FaSSGF, pH 1.6) which reflects the average pH in the stomach during the gastric emptying of glass of water in the fasted state, i.e. it is lower than the pH expected to be in stomach early after administration of a glass of water (about 2.5, e.g. Kalantzi et al. 2006). The lower than physiological pH at early times after initiation of the experiment accelerates ABZ free base dissolution and, therefore,

concentrations in gastric and, subsequently, in duodenal compartment are increased, compared with concentrations in the human upper gastrointestinal lumen at early times, after administration. When using Susp-III A or Susp-IV, the discrepancy between *in vitro* and luminal concentrations at early times was not apparent (Figures 2c and 2d). The fact that ABZ concentration in Susp-III A and in Susp-IV administered to adults was 9 and 6 times, respectively, higher than ABZ concentration in the administered Susp or Susp-HPMC (Kourentas et al. submitted) could be a reason for the similarity of *in vitro* with luminal data of Susp-III A and of Susp-IV at early times.

In all cases, ABZ concentrations in the duodenal compartment after about 5 minutes post experiment start generally matched the average data in upper small intestine during the first 50 minutes, after administration of all four tested ABZ free base suspensions to fasted adults (Figure 2).

Solubility values in duodenal compartment of BioGIT were in line with previously measured values in the lumen of upper small intestine of healthy adults (Figure 3). Occasionally, limited underestimation was observed, primarily in the case of Susp-III A (Figure 3c).

Contents of the duodenal compartment of BioGIT were significantly supersaturated in case of Susp, Susp-HPMC, and Susp-III A at all sampling times but one, after initiation of the experiment (Table 1). In case of Susp-IV, significant supersaturation was observed only occasionally (Table 1). Recently reported data from the upper small intestine of healthy adults suggest that significant supersaturation occurs only in case of Susp-HPMC, Susp-III A, and Susp-IV and at specific times post administration (Kourentas et al. submitted). The discrepancy between *in vitro* and luminal supersaturation could be attributed to the higher concentrations in BioGIT at early times (diamonds vs. boxes in Figures 2a, 2b, and 2c), the lower solubility in the duodenal compartment measured occasionally (Figure 3c), and the



high variability of luminal data which led to high average degrees of supersaturation but of borderline statistical significance (Kourentas et al. submitted). Overall, the BioGIT data, consistent with the human data (Kourentas et al. submitted) indicates that the lipid based formulations may be slightly less effective than formulations containing HPMC E5 in maintaining supersaturation in upper small intestine (Table 1).

#### BioGIT data using ABZ free base and ABZ sulfate suspensions

Total ABZ amount per volume in the duodenal compartment of BioGIT did not change substantially when S-Susp or S-Susp-HPMC was used in the gastric compartment (Figure 4a vs. Figure 1a and 1b). ABZ concentrations in the duodenal compartment also remained substantially unaffected, apart from at 5 minutes; when using S-Susp-HPMC concentration at 5 min was almost doubled, compared with the concentration measured when using Susp-HPMC (Figure 4b vs. Figure 2b).

Since solubility of ABZ sulfate was not affected by the presence of HPMC E5 (Figure 4b), the higher supersaturation when using S-Susp-HPMC could be attributed to the increased concentrations in presence of HPMC E5, especially at early times, after initiation of the experiment (Figure 4b). Although mean values of the degree of supersaturation were higher with S-Susp-HPMC than with S-Susp (Table 2), values were smaller than those estimated using Susp-HPMC and Susp, respectively (Table 2 vs. Table 1).

Regardless of the presence of HPMC E5, BioGIT data suggest that, compared with ABZ free base, ABZ sulfate will not lead to significantly more drug available for absorption. This is in line with literature data suggesting that usefulness of salts in increasing the absorption of

lipophilic weak bases has not been well documented in humans (Verbeeck et al. 2006) and may be overestimated (Erceg et al. 2012; Dimopoulou et al. in press).

