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Research paper

Exploring the impact of real-life dosing conditions on intraluminal and systemic concentrations of atazanavir in parallel with gastric motility recording in healthy subjects

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ABSTRACT

This work strived to explore gastrointestinal (GI) dissolution, supersaturation and precipitation of the weakly basic drug atazanavir in humans under different 'real-life' intake conditions. The impact of GI pH and motility on these processes was thoroughly explored. In a cross-over study, atazanavir (Reyataz®) was orally administered to 5 healthy subjects with (i) a glass of water, (ii) a glass of Coca-Cola® and (iii) a glass of water under hypochlorhydric conditions (induced by concomitant intake of a proton-pump inhibitor (PPI)). After intake, GI fluids were aspirated from the stomach and the duodenum and, subsequently, analyzed for atazanavir. In parallel, blood samples were collected to assess systemic concentrations. In general, the results of this study revealed that the acidic gastric pH in combination with gastric residence time played a crucial role in the dissolution of atazanavir along the GI tract. After intake of atazanavir with a glass of water (*i.e.,* reference condition), complete gastric dissolution was observed. After GI transfer, supersaturation was noticed for a limited amount of time (1.25 h). With respect to the Coca-Cola® condition, complete gastric dissolution was also observed. A delay in gastric emptying, highly likely caused by the caloric content (101 kcal), was responsible for delayed arrival of atazanavir into the upper small intestine, creating a longer time window of supersaturated concentrations in the duodenal segment (3.25 h) compared to the water condition. The longer period of supersaturated concentrations resulted in a slightly higher systemic exposure of atazanavir compared to the condition when atazanavir was taken with a glass of water. A remarkable observation was the creation (when the drug was given in the migrating motor complex (MMC) phase 2) or maintenance (when the drug was given in MMC phase 1) of a quiescent phase for up to 80 min. With respect to the PPI condition, negligible gastric and intestinal concentrations were observed, resulting in minimal systemic exposure for all subjects. It can be concluded that gastric pH and residence time play a pivotal role in the intestinal disposition of atazanavir in order to generate sufficiently high concentrations further down in the intestinal tract for a sufficient period of time, thus creating a beneficial driving force for intestinal absorption.

1. Introduction

A thorough understanding of gastrointestinal (GI) drug behavior is essential to improve formulation development in the pharmaceutical industry. After oral intake, a drug product faces numerous physiological barriers that all have the potential to affect the life-cycle of the drug along the GI tract [1]. In the case of ionizable compounds, the prevailing pH at the time the drug passes by dictates the fraction of drug dissolved and, consequently, the driving force for absorption. Moreover, the time of drug intake in relation to the motility cycle may affect the rate of gastric emptying [2,3]. *In vitro* data clearly illustrate the impact of the transfer rate on the obtained concentrations of weakly basic compounds in the intestinal compartment of the transfer model, as presented by Kostewicz *et al.* [4]. A faster transfer rate resulted in higher supersaturated concentrations in the intestinal compartment. Nevertheless, once precipitation started, drug concentrations decreased noticeably faster at higher transfer rates. This opens the debate on the impact of gastric emptying on the obtained intestinal drug levels that serve as a driving force for intestinal absorption. The importance of the transfer rate has also been shown for a weakly basic compound using a physiologically-based pharmacokinetic (PBPK) simulation software tool: a parameter sensitivity analysis (PSA) demonstrated the impact of

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gastric emptying on systemic exposure for the weak base posaconazole [5].

