Disposition of two highly permeable drugs in the stomach and the upper small intestine of healthy adults after a standard high-calorie, high-fat meal

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Graphical abstract
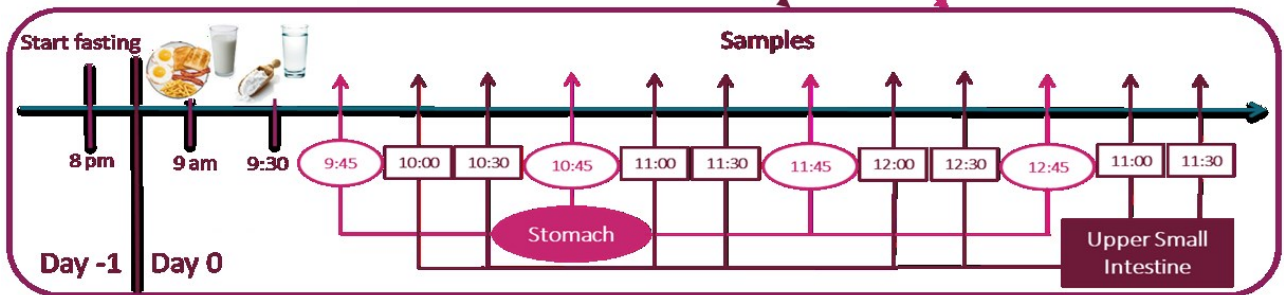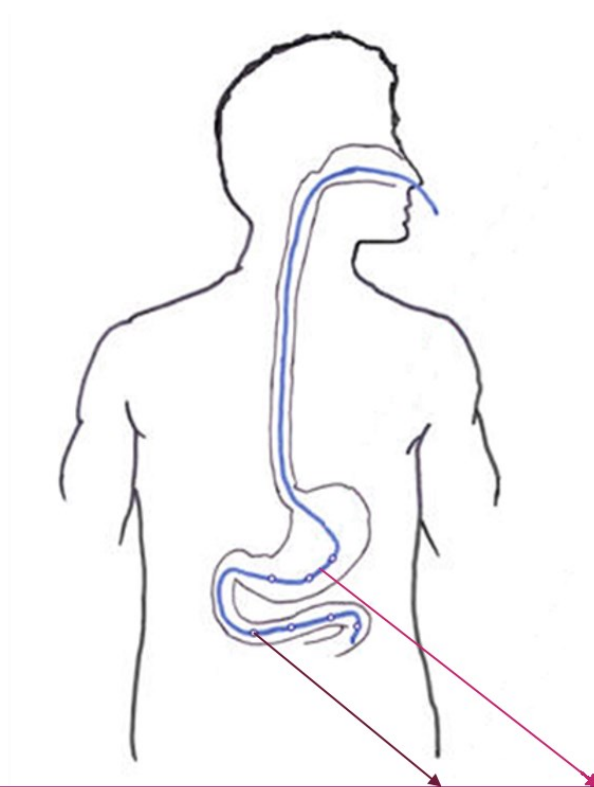
ABSTRACT

Objectives: To quantify the presence of two model highly permeable drugs, paracetamol and danazol, in the upper gastrointestinal lumen under conditions simulating the situation after disintegration of immediate release dosage forms administered in bioavailability/bioequivalence studies in the fed state. To understand the drug transfer process from the antral contents through the upper small intestine using the luminal drug data.

Methods: 8 healthy male adult volunteers participated in a randomized, single dose, two-phase, crossover study. After evaluating the impact of homogenization on meal's viscosity and particle size, the meal, containing phenol red as non-absorbable marker, was administered to the antrum via the gastric lumen of a naso-gastro-intestinal tube. The drugs were administered in solution form (Phase I) and in suspension form (Phase II) with a glass of tap water to the antrum of the stomach, 30 min after the initiation of meal administration. Samples were aspirated from the antrum and the upper small intestine up to 4 hours post drug administration.

Results: Apparent concentrations in the aqueous contents of the antrum were higher than apparent concentrations in micellar contents of the upper small intestine for paracetamol; the opposite was observed for danazol. Based on total drug amount per volume data in contents of the upper gastrointestinal lumen, the transfer of paracetamol (aqueous solution or suspension) and danazol (aqueous suspension) through the upper small intestine could be described as an apparent first-order process. Transfer of a long-chain triglyceride solution of danazol was highly variable.

Conclusions: Concentrations in the aqueous/micellar phase of luminal contents and values of parameters controlling the transfer from bulk gastric contents through the upper small intestine after a high-calorie, high-fat meal, were reported for the first time for highly permeable drugs. Data are expected to enhance the development of biorelevant *in vitro* and physiologically based biopharmaceutics modelling methodologies.

1. **INTRODUCTION**

Disposition of drug products in the upper gastrointestinal (GI) lumen after a high-calorie, high-fat meal varies with the type of ingested dosage form.

After a high-calorie, high-fat meal, aqueous drug solutions or aqueous drug suspensions will likely slide down the lesser curvature and empty rapidly from the stomach, similarly to water (Koziolek et al., 2014). Based on early exposure data, this behaviour has been recently confirmed for a paracetamol suspension (Statelova et al., 2019). In contrast, solid drug dose units will be deposited in the proximal stomach e.g. (Weitschies et al., 2008).

For immediate release (IR) tablets or IR layers of modified release tablets, time for complete disintegration in the human stomach after a high-calorie, high-fat meal has been reported to range from slightly more than 10 min to about 1 h, on average (Kelly et al., 2003; Weitschies et al., 2008; Rubbens et al., 2019). For hard gelatine capsules, rupture time in the fed state is typically slightly longer than 10 minutes (Digenis et al., 2000). As a result, substantial drug concentrations in stomach and drug emptying from the stomach are not expected immediately after ingestion, whereas during the disintegration/rupture period the solid and/or dissolved drug is mixed with bulk gastric contents.

Data on the drug presence and/or the drug transfer process from the stomach into the duodenum when IR tablets or capsules are administered after a high-calorie, high-fat meal i.e. in drug bioavailability/bioequivalence (BA/BE) studies performed in the fed state have been very limited. Early data for capsules collected by using gamma scintigraphy are not useful because the dosing conditions were not controlled (Davis et al., 1984) or the protocol for inducing fed state conditions (Theodorakis et al., 1980; Hunter et al., 1983) was much different than that applied in BA/BE studies during the last three decades (Department of Community Services and Health, Australia 1989; U.S. FDA 1992; U.S. FDA 2002; EMA 2010; U.S. FDA 2019). On the other hand, studies involving direct sampling of luminal contents after a high-calorie,

high-fat meal to date have primarily aimed to understanding intraluminal processing of food (Fordtran and Locklear, 1966; Malagelada et al., 1976; Camilleri et al., 1985). The first study aiming to evaluate disposition of an orally ingested IR product after administration of the high-calorie, high-fat meal suggested by regulatory agencies to be used in drug BA/BE studies in the fed state (standard meal) (EMA, 2010; U.S. FDA, 2019) has been published very recently (Rubbens et al. 2019). Aspiration studies for evaluating drug product performance after administration of the standard meal are challenging for two reasons. Firstly, the drug must be administered safely after the positioning of the gastro-intestinal aspiration tube and 30 minutes after the initiation of meal intake. The second concern relates to the tube passing through the pylorus; it should not restrict the drug transfer process into the duodenum and it should not create difficulties in aspirating samples from the upper small intestine which may contain drug and meal solid particles and/or have increased viscosity.