Equilibrium solubility data vs. BioGIT data for evaluating the impact of free base vs. salt on ABZ concentrations in upper small intestine

ABZ free base equilibrium solubility in Level III FaSSGF (mean: 128 µg/ml, Figure 5a) is in line with its pH - solubility profile (Paulekuhn et al. 2013). Solubilities in Level III FaSSGF and in HGF as well solubilities in Level II FaSSIF and in HIF are significantly higher when ABZ sulfate is used; compared with mean solubility of ABZ free base, mean solubility of ABZ sulfate is 3.8 times higher both in Level III FaSSGF and in HGF (Figure 5a). Similarly, compared with data when ABZ free base was used, solubility in equilibrium with ABZ sulfate is 2.3 and 3.1 times higher in Level II FaSSIF and in HIF, respectively (Figure 5b).

Although ABZ sulfate is more soluble in contents and in simulated contents of upper gastrointestinal lumen (Figure 5), concentrations in duodenal compartment of BioGIT when using Susp and when using S-Susp were similar (Figure 2a vs. Figure 4b).

## **Concluding remarks**

BioGIT was demonstrated to be a useful tool for mimicking the average gastrointestinal transfer process and concentrations in upper small intestine observed after administration of various ABZ free base suspensions to fasted adults. However, compared with luminal data, a greater extent of supersaturation in the duodenal compartment in the BioGIT was typically observed.

BioGIT data indicate that ABZ sulfate, despite its improved equilibrium solubility in contents of stomach and upper small intestine, leads to only a minimal, transient increase of concentrations of ABZ in upper small intestine (only in the first 10 minutes after administration and enhanced by the presence of HPMC E5). These data suggest that equilibrium solubility data should be used to evaluate the luminal concentrations cautiously in situations where supersaturation and precipitation is likely to occur.

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Table 1: Mean±SD (n=3) values for the Degree of Supersaturation (DS) in samples collected from the duodenal compartment of BioGIT at times corresponding to specific time intervals, after initiation of the experiment when using various ABZ free base suspensions in the gastric compartment.

Time interval (min)	Susp	Susp-HPMC	Susp-III A	Susp-IV
0-10	4.66±0.52*	5.52±0.87*	3.11±0.40*	1.25±0.05*
10-20	4.93±0.59*	5.90±0.44*	3.84±0.57*	1.29±0.22
20-30	5.07±0.66*	5.93±0.76*	4.08±0.80*	1.22±0.11
30-40	5.06±0.38*	4.09±0.85*	3.30±0.80	1.38±0.15*
40-50	4.15±0.40*	3.81±0.29*	3.16±0.48*	1.21±0.11

\*Statistically different from 1 at the 0.05 level of significance



Table 2: Mean±SD, (n=3) values for the Degree of Supersaturation (DS) in samples collected from the duodenal compartment of BioGIT at times corresponding to specific time intervals, after initiation of the experiment when using various ABZ sulfate suspensions in the gastric compartment\*.

Time interval (min)	S-Susp	S-Susp-HPMC
0-10	2.99±0.08	4.90±0.07
10-20	1.77±0.06	2.39±0.15
20-30	1.89±0.14	2.89±0.07
30-40	2.34±0.05	2.51±0.35
40-50	2.20±0.04	2.25±0.17

\*All mean values were statistically different from 1 at the 0.05 level of significance

## Figure captions

### *Figure 1*

Mean $\pm$ SD (n=3) values (diamonds) for the total ABZ amount per volume in the duodenal compartment vs. time after initiation of the experiment with BioGIT when using Susp (a), Susp-HPMC (b), Susp-IIIA (c) and Susp-IV (d) in the gastric compartment. For comparative purposes, values estimated using the transfer model equation (continuous lines, Kourentas et al. 2016), and recently reported total ABZ amount per volume in the contents of upper small intestine, after administration of the corresponding suspensions to healthy adults are also presented (box-whisker plots with dotted lines indicating the means, Kourentas et al. submitted).