The rate of gastric emptying can be tremendously altered after the concomitant intake of food [6,7]. As recently shown by Paixão and co-workers, the amount of ingested calories is a crucial trigger in the delayed onset of phase 3 contractions of the migrating motor complex (MMC), better known as the '*house-keeper wave*', responsible for transferring remaining residuals (*e.g.,* drug particles/food content) out of the stomach directly into the small intestine [8]. GI motility is defined by the different contractile phases of the MMC including periods ranging from absent activity (*i.e.,* MMC phase 1) towards periods with powerful, high-frequency contractile bursts that promote gastric emptying of contents (*i.e.,* MMC phase 3) [9,10]. MMC phase 3 contractions only occur in the fasted state and are absent in the fed state subjects. A recent study illustrated that the rapid onset of these MMC phase 3 contractions resulted in higher plasma C_{max} concentrations for ibuprofen, a weakly acidic drug, categorized as a BCS class 2a drug [8,11,12]. Rapid transfer to the neutral environment of the small intestine may be beneficial for dissolution and absorption of weakly acidic drugs. In contrast, in the case of weakly basic compounds, it may be more favorable for the drug to have a prolonged gastric residence time to promote gastric dissolution, resulting in higher intestinal drug levels upon transfer. From that perspective, the use of acidic beverages (*e.g.,* Coca-Cola®, pH 2.5) has shown to be beneficial in increasing the absorption of ketoconazole and posaconazole compared to the experimental condition when the drug was taken with a glass of water (*i.e.,* fasted state conditions) in healthy and hypochlorhydric subjects [13,14]. Moreover, concomitant intake of a proton-pump inhibitor (PPI) clearly demonstrated a decrease in plasma C_{max} and AUC compared to the fasted state condition. Walravens and colleagues [14] hypothesized that the increased systemic exposure of posaconazole after intake with Coca-Cola® and concomitant intake of a PPI was due to a prolonged gastric residence time caused by the present calories (101 kcal), and not only because of a substantial drop in gastric pH after administration of the drug with the acidic beverage as mentioned by Chin *et al.* [13]. Nevertheless, this points out that there is a need to have a better understanding of the impact of gastric residence time on the obtained drug levels in the intestine when a basic drug is taken with a glass of Coca-Cola® or with concomitant intake of a PPI. A recent study - in which a questionnaire was conducted among 895 adults (< 16 years old) in Flanders, Belgium – has reported about their medication intake conditions and the volume of water used and whether they respect the standardized clinical guidelines by taking the drug product with 240 mL of water in case of fasted state conditions (*i.e.,* following the guidelines of the US FDA for pharmaceutical companies to test their study medication in the clinical stage of drug development) [15,16]. Remarkably, 12.9% of the participants used soda for the intake of their medicines. In particular for azoles, results from this study showed that 18.5% of the study population took this class of drugs with acidic beverages [16]; in addition, 24% of the participants took PPIs at the same time. An improved understanding of the impact of these 'real-life' dosing conditions on systemic exposure may serve as a basis to develop guidelines for appropriate use with respect to these classes of drugs. The examples so far illustrate the importance of pH (posaconazole) and motility (ibuprofen/fosamprenavir) on intraluminal behavior and systemic concentrations for an orally administered drug [11,17,18]. As pH and motility can be triggered by the concomitant intake of beverages and medication, it is of utmost interest to investigate these phenomena on GI drug behavior and systemic concentrations. The aim of this study was to investigate differences in systemic exposure when the same drug product is given under different intake conditions (intake with water, Coca-Cola® or with water under achlorhydric conditions) and to link these differences in the plasma concentration-time profiles with the underlying luminal behavior. Atazanavir, categorized as a BCS class 2b drug [19], was selected as model drug and explored under different 'real-life' dosing conditions. To this end, five healthy subjects were recruited and 150 mg of atazanavir was orally administered under predefined conditions: (i) with 240 mL of water, (ii) with 240 mL of Coca-Cola® and (iii) with the concomitant intake of a PPI. After positioning of a gastric and intestinal catheter, atazanavir concentrations were profiled in the stomach and the upper small intestine. In parallel, blood samples were collected to assess the systemic exposure of the drug. A high-resolution manometry (HRM) catheter recorded gastric contractions after intake of the formulation to explore whether the drug was taken in MMC phase 1 or 2; as the duration of an MMC phase 3 period is rather short and its occurrence is less predictive, the administration of atazanavir in MMC phase 3 was not attempted.

2. Materials & methods

2.1. Chemicals and study medication

Atazanavir sulfate was kindly provided by Nanjing Legend Pharmaceutical & Chemical Co., LTD (Nanjing, China, batch number: 10052102). $^{2}H_{6}$-atazanavir was purchased from Alsachim (Illkirch-Graffenstaden, France). The marketed immediate-release capsules of atazanavir sulfate (Reyataz®, 150 mg, Bristol-Myers Squibb Pharma EEIG) and the gastro-resistant tablets of esomeprazole (Nexiam®, 40 mg, AstraZeneca) were purchased from the University Hospitals Leuven (Leuven, Belgium). Dimethyl sulfoxide (DMSO) and methanol (MeOH) were received from Acros Organics (Geel, Belgium), while BHD Laboratory Supplies (Poole, UK) provided HCl and NaOH. Acetonitrile was purchased from Fisher Scientific (Leicestershire, UK). Sodium acetate and acetic acid were purchased from VWR (Leuven, Belgium). Coca-Cola® was provided by The Coca-Cola Company (Atlanta, GA, USA). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

2.2. Design of the clinical study

Five healthy volunteers (HVs) were recruited and a cross-over study was performed with three experimental conditions. Three women and two men participated in this study, aged between 24 and 49 years old. Exclusion criteria (*i.e.,* GI disorders, infection with hepatitis B, hepatitis C or HIV, use of medication, pregnancy and frequent X-ray exposure) were checked during a medical examination. All volunteers provided informed consent to participate in the clinical study. Following the tenets of the Declaration of Helsinki, the clinical study was approved by the Committee of Medical Ethics of the University Hospitals Leuven (Leuven, Belgium), and the Federal Agency of Health and Medicines (FAHM, Brussels, Belgium). The study has been saved in the European Clinical Trials Database (EudraCT) with reference number 2017-004579-29. After an overnight fasting period of at least 12 h, volunteers were asked to come to the hospital.