In the present study, we initially addressed the impact of homogenization on the viscosity and size of meal particles so that the meal and drugs were administered directly into the antrum by using a tube that allows aspirating samples from the stomach and especially from the upper small intestine, 30 minutes after initiation of administration of the standard meal, without restricting the physiological GI transfer process. Then, we set two objectives. The first objective was to measure the total drug amount per volume of contents aspirated from the stomach and from the upper small intestine, as well as the apparent drug concentrations in the aqueous phase of contents aspirated from the stomach and in the micellar phase of contents aspirated from the upper small intestine, at various times after administration of drug solutions and suspensions to the antrum, i.e. under dosing conditions simulating the situation after disintegration of orally administered IR dosage forms in BA/BE studies. The second objective was to understand the drug transfer process from the bulk gastric contents through the upper small intestine based on the total drug content per volume of samples aspirated from upper GI lumen at various times post administration.

Two model highly permeable drugs were employed to achieve the objectives of this study; paracetamol (pka 9.5, acidic, BCS Class I compound) and danazol (non-ionized, BCS Class II compound) (Kalantzi et al., 2006a;

Tsume et al., 2014). Since no pharmaceutical interaction is expected, for ethical reasons paracetamol and danazol were co-administered. The following two dosing conditions were applied:

1. Solutions of paracetamol (12.5mg per mL of tap water; 500mg in 40mL) and of danazol (6.25mg per gram of sunflower oil; 100mg in 16g) co-administered with a glass of tap water, 30 minutes after initiation of administration of standard meal.

2. Suspensions of paracetamol (500mg in 10mL tap water) and of danazol (100mg in 10mL tap water) co-administered with a glass of tap water 30 minutes after initiation of administration of the standard meal.

2. MATERIALS AND METHODS

2.1. Materials

Paracetamol for human use was from Lianyungang Kangle Pharmaceutical Co., Ltd, CHINA (Batch No. CW-1704010M), micronized. Danazol for human use was from Coral Drugs Pvt. Limited, INDIA (Batch No. 5201-B-17009), micronized/mean particle size of 6.75 μm. Phenol red (phenolsulfophthalein sodium salt) was purchased from Sigma-Aldrich Chemie GmbH). All chemicals used were of analytical grade and purchased from Sigma Aldrich Chemie GmbH or E. Merck (Germany). All solvents were of HPLC grade. Composition and caloric breakdown of the meal is presented in Table 1.

2.2. The impact of homogenization on the texture of the standard meal

The impact of homogenization on meal's texture was evaluated by comparing the particle size distribution and the viscosity of the meal, after homogenization with Multi Pyrex food processor (3 meal preparations) and after chewing (3 adult volunteers chewed the meal within 20 minutes and collected it back instead of swallowing).

For the particle size measurements, the total volume of the meal was initially measured. The meal was then let pass sequentially through a 2 mm mesh diameter and a 1 mm mesh diameter sieve. The volume of meal with particles larger than 2 mm, between 1 and 2 mm, and less than 1 mm was estimated. A sample from the latter portion of the meal was transferred in a Malvern Mastersizer S particle size analyzer (Malvern Panalytical Ltd, UK) where the particle size distribution was measured.

Meal viscosity was measured using a cone and plate rotational viscometer (RM 100 CP 2000 PLUS, LAMY Rheology, France) at ascending shear rates of 50 s^{-1}, 100 s^{-1} and 200 s^{-1} at two different temperatures: 25 °C and 37 °C.

2.3. The human aspiration study

2.3.1. Clinical center, approvals and study design

The study was performed at the Red Cross Hospital of Athens under a bilateral agreement between the National and Kapodistrian University of Athens and the Hospital (AP 047492/13-6-2018), after receiving approval by the Scientific and the Executive Committee of the Hospital (AP 20505/13-09-17) and according to the currently applied EU regulations (EMA, 2001). It was a randomized, single dose, crossover, two-phase study.

Phase 1: 30 min after initiation of administration of the meal, paracetamol aqueous solution and danazol solution in sunflower oil were administered with a glass of tap water.

Phase 2: 30 min after initiation of administration of the meal, paracetamol aqueous suspension and danazol aqueous suspension were administered with a glass of tap water.

2.3.2. Volunteers

Inclusion Criteria: Willingness of the subject to participate was indicated by his signed informed consent, age 18-60 years, weight within 15% of ideal body weight as determined by Metropolitan Life Tables, verification of suitability by a general physical examination and ability to abstain from smoking, alcohol, and over-the-counter and prescription medication(s) for 3 days prior to and throughout the experimental day. In addition, a blood sample was taken to assess electrolyte balance, kidney and liver function, blood morphologic characteristics, and lipid levels. The subject had to be found healthy in all of these examinations to qualify.

Exclusion Criteria: The existence of a major health problem and/or existence of any condition requiring prescription drug therapy, recent history of GI symptoms regardless of the severity, receipt of an investigational agent (new or generic) within 30 days prior to the initiation of study, the presence of antibodies indicating active acute or chronic HIV, HBV, or HCV infection, use of medication that may affect

GI function (including antibiotics) within 30 days of the study and irregular bowel habits were exclusion criteria.

Ten healthy male adult volunteers were recruited according to the inclusion and exclusion criteria. Two subjects were eliminated, because, during their first visit, one could not be intubated for reasons relating to the anatomy of the nasal cavity/stomach and another decided to terminate his participation, immediately after administration of the meal for personal reasons. Eight healthy male adult volunteers completed both phases of the study. Subjects were between 21 and 48 years old and deviated by not more than 11% from their ideal body mass index.

2.3.3. The naso-gastro-intestinal tube for aspirating samples from stomach and small intestine

As in previous aspiration studies performed in our laboratory in the fasted state (Psachoulias et al., 2011; Kourentas et al., 2016a), in this study we used a sterile Freka Trelumina Ch/Fr 16/9, 150 cm, two lumen naso-gastro-intestinal tube for aspirations both from the antrum and the upper small intestine. The outside diameter (OD) of the gastric lumen of the tube is 5.3 cm and the inside diameter (ID) is 4.1 cm. The gastric lumen of the tube contains the intestinal tube and the thickness of the ring through which samples from the contents of the antrum were aspirated is 0.6 mm. The intestinal lumen of the tube (passing through the pylorus) has an OD of 2.9 cm and an ID of 1.9 mm. Longstreth et al. (Longstreth et al., 1975) have shown that a 4mm OD transpyloric tube does not significantly affect gastric emptying of contents after administration of a meal with composition similar to the one employed in the present study. Mueller-Lissner et al. (Mueller-Lissner, Fimmel et al., 1982) showed similar data for a 5mm OD, after administration of liquid meals, unlike the situation where more than one tubes are passing through the pylorus (Read et al., 1983). Therefore, the intestinal lumen of the tube employed in the present study should not interfere significantly with the physiological GI transfer process. A series of holes (55 - 65 cm proximal to the end of the tube) were used to access the antrum of the stomach and a further series of handmade holes (13.5 - 23.5 cm proximal to the

end of the tube) proved to be adequate for aspirating (not without difficulties at often times) samples with increased viscosity from the upper small intestine.