### *Figure 2*

Mean $\pm$ SD (n=3) values (diamonds) for ABZ concentration in the duodenal compartment vs. time after initiation of the experiment with BioGIT when using Susp (a), Susp-HPMC (b), Susp-IIIA (c) and Susp-IV (d) in the gastric compartment. For comparative purposes, recently reported ABZ concentrations in the contents of upper small intestine of healthy adults, after administration of the corresponding suspensions are also presented (box-whisker plots with dotted lines indicating the means, Kourentas et al. submitted).

### *Figure 3*

Mean $\pm$ SD (n=3) values (triangles) for ABZ equilibrium solubility in the duodenal compartment at various times after the initiation of the experiment with BioGIT using Susp (a), Susp-HPMC (b), Susp-IIIA (c) and Susp-IV (d) in the gastric compartment. For comparative purposes, recently reported ABZ equilibrium solubilities measured in the contents of upper small intestine of healthy adults, after administration of the corresponding suspensions are

also presented (box-whisker plots with dotted lines indicating the means, Kourentas et al. submitted).

*Figure 4*

(a) Mean $\pm$ SD (n=3) values for total ABZ amount per volume in the duodenal compartment of BioGIT; (b) Mean $\pm$ SD (n=3) values for ABZ concentrations in duodenal compartment of BioGIT (symbols) and mean (n=3) values for equilibrium solubility (lines).

Key: S-Susp, squares and continuous line; S-Susp-HPMC, triangles and dotted line.

*Figure 5*

Mean $\pm$ SD (n=3) values for equilibrium solubility in (a) HGF and Level III FaSSGF, and (b) HIF and Level II FaSSIF. Key: ABZ free base, grey bars; ABZ sulfate, white bars.