Determination of the different phases of the MMC cycle was performed by positioning a high-resolution manometry (HRM) catheter (36 circumferential pressure-sensing channels, spaced at 1 cm intervals; Manoscan 360, Sierra Scientific Instruments, Los Angeles, CA, USA) as close to the pylorus as possible, covering the stomach's curvature. Prior to intubation, the HRM catheter was calibrated to assure accurate and precise pressure and measurements at body temperature (37 °C) by performing pressure measurements at predefined values ranging from 0 to 150 mmHg. Intubation of this HRM catheter occurred via the nose. To aspirate fluids from the stomach and upper small intestine, two aspiration catheters were intubated via mouth and nose or both via nose, depending on the volunteer's preference. A first double-lumen polyvinyl catheter (Argyle Salem Sump Tube, 14 Ch (external diameter 4.7 mm); Sherwood Medical, Tullamore, Ireland) was introduced via the mouth/nose and positioned in the duodenum (D2/D3) of the small intestine. A second single-lumen polyurethane catheter (Enteral Feeding Tube Wide Bore, external diameter 3.3 mm (10FR), 100 cm length, Eurosteriel Medical, Dronten, The Netherlands) was positioned

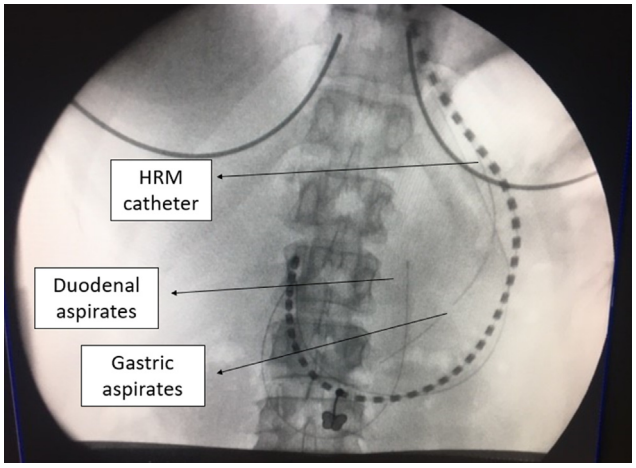

Fig. 1. Fluoroscopic image of HV 5 showing the positioning of the three catheters.

in the antrum of the stomach (*i.e.,* lowest part of the stomach). The position of the catheter was checked by applying non-continuous fluoroscopy imaging until the catheter reached the region of interest. A representative image of the positioning of the catheters is depicted in Fig. 1. Hereafter, the participants were asked to take place in a bed in semi-recumbent position for the remainder of the experiment.

Three experimental conditions were explored in a cross-over study design with a minimum 7 day wash-out period between each test condition:

(i) Oral administration of 1 capsule of Reyataz® with 240 mL of tap water;
(ii) Oral administration of 1 capsule of Reyataz® with 240 mL of Coca-Cola® (101 kcal);
(iii) Oral administration of 1 capsule of Reyataz® with 240 mL of water under hypochlorhydric conditions (induced by concomitant PPI intake).

It should be noted that the drug product itself was not manipulated, only the co-administration of the drug changed among the different arms of the study. With respect to the third experimental condition, one tablet of Nexiam® (40 mg esomeprazole; PPI) was taken in the early morning of the test day as well as three days prior to the study day (1 tablet/day). Based on previous research from our group, an optimal elevation in gastric pH was observed when Nexiam® was administered based on the protocol as described above [20]. Also, an optimal elevation in gastric pH was observed in this study by measuring the pH of gastric aspirates after being pretreated with esomeprazole for several days. A comparative study of different PPIs (esomeprazole, omeprazole, lansoprazole, pantoprazole) demonstrated that esomeprazole may be more effective than omeprazole, lansoprazole, and pantoprazole for the rapid relief of heartburn symptoms and acid reflux symptoms in patients with reflux esophagitis [21]. Due to practical issues with the positioning of the aspiration catheters, no duodenal aspirates were obtained from HV 4 for any condition.