2.3.4. Study Protocol

For each phase the subject reported to the clinic in the morning after fasting for at least 12 h. The subject was then intubated nasally using the naso-gastro-intestinal double lumen tube. Insertion of the tube was assisted by a guiding wire and its position was monitored fluoroscopically. After reaching its final position, the wire was removed and the subject laid semi-supine. Body posture may affect distribution of contents but does not seem to affect gastric emptying rates (Steingoetter et al., 2006). The stomach was emptied from mucus secretions induced in response to the tube insertion/positioning procedure, as confirmed by the pH values of two preliminary samples aspirated from the stomach and the upper intestine, respectively. Just before administration, the meal was homogenized using a Multi Pyrex food processor. 50 mL water containing 100 mg phenol red, a non-absorbable water flux indicator, were added to the mixture. The physicochemical characteristics of the administered meal are presented in Table 2. Previously reported values of a meal with similar composition (Klein et al., 2004) are generally in line with data in Table 2. Small deviations could be attributed to the inclusion of 50 mL aqueous solution of phenol red in this study.

The meal was administered via the gastric port of the tube to the antrum using 60 mL-capacity syringes within 15-20 minutes. Thirty minutes after initiation of administration of the meal, 500 mg paracetamol and 100 mg danazol were co-administered via the gastric port of the tube with 200 mL tap water i.e. with approximately a "glass of water" after taking into account the 50 mL aqueous phenol red solution included in the meal.

Phase 1: 500 mg paracetamol were dissolved in 40 mL water; 100 mg danazol were dissolved in 16 g sunflower oil [long chain triglyceride (Orsavova et al., 2015)]; administration of the two solutions was followed by administration of 160 mL tap water.

Phase 2: 500 mg paracetamol were suspended in 10 mL water; 100 mg danazol were suspended in 10 mL water; administration of the two suspensions was followed by administration of 180 mL tap water.

Samples (up to 18 mL) were aspirated from the antrum and the end of the duodenum at various times after administration of the drugs as shown in Figure 1. The volume, pH, and buffer capacity were measured immediately upon each aspiration. Six hundred microliters of the aspirated sample were transferred in six vials, 100 µL in each vial, for assaying the total content of phenol red, paracetamol, and danazol, in duplicate. A cocktail of lipase/protease inhibitors consisting of 50 mM diisopropylfluorophosphate, 50 mM diethyl(p-nitrophenyl)phosphate, 50 mM acetophenone, and 250 mM phenylboronic acid was added at 2% v/v to the remaining sample which was then divided in two sub-samples. The first was stored at -70°C for further analyses. The second was immediately centrifuged (11000 g, 37 °C, 10 min) at the hospital to eliminate remaining solid API particles in the case of suspensions administration. The centrifuged samples were subsequently ultracentrifuged (410174 g, 37 °C, 2 h) to obtain the aqueous phase i.e. whatever is dissolved in the aqueous/micellar phase and assay the concentration of phenol red, paracetamol, and danazol in the aqueous/micellar phase of each aspirated sample. Four vials were prefilled with 100 µL of the aqueous phase for the analysis of phenol red and paracetamol, each in duplicate, and two vials were prefilled with 200 µL of the aqueous phase for the analysis of danazol, in duplicate. The remaining volume of the aqueous phase was stored at -70 °C. At the end of each collection period the tube was removed and, after a brief examination, the subject was discharged from the clinic.

The pH and buffer capacity data measured immediately upon aspirations as well as other characteristics of luminal contents will be reported in a subsequent manuscript.

2.4. Analytical methods applied to the aspirated samples

Phenol red content in aspirated samples and their aqueous/micellar phases was estimated based on a previously described assay methodology (Oberle et al., 1990). The analytical column was a Hypersil BDS C18 (150 × 3.0 mm, 5 µm). The mobile phase consisted of phosphate buffer 0.1M, pH 3: methanol - 50:50 v/v and the flow rate was 0.5 mL/min. The detection wavelength was 423 nm. The injection volume was 50 µL and the retention time was about 7 min. The lower limit of quantification (LLOQ) of the method was 50 ng/mL.

Paracetamol content in aspirated samples and their aqueous/micellar phases was estimated based on a previously described assay methodology (Vertzoni et al., 2003). The analytical column was a Hypersil BDS C18 (250 mm × 4.6 mm, 5 μm). The mobile phase was gradient phosphate buffer 0.05M, pH 6.5: methanol - 80:20 v/v to 24:76 v/v. The flow rate was 0.8 mL/min. The detection wavelength was 242 nm. The injection volume was 50 μL and the retention time was about 7 min. LLOQ was 100 ng/mL.

Danazol content in aspirated samples and their aqueous/micellar phases was estimated using a previously described methodology (Vertzoni et al., 2012). The analytical column was a Hypersil BDS C18 (150 × 3.0 mm, 5 μm). The mobile phase consisted of acetonitrile:water (70:30 v/v) and the flow rate was 0.5 mL/min. The detection wavelength was 286 nm. The injection volume was 50 μL and the retention time was about 7 min. LLOQ was 5 ng/mL.

2.5. Data treatment

Raw data are presented as Box-Whisker plots showing the median value, the 10th, 25th, 75th, and 90th percentiles and the individual outlying data points. The number of individual data points used for the construction of a box is indicated on top of the box. Within each box, horizontal solid lines indicate median values and horizontal dotted lines indicate mean values. Individual raw data are also presented together with the corresponding best fitted lines, assuming first-order kinetics (Kourentas et al., 2016b).

The following two equations were fitted to the individual data using Phoenix WinNonlin 8.2 (Certara USA, Inc., Princeton, USA):

$$f(t) = \frac{Dose}{V_G} \cdot e^{-k_G \cdot t} \tag{1}$$

$$f(t) = \frac{Dose}{V_I} \cdot \frac{k_G}{(k_G - k_I)} \cdot (e^{-k_I \cdot t} - e^{-k_G \cdot t}) \tag{2}$$

where k_G is the apparent first order gastric emptying rate constant, V_G is the apparent volume of gastric contents, k_I is the apparent duodenal elimination rate constant, and V_I is the apparent volume of duodenal contents. Since data after the administration of paracetamol solution and suspension were similar for total amounts per volume and concentrations in the stomach and the upper small intestine (p>0.05, paired t-test or Wilcoxon test depending on data normality), individual data were considered simultaneously.

3. **RESULTS AND DISCUSSION**

3.1. The impact of homogenization on the texture of the standard meal

On average, chewing of the meal led to salivary secretions of about 90mL (622 mL vs. 532 mL, Table 3). Compared to the homogenized meal administered to the volunteers, in the chewed meal the % volume with particles > 2mm was 15% higher (Table 3). The % volume with particles between 1mm and 2mm was similar in the homogenized and the chewed meal (about 14%, Table 3). The % volume with particles < 1mm was 16% lower in the chewed meal (61% of total vs. 77% of total, Table 3); in this portion of the meal, particles with size between 100 µm and 1mm were 17% less in the chewed meal (21% of total vs. 38% of total, Table 3).