Figure 1

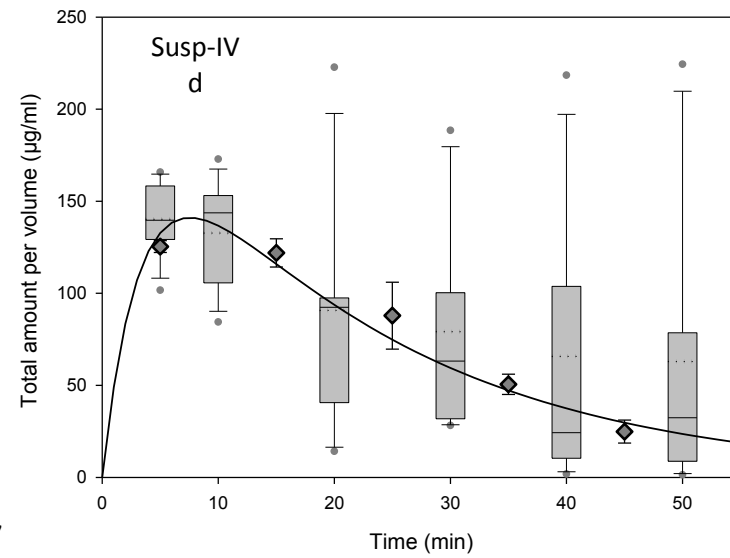
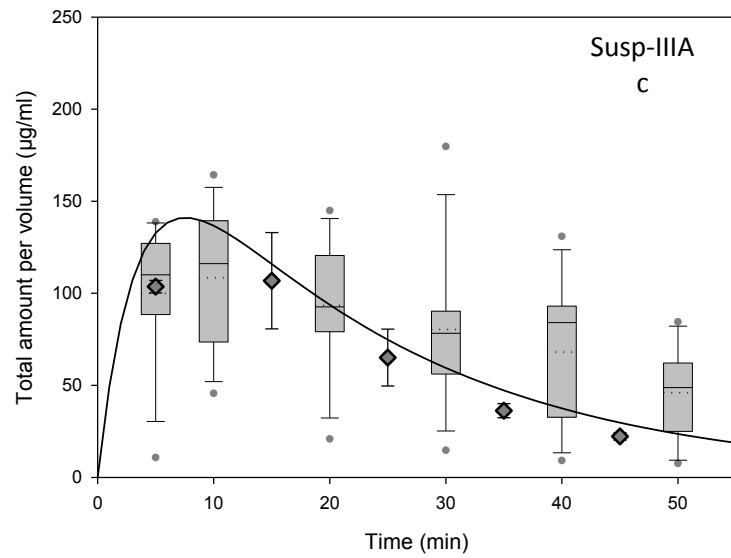
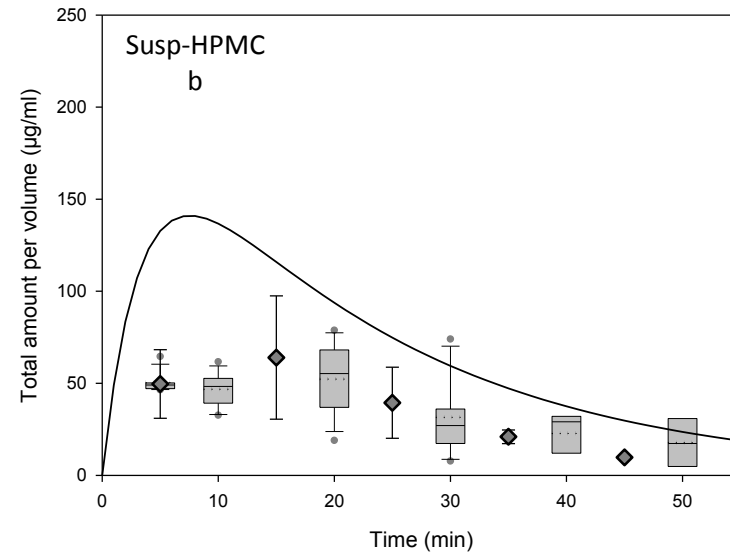
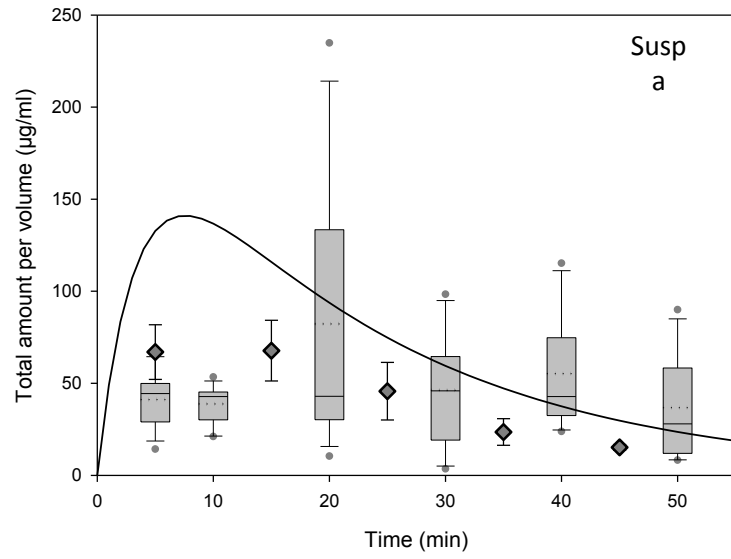