The intake of the capsule was done randomly and not during a specific phase of the MMC cycle. After administration, antral and duodenal fluids were aspirated for 4 h; samples were taken each 15 min. No blank GI fluids nor blank blood samples were collected prior to drug administration, assuming that no atazanavir was present in the GI fluids or plasma samples of the participating volunteers. The sampling volume was kept as small as possible (< 4 mL per time point). Immediately after aspiration of fluids, pH was measured (Hamilton Knick Portamess®, Bonaduz, Switzerland) and the determination of dissolved and total atazanavir was initiated (see below). Blood samples were collected in heparinized tubes (BD Vacutainer Systems, Plymouth, UK) at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h after administration. Blood samples were centrifuged (2880 g, 10 min, 4 °C) and the obtained plasma was stored at −26 °C until analysis. The MMC activity was measured with the use of the ManoView analysis software version 2.0 (Sierra Scientific Instruments, Los Angeles, CA, USA). After 4 h, the three catheters were removed and the subject was allowed to drink and eat again. After collection of the final blood sample and removing the cannula that was placed in the forearm vein, the volunteer was allowed to leave the hospital.

2.3. Treatment of the aspirated GI fluids: solution versus total concentrations

In order to determine the apparent solution concentrations (*i.e.,* free and solubilized drug by colloidal species (*e.g.,* micelles/lipid vesicles)) of atazanavir in the gastric and duodenal fluids, the aspirates were immediately centrifuged (20817*g*, 5 min) and the supernatant was 9-fold diluted in an equal mixture of MeOH and purified water (50:50 v/v). In case of determining total concentrations (*i.e.,* solute + solid), GI aspirates were *directly* (*i.e.,* no centrifugation step) 9-fold diluted in an equal mixture of methanol and purified water (50:50 v/v). Precipitated proteins in the samples were separated with an additional centrifugation step (20817*g*, 5 min). Samples were analyzed by HPLC-UV at a wavelength of 265 nm. A Hitachi LaChrom Elite HPLC system was used consisting of an L-2130 pump and an L-2200 autosampler. After 50 µL injection, separation was performed using a Novapak C18 column (pore size 60 Å, particle size 4 µm, 8 mm i.d. ×100 mm, Waters, Milford, USA) under radial compression. The mobile phase consisted of 25 mM acetate buffer (pH 5.5) (A) and MeOH (B). Elution at a constant flow rate of 1 mL/min was performed as follows: an isocratic run with A and B (25:75 v/v) was performed for the first 5 min followed by a rinsing step making use of acetonitrile and water/MeOH (90:10 v/v) for 2 min to rinse the column. Subsequently, the column was re-equilibrated with the mobile phase. Calibration curves were made in an equal mixture of MeOH and purified water (50:50 v/v) based on a stock solution of atazanavir in DMSO (7 mM). Linearity was observed between 40 µM and 78 nM. The method was validated in human gastric and intestinal fluids, assuring accuracy and precision at three relevant concentrations. The accuracy and precision errors were less than 10%, meeting the FDA standard guidelines for bioanalysis methods [22]. Quality control samples were analyzed together with the samples of the *in vivo* study, resulting in a relative standard deviation and accuracy error of less than 10% for both cases.

2.4. Measuring thermodynamic solubility of atazanavir in the aspirated GI fluids

To express the degree of supersaturated concentrations in the intestinal tract for atazanavir, a comparison needs to be made between the apparent solution concentration at the time of aspiration and the apparent equilibrium solubility of atazanavir in the corresponding aspirate. A supersaturated solution refers to a state where dissolved concentrations of atazanavir exceed its thermodynamic solubility in the corresponding media. The degree of supersaturation (DS) is expressed as:

$$DS = \frac{C}{C_{eq}} \quad (1)$$

where C stands for the dissolved concentration of atazanavir in the gastric or intestinal fluid at the time of aspiration and C_{eq} stands for the thermodynamic solubility of the drug in the corresponding gastric or intestinal fluid. A solution can be defined as supersaturated, unsaturated or saturated whenever DS > 1, DS < 1 or DS = 1, respectively. The thermodynamic solubility of atazanavir was determined in all aspirated gastric or duodenal samples, by adding an excess of