Meal viscosity was in line with previously reported data for the viscosity of the standard meal (Pao et al. 1998). It decreased with increasing shear rates in all cases (Table 4), indicating pseudo-plastic characteristics. For a given temperature and shear rate, viscosity of the chewed meal was slightly higher (on average) and more variable than the homogenized meal (Table 4). Apparently, salivary secretions counterbalance the higher percentage of bigger particles in the chewed vs. the homogenized meal, resulting in similar viscosities.

Normally, after chewing, food enters the stomach through the cardia and slides down the lesser curvature with subsequent boluses stacking up like funnels. Food layers in the stomach and, layer by layer, the gastric contents become pasty and then liquid as they approach the pylorus (Schulze et al., 2006). However, based on MRI images, heterogeneity of gastric contents is decreased 30 min after initiation of meal consumption (Koziolek et al., 2014).

Based on the above, 30 min after administration of the homogenized meal in this study, deviations from the actual conditions 30 min after chewing and swallowing the meal should be minimal.

3.2 Phenol red data

In line with previous data (Malagelada et al., 1976; Koziolek et al., 2014), total phenol red amounts per volume of contents indicated substantial secretions and practically no major changes in intragastric volume 15-195 min post drug administration; mean values ranged from 41.8 µg/mL to 72.4 µg/mL (Figure 2A) whereas based on the administered meal volume (≈532 mL), assumed resting gastric volume (≈30mL), and the glass of water co-administered with the drugs (≈200mL), nominal values should have been ≈130 µg/mL if no water flux had occurred in the stomach.

In the upper small intestine, mean values of total phenol red amounts per volume of contents 30-210 min post drug administration were slightly lower than in the stomach (range: 25.1-59.2 µg/ml, Figure 2B), suggesting additional limited water secretion in the duodenum.

3.3. Paracetamol in the upper GI lumen after administration in the antrum

3.3.1. Aqueous solution

Total paracetamol amounts per volume of contents decreased exponentially in the stomach (Figure 3A) and the upper small intestine (Figure 3B). As expected for a highly soluble drug, mean apparent concentrations in the aqueous phase of gastric contents were almost superimposable to the mean total amounts per volume of gastric contents (Figure 3A). Similar observations were made for the data in upper small intestine (Figure 3B). Apparent concentrations in the micellar phase of contents of the upper small intestine were lower than concentrations in the aqueous phase of gastric contents.

3.3.2. Aqueous suspension

Total amounts per volume decreased exponentially in the stomach (Figure 3C) and the upper small intestine (Figure 3D). Interestingly, inter-subject variability seems to be lower compared to the aqueous solution; at

every aspirating time point the box was smaller (Figures 3C vs. 3A and Figures 3D vs. 3B). Mean apparent concentrations in the aqueous phase of contents in stomach and in the micellar phase of contents in the upper small intestine were almost superimposable to the corresponding mean values of total amounts per volume (Figures 3C and 3D), reflecting the high solubility characteristics of paracetamol. Apparent concentrations in the micellar phase of contents of upper small intestine were lower than in the aqueous contents of stomach.

3.4. Gastrointestinal paracetamol transfer after the standard meal

Individual total paracetamol amount per volume of intestinal contents vs. time data were considered for estimating GI transfer parameters, as previously done for similar data collected after administration of drug solutions in the fasted state (Kourentas et al., 2016b). The lack of data at times earlier than 30 min (Figures 3B and 3C), however, did not allow for successful fitting of the bi-exponential first-order model to the data collected in the present study. Phenol red data suggest practically unchanged volume of gastric contents (Figure 2). Also, based on previous data, gastric secretions in response to a similar type of meal follow apparent zero-order kinetics (Fordtran and Walsh, 1973). Using simple solutions, it has been shown that when the volume of a drug solution in a beaker is maintained constant and there is simultaneous constant inflow of solution not containing the drug and constant outflow of the drug solution from the beaker, the drug concentration in the beaker over time reflects the first-order emptying process of the drug from the beaker (Rowe and Morozowich, 1969).

Total paracetamol content per volume of gastric contents decreased continuously with time (Figures 3A and 3C) and based on the above, the gastric profiles reflect in essence the paracetamol gastric emptying process. Individual total drug amount per volume of contents vs. time data in the stomach and the upper small intestine were modelled by simultaneous fitting of equations 1 and 2. Individual data sets showed continuously decreasing pattern in stomach and in upper small intestine and individual total amounts per volume as well as the best fitted lines are presented in Figure 4. The values of estimated parameters are

presented in Table 5. The validity of the modelling approach was additionally confirmed by estimating the rate constant for the terminal phase of the data in upper small intestine. By using individual data sets at 150, 180, 210 and 240min, the rate constant was estimated to be 0.018 (0.002) min^{-1}. If gastric emptying is assumed to be much slower than the elimination from upper small intestine (Table 5), the terminal phase of the data in upper small intestine should practically reflect the input process in the small intestine, i.e. the gastric emptying process [flip-flop kinetics (Shargel and Yu, 1999)]. Indeed, the rate constant estimated from the terminal phase of the data in the small intestine was identical to the estimated k_G value in Table 5. Based on Table 5, the estimated half-life for paracetamol gastric emptying is 38.5 min, more than two times longer than the estimated half-life for drug gastric emptying after administration of an aqueous drug solution in the fasted state (Kourentas et al., 2016b). Estimated apparent volume of gastric contents 30 min post meal administration (468 mL) is in line with previous studies indicating that the intragastric volumes are similar to meal volumes at this time point (Malagelada et al., 1976; Koziolek et al., 2014).

3.5. Danazol in the upper GI lumen after administration in the antrum

3.5.1. Sunflower oil solution

Total amounts per volume of gastric contents were highly variable (Figure 5A, lined boxes). Similar observations were made for the total amounts per volume in the upper small intestine (Figure 5B, lined boxes). High variability of data in the lumen of the upper small intestine after administration of a simple danazol solution in olive oil with a liquid meal has also been observed previously (Vertzoni et al., 2012). Apparent concentrations in the aqueous phase of gastric contents were lower than total amounts per volume of gastric contents (Figure 5A). Concentrations of danazol in the aqueous phase of antral contents ranged from 0.1 to 1.6 μg/mL i.e. close to solubility of danazol in water: 1μg/mL (Sunesen et al., 2005). Similar observations were made for the apparent concentrations in the micellar phase of contents of upper small intestine; they ranged from 0.04 to 32 μg/mL i.e. only slightly lower than the solubility in the micellar phase of intestinal aspirates measured previously: 40±43μg/mL, n=44, 37°C (Vertzoni et al., 2012). In contrast with

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paracetamol data, apparent danazol concentrations in the micellar phase of contents in the upper small intestine were higher than in the aqueous phase of gastric contents, reflecting the efficient transfer of lipophilic danazol from the long chain triglyceride solution into the bile salt micelles so that apparent concentrations were maintained close to saturation. It should be noted that, based on danazol solubility in the total and micellar contents of upper small intestine in the fed state, precipitation due to lipid digestion in the upper small intestine is unlikely.