Figure 2

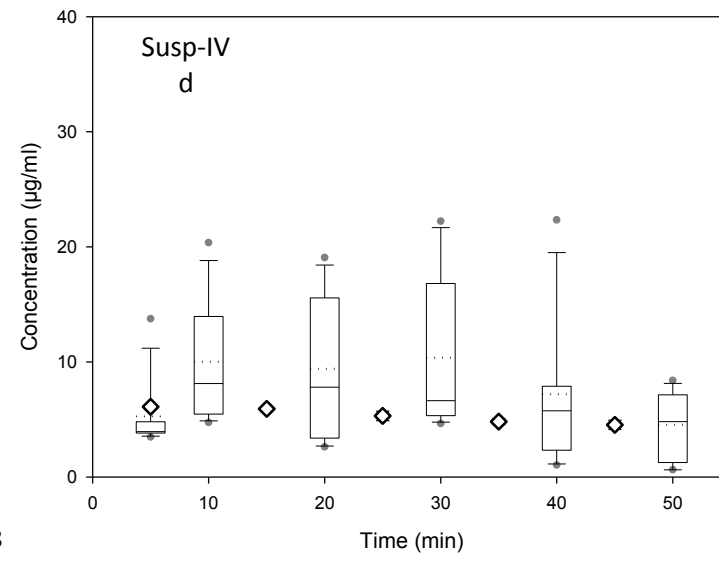
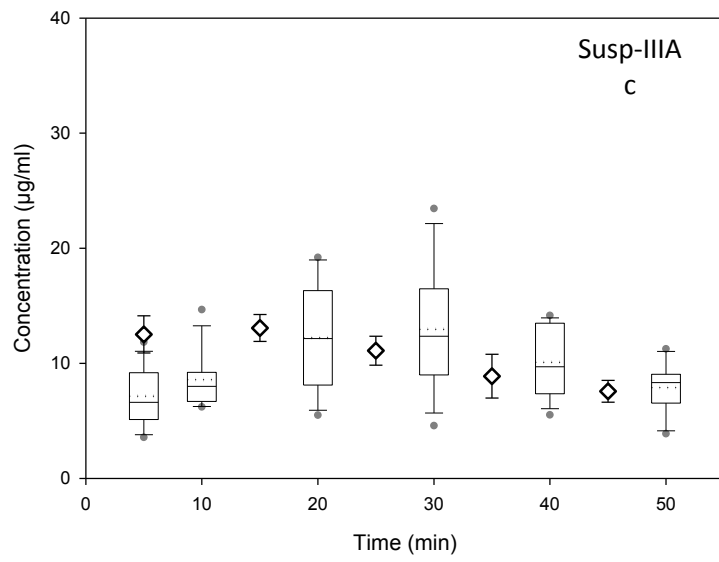
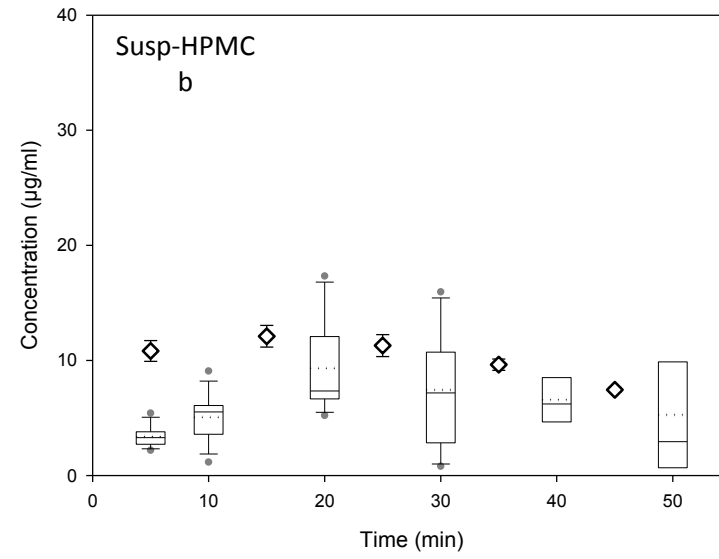
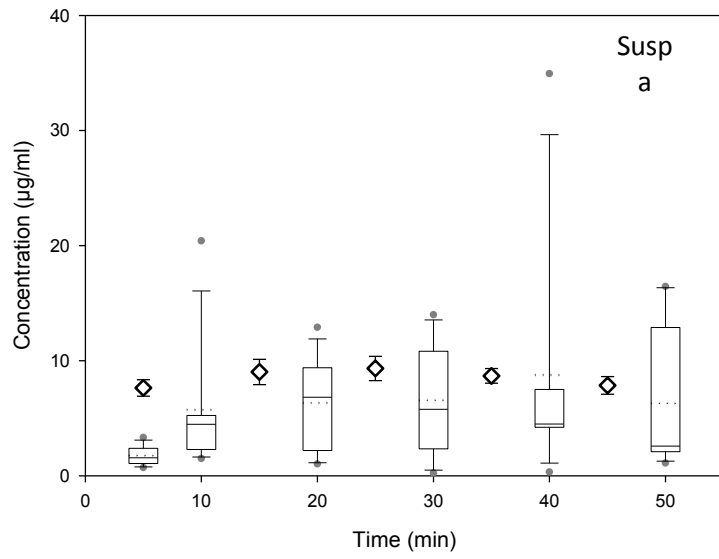