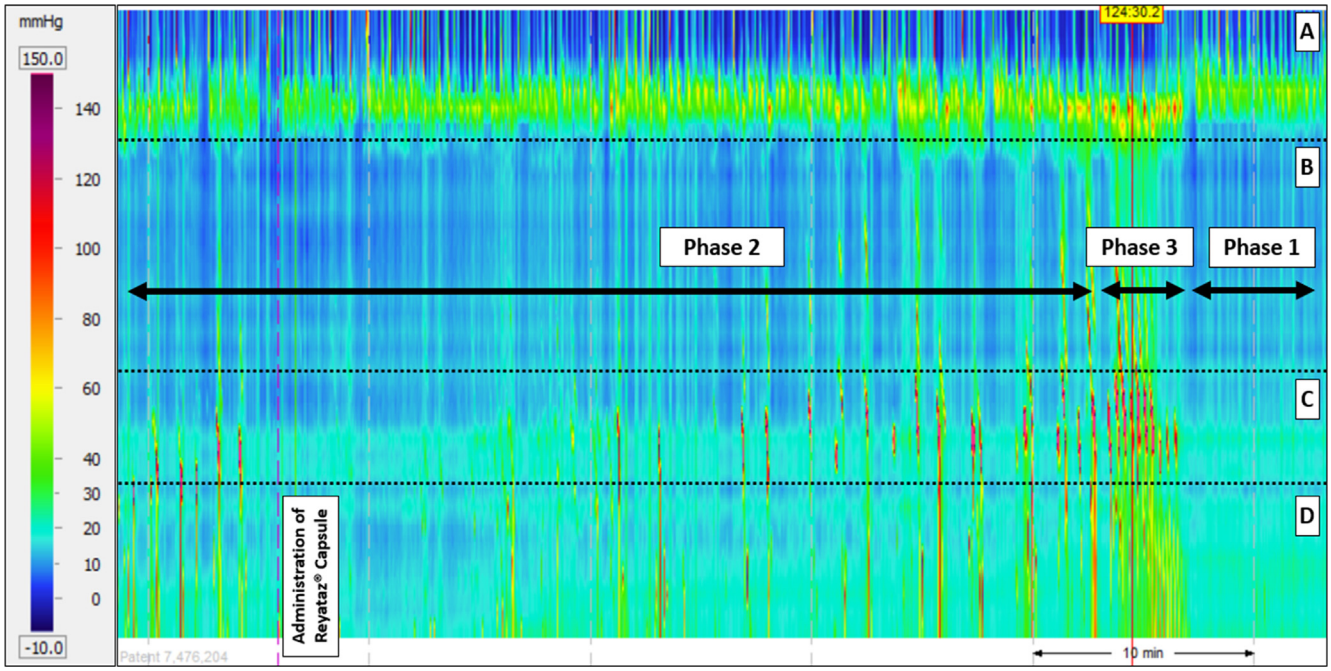

Fig. 2. High-resolution pressure map as a function of time generated using high-resolution manometry (HRM), depicting the different phases of the migrating motor complex (MMC). The different colors refer to a different contractile activity, expressed as pressure (mmHg). The different regions can be defined as: A. Gastro-esophageal junction, B. Corpus, C. Antrum, D. Duodenum. These regions are separated by the horizontal dotted lines. The vertical dashed line represents the time point when HV 2 ingested the capsule with a glass of water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

atazanavir sulfate powder to 800 µL of fluid. After 24 h of shaking in a 37 °C prewarmed incubator (175 rpm) (KS4000i incubator, Ika, Staufen, Germany), fluids were centrifuged (20817*g*, 15 min, 37 °C). After removing the surface layer of the fluid, the supernatant was diluted 9 times with a 50/50 mixture of MeOH and purified water. Precipitated proteins in the samples were discarded by performing an additional centrifugation step (20817*g*, 5 min, 37 °C). Subsequently, 50 µL of the supernatant was injected into the HPLC system and analyzed for atazanavir as described in the previous section.

2.5. Assessment of precipitation and solid amount of atazanavir in the aspirated GI fluids

In order to express the precipitated fraction (π) of atazanavir in the aspirated duodenal samples after oral administration of atazanavir with 240 mL of tap water or Coca-Cola® (test condition (1) and (2), respectively), the following equation was used, as previously reported by Psachoulias and co-workers [23]:

$$\pi = 1 - \frac{C}{C_t} \tag{2}$$

where C stands for the apparent dissolved concentration of atazanavir in the aspirated sample at a specific time point and C_t stands for the total concentration (*i.e.,* dissolved and precipitated) of atazanavir at that same time point. Given the fact that atazanavir was completely dissolved in the gastric fluids for both test conditions, it is justified to mention that the solid amount of atazanavir present in the duodenal fluids represents the amount of atazanavir that precipitated upon entry into the upper small intestine. In case of the PPI test condition (*i.e.,* test condition (3)), atazanavir did not completely dissolve in the gastric fluids so that the solid amount of atazanavir present in the duodenal fluids represents solid particles from the dosage form and/or precipitated drug particles caused by the GI transfer. In order to express the fraction of solid atazanavir in the aspirated duodenal samples after administration of atazanavir during the PPI test condition, the parameter ϒ is used (Eq. (3)), as recently introduced by Hens and colleagues [24]:

$$\gamma = 1 - \frac{C}{C_t} \tag{3}$$

where C is the apparent dissolved concentration of atazanavir at a specific time point and C_t is the total concentration (*i.e.,* dissolved and solid amount (precipitated and never dissolved) of atazanavir at the same time point).