3.5.2. Aqueous suspension

Inter-subject variability was lower compared to the situation where sunflower solution was initially in the antrum (Figures 5C vs. 5A and Figures 5D vs. 5B; lined boxes). As with paracetamol, total amount per volume decreased exponentially in stomach (Figure 5C) and in upper small intestine (Figure 5D). Unlike the corresponding paracetamol data, apparent concentrations were much lower than the total amounts per volume, both in stomach and in upper small intestine. Apparent concentrations in the aqueous phase of antral contents were very low (Figure 5C), ranging from 0.2 to 1.7 µg/mL (solubility in water ≈1 µg/mL (Sunesen et al., 2005). In the upper small intestine (Figure 5D), apparent concentrations in the micellar phase ranged from 0.03 to 9 µg/mL, i.e. they were much lower than the apparent solubility in the micellar phase of intestinal aspirates, 40±43µg/mL (n=44, 37°C, Vertzoni et al., 2012) and much lower than apparent concentrations after the sunflower oil solution, emphasizing the slower transfer of lipophilic danazol from solid particles to the micellar phase than from the sunflower solution to the micellar phase.

3.6. Gastrointestinal danazol transfer after the standard meal

Danazol sunflower oil solution data could not be modelled due to the high variability. A simultaneous fitting of equations 1 and 2 to the total amounts per volume of contents of the danazol suspension data was performed but the standard errors of estimates for k_I and V_I were very high (data not shown). Therefore, only equation 1 was used to describe the gastric emptying process after administration of the suspension in the

antrum. As with paracetamol, individual data decreased continuously with time and the individual total amounts per volume as well as the best fitted line are presented in Figure 6. Estimated values for k_G and V_G were 0.0145 (0.0046) min^{-1} and 386.9 (68.0) mL, respectively. The estimated half-life for gastric emptying is 47.8 min, somewhat longer than that estimated for paracetamol. It has been reported that hydrophobic solid drug particles administered in an aqueous suspension form in the antrum in the fasted state tend to agglomerate in the gastric contents and/or adhere on the gastric mucosa resulting to delayed gastric emptying rates (Kourentas et al., 2016a). A similar phenomenon could be assumed for the fed state too. Estimated apparent volume of gastric contents, V_G, was lower than the one estimated after the paracetamol potentially reflecting differences between paracetamol and danazol particles distribution in stomach.

4. CONCLUSIONS

Based on the data collected in this study and previous data on disintegration times of IR tablets and capsules in the stomach and the upper small intestine in the fed state, the following remarks could be made.

For non-ionizable BCS Class I drugs administered as IR solid dosage forms after the standard meal, apparent concentrations in the aqueous contents of the stomach are higher than apparent concentrations in the micellar contents of the upper small intestine. Gastric emptying follows apparent first-order kinetics and rates are slower than in the fasted state.

For non-ionizable BCS Class II drugs administered as IR solid dosage forms after the standard meal, apparent concentrations in the aqueous contents of the stomach are lower than apparent concentrations in the micellar contents of the upper small intestine. Gastric emptying is expected to be highly variable, after disintegration of a capsule containing the drug in long-chain triglyceride solution. If the dosage form disintegrates to solid particles, gastric emptying is expected to follow apparent first-order kinetics and rates are slower than BCS Class I drugs.

In combination with data on the characteristics of contents in the upper GI lumen and disintegration times in stomach, data collected in this study are expected to enhance the development of biorelevant *in vitro* and physiologically based biopharmaceutics modelling methodologies for the evaluation of oral IR solid dosage forms administered after the standard meal.

ACKNOWLEDGMENTS

TABLES

Table 1: Composition and caloric breakdown of the standard meal (EMA 2010; U.S. FDA 2019)

Ingredient	Quantity	Distribution and amount of calories (kcal)			
		Fats	Carbohydrates	Proteins	Total
Toasted bread	2 slices	13	94	18	125
Eggs	2, size Large	110	5	70	185
Bacon	2 strips	74	6	18	98
Fried potatoes	50 g	72	88	8	168
Whole milk	250 mL	79	46	33	158
Butter	10 g	90	-	-	90
Sunflower oil	16 g	165	-	-	165
Total amount of calories (% of total amount)		603 (61%)	239 (24%)	147 (15%)	989 (100%)

Table 2: Physicochemical characteristics of the standard meal (containing in addition 50 mL aqueous phenol red solution) after homogenization*

Mass (g)	543.1 ± 0.4
pH	6.16 ± 0.05
Buffer capacity (mmol/L/ΔpH) using 0.1N NaOH	26.8 ± 2.3
Osmolarity (mOsm/kg)	438.0 ± 4.5
Surface tension (mN/m) of supernatant after ultracentrifugation	43.66 ± 0.86

*Values are mean ± SD (n=3 meal preparations); buffer capacity, osmolality, and surface tension were measured as described previously (Kalantzi et al., 2006b).

Table 3: Particle size distribution on a volume basis (mean ± SD, n=3) and particle size distribution on % volume basis (mean, n=3) for the standard meal (containing in addition 50mL aqueous phenol red solution) after chewing and after homogenization.

	Meal after chewing		Meal after homogenization	
	Volume (mL)	% total	Volume (mL)	% total
Total	622 ± 67	(100)	532.0 ± 1.6	(100)
> 2 mm	155 ± 33	25	52 ± 11	10
1 -2 mm	90 ± 18	14	70 ± 19	13
100µm – 1mm	377 ± 26	21	411±19	38
10µm – 100µm		30		24
1µm – 10µm		7		13
< 1µm		3		2

Table 4: Viscosity (mean ± SD, n=3) of the standard meal (containing in addition 50mL aqueous phenol red solution) at 25 °C and at 37 °C, after chewing and after homogenization.

Shear rate (s^{-1})	Meal after chewing		Meal after homogenization	
	25 °C	37 °C	25 °C	37 °C
50	1894 ± 1300	1862 ± 861	1731 ± 225	1729 ± 80
100	1724 ± 794	1648 ± 594	1605 ± 228	1582 ± 166
200	1000 ± 287	877 ± 197	945 ± 107	938 ± 65

Table 5: Estimated apparent gastric emptying rate constant of paracetamol, k_G, apparent volume of gastric contents, V_G, apparent rate constant for paracetamol elimination from the upper small intestine, k_I, and apparent volume of contents in the upper small intestine, V_I, and for paracetamol administered 30 minutes after the standard meal, based on the results of the simultaneous fitting of equation 1 and equation 2 to the individual total paracetamol amounts per volume of antral contents and contents of upper small intestine*.

k_G (min^{-1})	0.018 (0.003)
V_G (ml)	451 (50)
k_I (min^{-1})	0.091 (0.075)
V_I (ml)	301 (268)

* Standard error of estimation in parentheses; n=60 individual time points for the fitting of equation 1 and n= 121 individual time points for the fitting of equation 2; R^2 = 0.6 (p <0.0001)

FIGURE CAPTIONS

Figure 1: Schematic representation of the clinical protocol and aspiration time points after drug administration.

Figure 2: Total phenol red amount per volume of aspirated antral contents (A) and contents from the upper small intestine (B) after drug administration. Lined boxplots show total amount per volume of aspirated sample and empty boxplots show the respective apparent concentration in the aqueous/micellar phase. Continuous line shows the mean total amount per volume values and dashed line shows the mean apparent concentration in the aqueous/micellar phase.

Figure 3: Paracetamol in the antral contents and in contents of upper small intestine after administration of aqueous solution (A and B, respectively) and aqueous suspension (C and D, respectively) in the antrum. Lined boxplots show total amount per volume of aspirated sample and empty boxplots show the respective apparent concentration in the aqueous/micellar phase. Continuous line shows the mean total amount per volume values and dashed line shows the mean apparent concentration in the aqueous/micellar phase. The number of individual data points used for the construction of a box is indicated on top of the box.