Figure 3

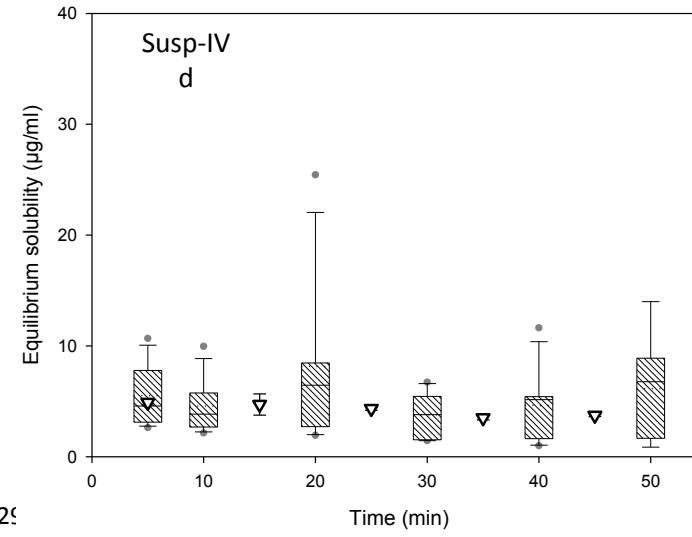
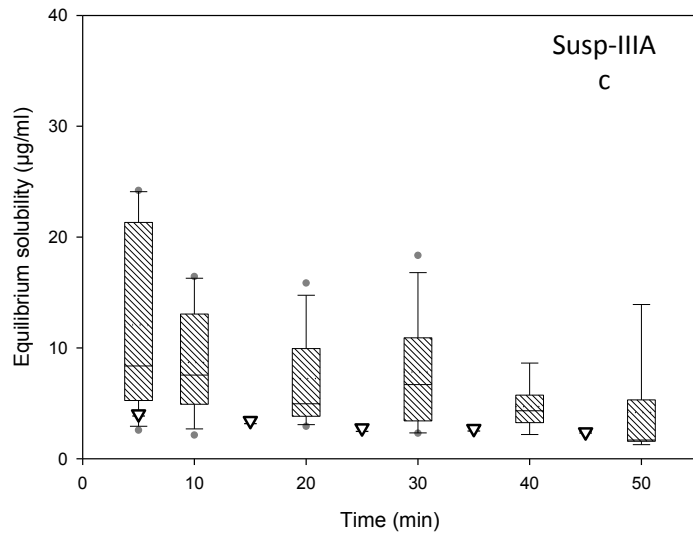
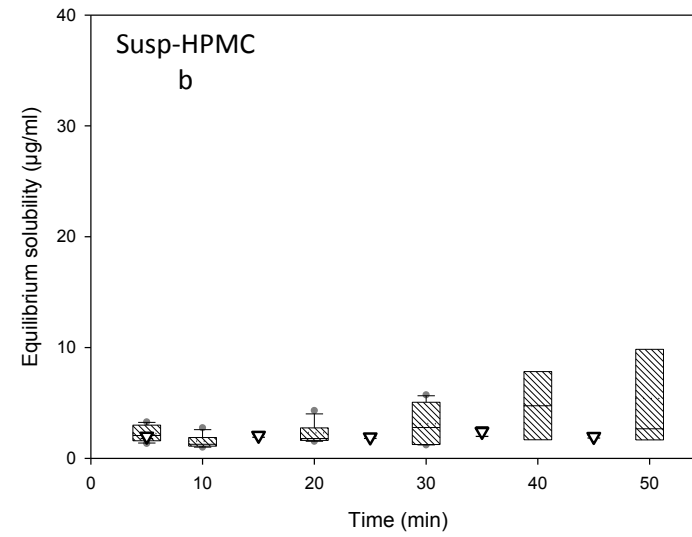
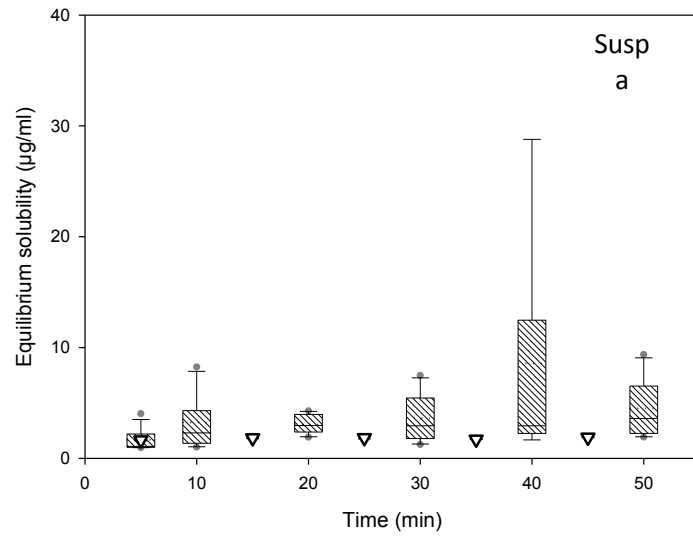