2.6. Determination of the different MMC cycle phases

GI motility is defined by the different contractile phases of the MMC [10]: MMC phase I is an inert period with negligible activity; MMC phase II features sporadic contractions gradually ascending in magnitude (mean amplitude: 39.7 ± 14.4 mmHg); and phase III is characterized by powerful, high-frequency contractile bursts that promote emptying of contents where peak pressure recordings are observed (mean amplitude: 88 ± 31.7 mmHg) [9,25]. MMC phase III can either originate in the stomach (71%) or the upper small intestine (29%) [26,27] and is generally of short duration (2–6 min) characterized by a regular contraction frequency (2–3 contractions/min) [9,11,25]. For more information related to the MMC cycle and the crucial role of the stomach towards drug disposition, the reader is referred to external literature [28]. The HRM catheter (diameter 4.2 mm; Acertys, Aartselaar, Belgium) consisted of 36 pressure sensors spaced 1 cm apart. This catheter provides the possibility to measure regional pressures in both stomach and upper small intestine [29,30]. By connecting the outer end of the catheter to a computer console, specialized computer software (Manoview Analysis™, version 2.0.1, Los Angeles, CA, USA) was able to generate a high-resolution pressure map, which allows to distinguish the different phases of the MMC cycle based on the definition as described above (Fig. 2).

As this study aimed to assess a qualitative link between gastric motility and intraluminal behavior of the drug, it should be pointed out that quantification of the pressure events was not the topic of interest

for this study.

2.7. Analysis of atazanavir in plasma

To determine concentrations of atazanavir in plasma, 100 μL of plasma was added to 400 μL MeOH containing 100 nM $^2H_6$-atazanavir as an internal standard. After vortexing the mixture for 10 s, samples were centrifuged at 20238*g* for 5 min at 4 °C. The supernatant was transferred into a micro-vialthe followed by a 0.1 μL injection in an Acquity H-class UPLC system (Waters, Milford, MA, USA). The separation was performed using a Kinetex XB C18 column (50 × 2.1 mm, 2.6 μm; Phenomenex) held at 30 °C (Waters, Milford, MA, USA). The mobile phase consisted of a mixture of MeOH (solvent A) and 0.1% formic acid in water (solvent B) at a flow rate of 500 μL/min. Gradient elution was performed as follows: 68% of solvent A during 1 min, followed by 95% A for 1.5 min. Prior to the next injection, the column was re-equilibrated with 68% of solvent A during 1.5 min. Atazanavir eluted after 0.78 min. An MS/MS positive ionization mode was carried out with a HESI source on a Xevo TQ-S micro mass detector (Waters, Milford, MA, USA) with the following parameters: 150 °C source temperature, 1.50 kV capillary voltage, 15 V cone voltage, 10 L N_2/h cone flow, 800 L N_2/h desolvation gas flow, 300 °C desolvation temperature. The mass transitions used for the detection of the different compounds were *m/z* atazanavir 705.30 → 168.00 (collision energy: 45 V, dwell time: 150 ms) and *m/z* $^2H_6$-atazanavir 711.20 → 167.90 (collision energy: 45 V, dwell time: 150 ms). The UPLC eluent was guided into the MS using a divert valve within the time frame of 0.4–1.3 min and into the waste for the remaining time. A calibration curve was made in plasma by serial dilution. Linearity was demonstrated over the range of 5000 nM to 0.61 nM. For validation of the analysis, control plasma samples were prepared to contain 2500, 250, 25 and 2.5 nM atazanavir. Concentrations of atazanavir could be precisely and accurately determined (highest intraday variability relative standard deviation (RSD) 5.7 and 14.5%, respectively (n = 6)). During each run with samples from the clinical trials, a plasma quality control sample was analyzed, resulting in a precision error of < 7.9% (n = 4).