Figure 4: Individual data for total paracetamol amounts per volume (μg/mL) in antral contents (A, n=60) and in contents of upper small intestine (B, n=121) after administration of aqueous solution or aqueous suspension in the antrum. Grey circles, mean data; Continuous line, best fitted lines after simultaneous fitting of equations 1 and 2 to individual data. Estimated parameters and measures of fit are presented in Table 5.

Figure 5: Danazol in the antral contents and in contents of upper small intestine after administration of simple lipid solution (A and B, respectively) and aqueous suspension (C and D, respectively) in the antrum. Lined boxplots show total amount per volume of aspirated sample and empty boxplots show the respective apparent concentration in the aqueous/micellar phase. Continuous line shows the mean total amount per

volume values and dashed line shows the mean apparent concentration in the aqueous/micellar phase. The number of individual data points used for the construction of a box is indicated on top of the box.

Figure 6: Individual data for total danazol amounts per volume (µg/mL) in antral contents (n=28) after administration of aqueous suspension in the antrum. Grey circles, mean data; Continuous line, best fitted line to individual data (R^2 = 0.5; p <0.0001).
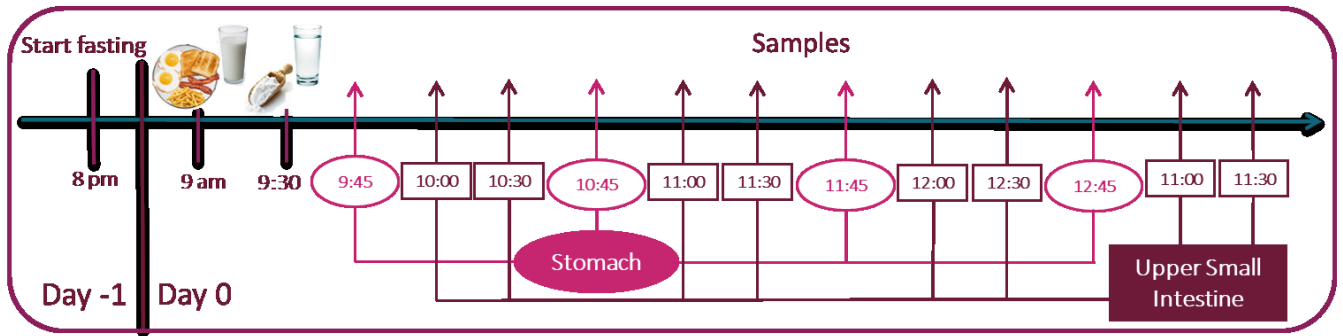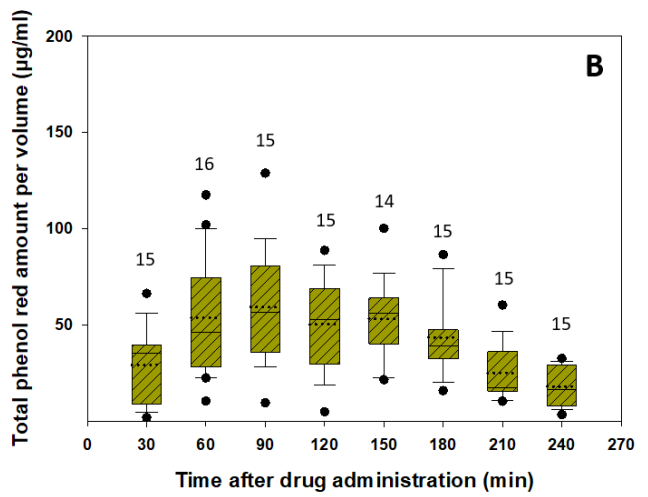
FIGURES

Figure 1

Start fasting   Samples

8 pm   9 am   9:30   9:45   10:00   10:30   10:45   11:00   11:30   11:45   12:00   12:30   12:45   11:00   11:30
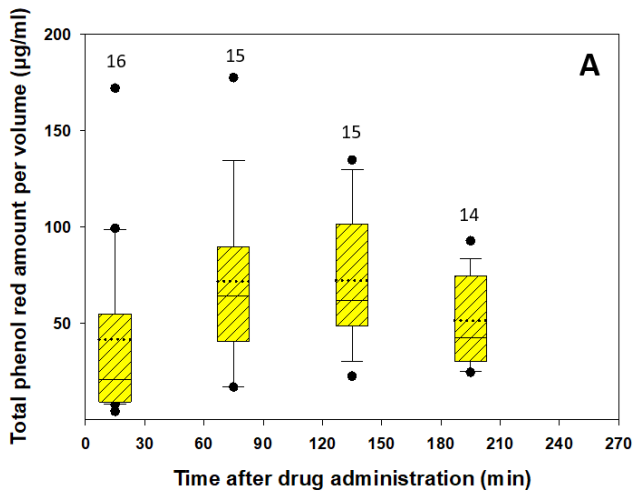
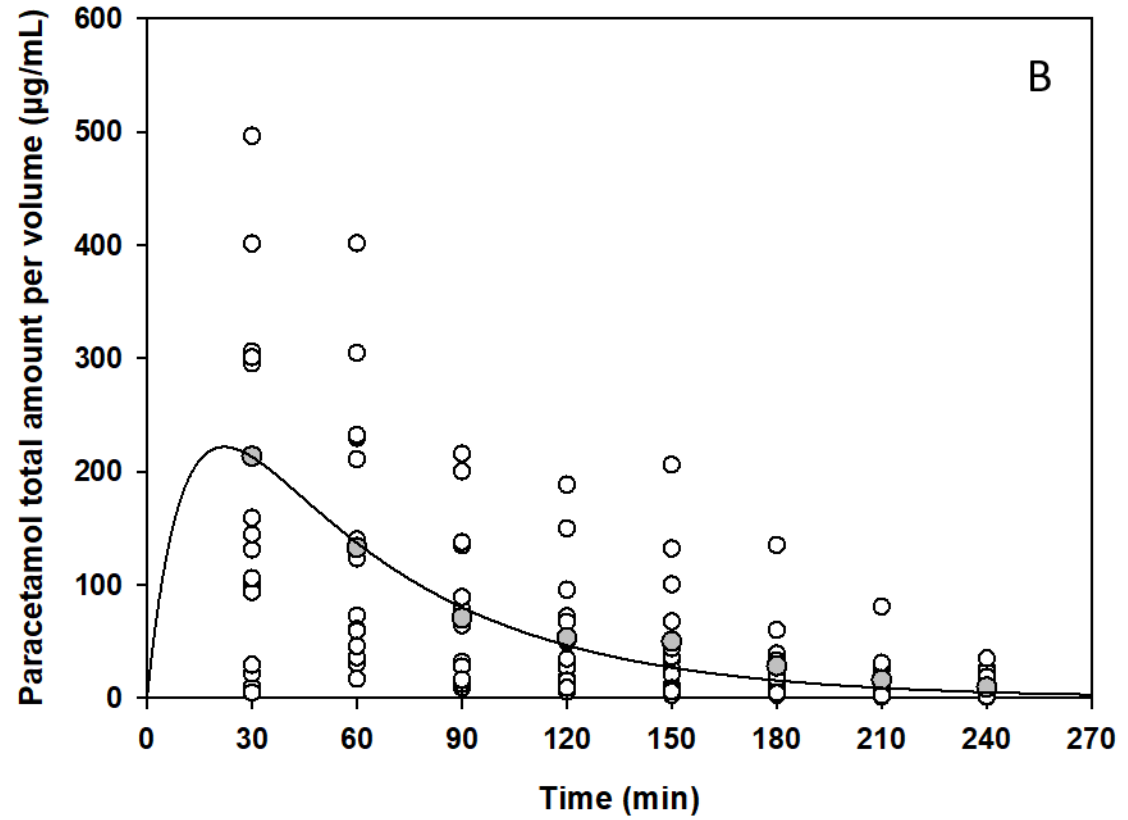Day -1   Day 0