Figure 4

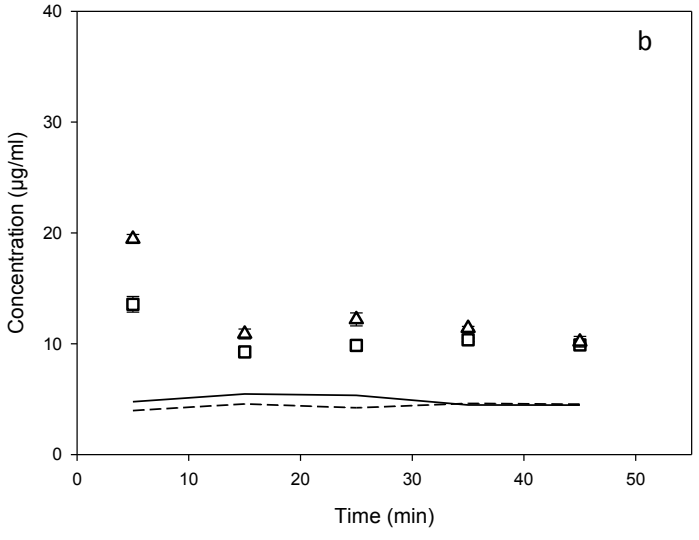
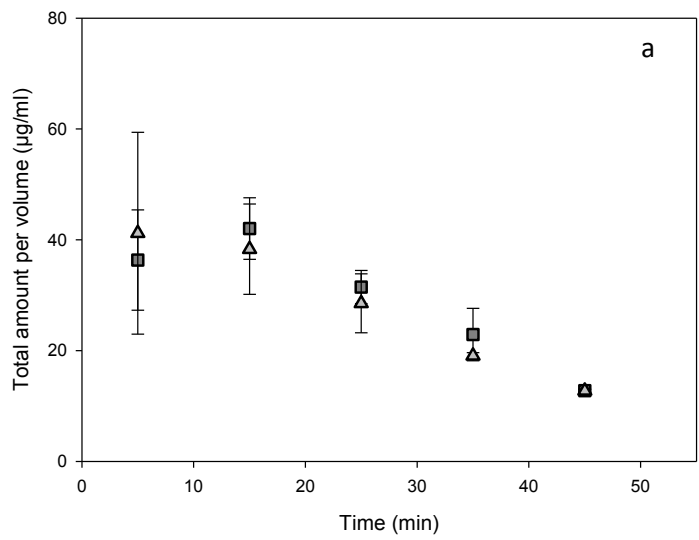


Figure 5