2.8. Data presentation and statistical analysis

Intraluminal and systemic concentration-time profiles are presented as the mean + standard deviation (SD) for all subjects. Pharmacokinetic parameters (plasma and duodenal C_{max}, T_{max} and area under the curve (AUC)) are reported as mean ± SD. Statistical tests were performed using a repeated-measures analysis of variance (ANOVA) test, followed by a Dunnett's multiple comparison test. These statistical tests were performed in GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, CA). Differences were considered statistically significant at $p < 0.05$. The control condition for this test was the intake of atazanavir with water. Linear regressions were executed in Microsoft® Office 365 Excel (Redmond, WA, USA) and were found significant at $p < 0.05$ (ANOVA). The length of each contractile phase and the frequency of occurrence are both reported as mean ± SD. The time to MMC phase 2 contractions post-dose is also depicted for each subject. Results that were outside the mean ± 3SD interval were considered as outlying results (examined by the SPSS statistical software package (IBM, Armonk, NY, USA)) and were not taken into account during the data analysis.

3. Results & discussion

3.1. Plasma concentration-time profiles of atazanavir

Fig. 3 shows the average plasma concentration-time profiles of atazanavir after oral intake of one Reyataz® capsule (150 mg of atazanavir) under different intake conditions.

Only a slight, non-significant increase in plasma C_{max} and AUC was observed after intake of atazanavir with a glass of Coca-Cola® compared to the control condition when atazanavir was co-administered with a glass of water ($p > 0.05$). Remarkably, the concomitant intake of PPIs resulted in extremely low systemic concentrations of atazanavir which were found to be statistically significant lower compared to the control condition. Pharmacokinetic disposition parameters for each individual are depicted in Table 1.

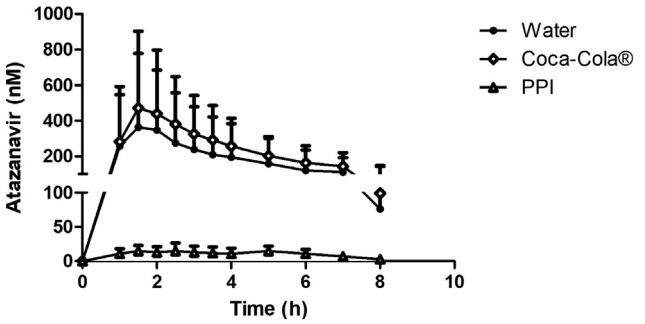

Fig. 3. Average plasma concentration-time profiles of atazanavir after oral intake of one Reyataz® capsule under different intake conditions. The diamonds (◊) represent the plasma concentration-time profile after intake of atazanavir with Coca-Cola®. The black dots (●) represent the plasma concentration-time after intake of atazanavir with water. The triangles (Δ) represent the plasma concentration-time profile after concomitant intake of atazanavir with a proton-pump inhibitor (mean ± SD, n = 5).

Compared to the intake with water, there was a slight increase in plasma C_{max} after intake of atazanavir with Coca-Cola® ($p > 0.05$). The plasma $AUC_{0\text{-}8h}$ was increased for all subjects after intake of atazanavir with Coca-Cola® whereas plasma T_{max} was delayed for three out of five subjects. With respect to the PPI condition, individual plasma C_{max} and AUC values were considerably lower compared to the other test conditions. To have a better understanding of these systemic profiles, the behavior of atazanavir was explored in the GI lumen where dissolution, precipitation and absorption take place.

3.2. Gastric concentration-time profiles of atazanavir

After oral administration of atazanavir under the predefined test conditions, solution and total concentrations of atazanavir were monitored in the antrum (*i.e.,* lowest part of the stomach). Fig. 4 depicts the mean gastric concentration-time profiles of atazanavir (solution versus total concentrations). In addition, the mean pH profiles of the gastric aspirates are depicted as an insert.

In general, a complete dissolution of atazanavir in the gastric fluids was observed since the solution and total concentrations were equal in case of the water and Coca-Cola® condition. The amount of drug that could be dissolved in the aspirated fluids is even higher than the measured solution concentrations at the time of aspiration (data not shown). Mean pH profiles were almost identical in both test conditions. In contrast, dissolution was incomplete in case of the PPI condition due to the elevated gastric pH that was observed. It should be noted that total concentrations of atazanavir that were measured during this arm of the study were remarkably lower compared to the other two conditions. *In vitro* results did not show any major difference in the disintegration of the capsule in fasted state simulated gastric fluid (FaSSGF) at pH 1.6 or 6.5 (data not shown). A pH of 6.5 was used to reflect the PPI condition. Rapid onset of dissolution of atazanavir was observed after intake with a glass of water showing an average C_{max} of 336 μM after 15 min. In the case of the Coca-Cola® condition, the delay in gastric emptying is likely responsible for an average C_{max} of 368 μM after 105 min. Descriptive parameters of the gastric solution concentration-time profiles are summarized in Table 2.

On average, there was a 26% increase in $AUC_{0\text{-}4h}$ for the Coca-Cola® condition compared to the control condition despite a lower gastric

Table 1
Individual and average disposition pharmacokinetic parameters for the systemic concentrations of atazanavir when orally taken under different conditions. The final row depicts the average values and standard deviations.