Stomach
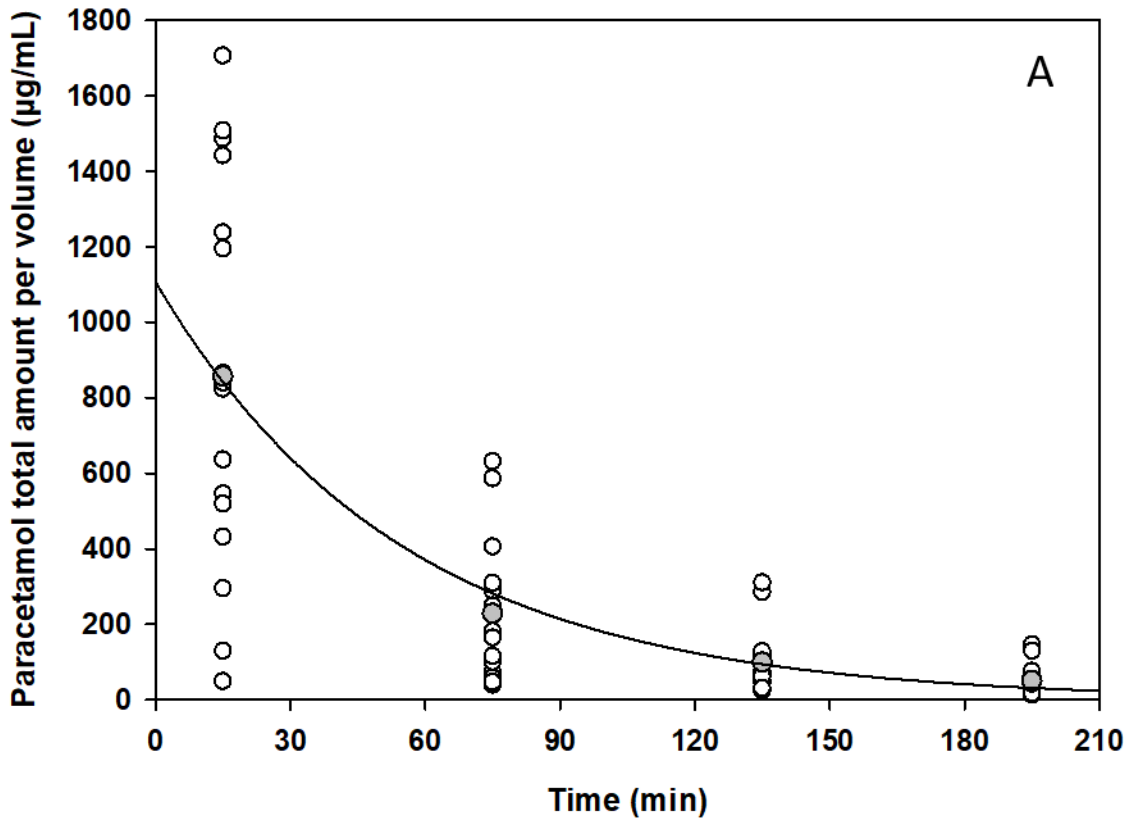
Upper Small Intestine
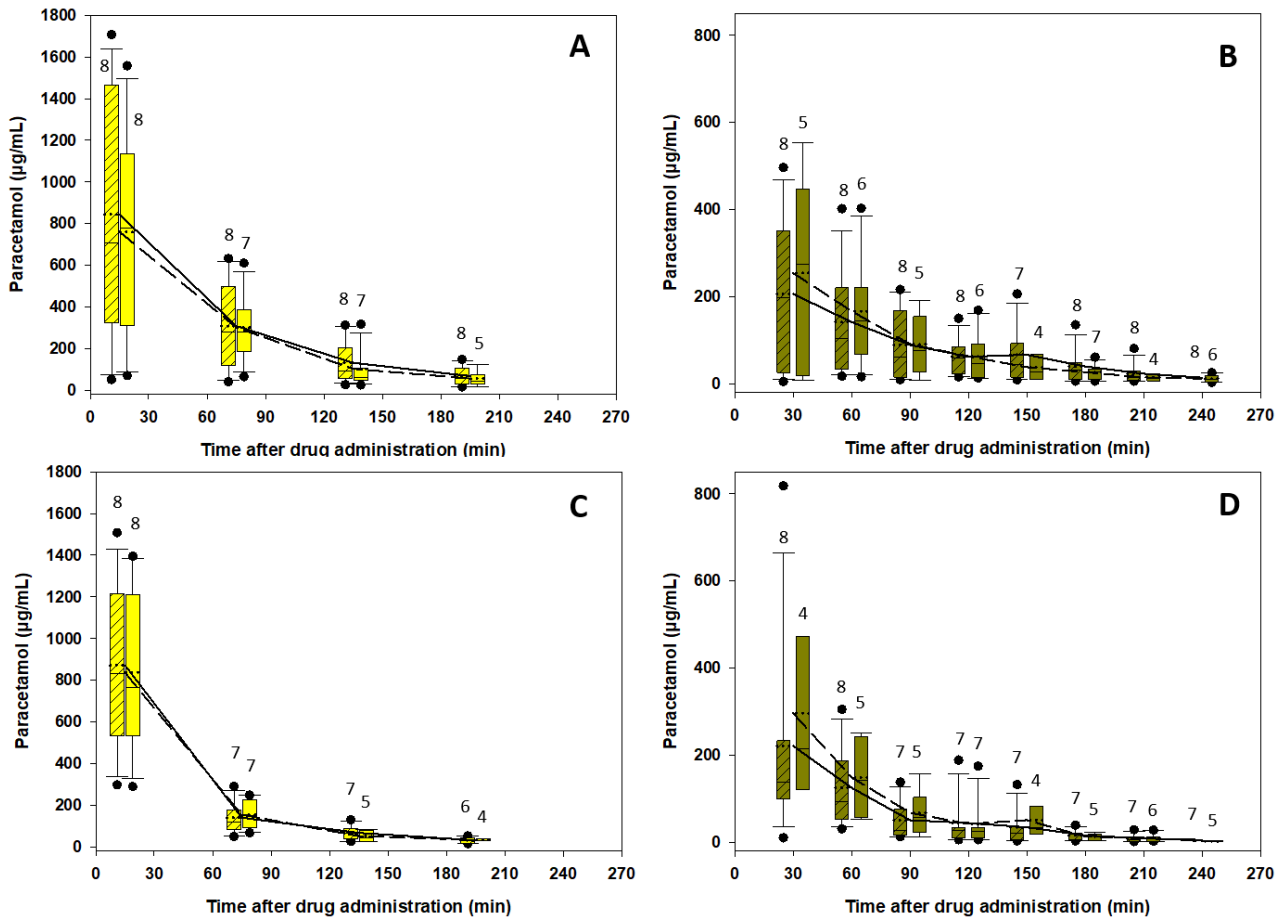
Figure 2

Figure 3

Figure 4

Figure 5

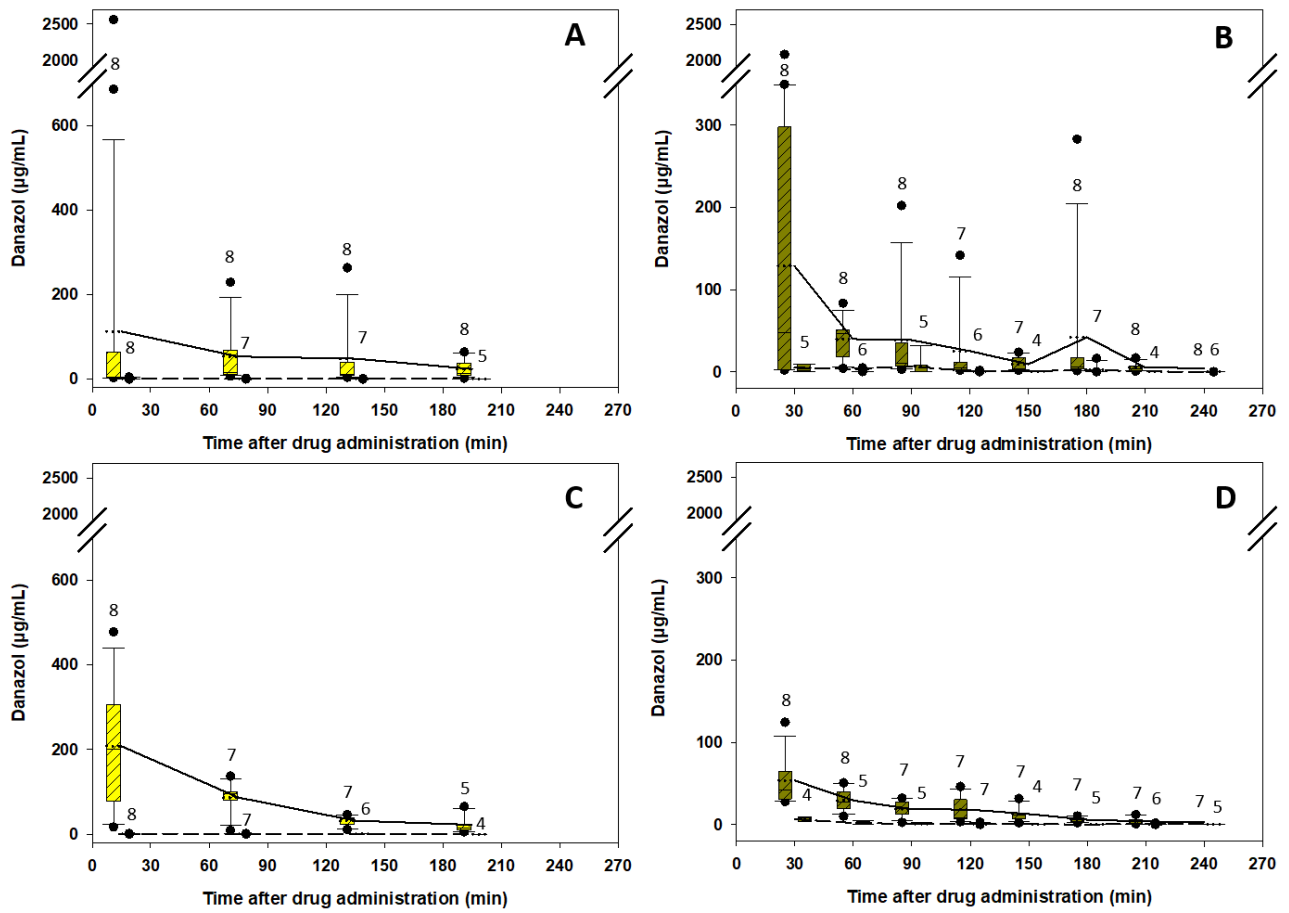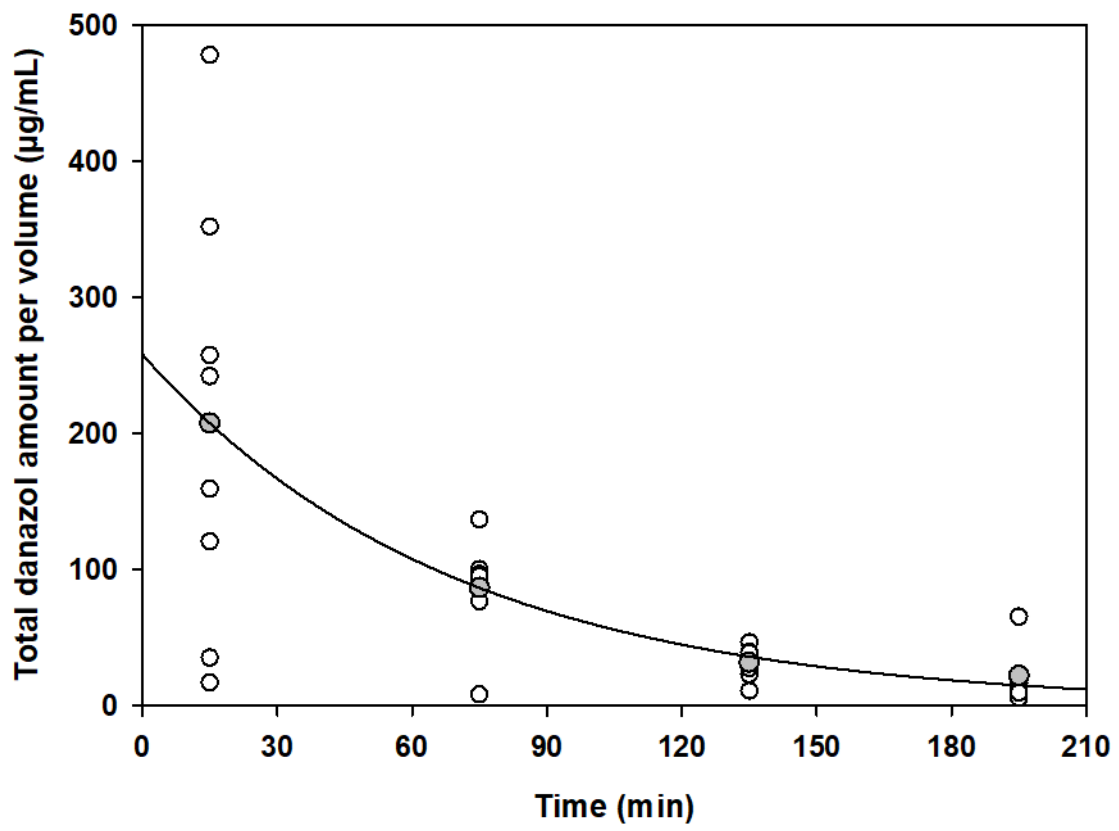Figure 6

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