	Water			Coca-Cola®			PPI		
	Plasma C_{max} (μM)	Plasma T_{max} (h)	Plasma $AUC_{0\text{-}8h}$ (μM min)	Plasma C_{max} (μM)	Plasma T_{max} (h)	Plasma $AUC_{0\text{-}8h}$ (μM min)	Plasma C_{max} (μM)	Plasma T_{max} (h)	Plasma $AUC_{0\text{-}8h}$ (μM min)
HV 1	1.0	1.5	188	0.90	1.5	190	0.031	2.5	7
HV 2	0.17	1.0	30	0.18	2.0	40	0.016	1.0	2
HV 3	0.12	2.0	11	0.10	2.5	24	0.008	1.5	3
HV 4	0.64	2.0	136	0.88	1.5	162	0.024	2.0	7
HV 5	0.10	1.0	28	0.51	1.5	102	0.007	3.0	3
Average ± SD	**0.41 ± 0.40**	**1.5 ± 0.50**	**79 ± 79**	**0.51 ± 0.38**	**1.8 ± 0.45**	**104 ± 73**	**0.017 ± 0.010**	**2.0 ± 0.79**	**4.4 ± 2.4**

C_{max} (520 μM versus 611 μM, respectively). Given the fact that for both test conditions (i) the drug was completely dissolved in the gastric fluids and (ii) assuming no gastric absorption of atazanavir, these descriptive parameters suggest a prolonged gastric residence time after intake of the drug product with Coca-Cola®. A delayed gastric T_{max} was indeed observed for four out of five healthy subjects. This pattern of delayed gastric emptying as frequently observed in fed state studies can be explained by the calories (101 kcal) present in Coca-Cola® slowing down the emptying of gastric content to the upper small intestine [6]. After concomitant intake of atazanavir with PPIs, the gastric T_{max} was in the same range as observed for the fasting state conditions; however, gastric exposure of the drug was negligible. Plotting gastric and systemic exposure resulted in a positive, significant, linear regression with a Pearson coefficient (R^2) of 0.73 (Fig. 5A). A significant linear regression was also observed between plasma C_{max} and gastric AUC (R^2 = 0.69) as depicted in Fig. 5B.

3.3. Looking beyond concentrations: gastric motility and pressure events

The impact of gastric motility on intraluminal drug behavior has already gained a lot of attention throughout the years. More and more *in vitro* models are focusing on the impact of pressure events on drug release. For Glumetza® 1000 and Madopar® HBS 125, two marketed dosage forms of metformin and levo-dopa, respectively, a pressure-sensitive drug release behavior was observed, potentially leading to intersubject differences in systemic exposure after intake of one of these drug products [31,32]. Also, clinical studies have shown (i) the effect of pressure events on drug release and (ii) the impact of gastric motility on the onset of drug absorption [11,18]. A recent study demonstrated the impact of transient pressure events on the fast disintegration of a paracetamol tablet after intake with a glass of sparkling water [33]. These transient pressure events were not seen when the tablet was taken with a glass of tap water. As paracetamol is widely recognized as an indirect marker to assess gastric emptying by monitoring plasma concentrations after oral intake [34], this study mapped the direct impact of transient pressure events on the disintegration and exposure of the drug. This fast onset of disintegration was accompanied by a fast onset of plasma T_{max} after intake of the tablet with sparkling water.

When atazanavir was given orally with a glass of water or a glass of Coca-Cola®, complete dissolution of atazanavir was established (Fig. 4). From that point of view and assuming that gastric absorption for the ionized form does not occur [35], a delay in gastric emptying was observed – based on the gastric T_{max} - when atazanavir was ingested with a glass of Coca-Cola®. Interestingly, the onset of gastric MMC phase 2 contractions post-dose was also delayed after administration of atazanavir with a glass of Coca-Cola® (Fig. 6).

Although the recipe of Coca-Cola® remains a best-kept secret, it is known that one can of Coca-Cola® (*i.e.,* 330 mL) contains 35 g of sugar (fructose or sucrose) as a sweetener. A recent study by Meyer-Gerspach and co-workers demonstrated the impact of caloric and non-caloric sweeteners on the antral motility [36]. After intragastric administration of 50 g of glucose (205 kcal) or 25 g of fructose (103 kcal) dissolved in 250 mL of water, antral contractions were interrupted for approximately 100 min. It was discussed that glucose in the duodenum inhibits the motor activity of the gastric antrum and proximal duodenum, and increases both pyloric tone and phasic pyloric activity, resulting in a slower gastric emptying process. This phenomenon was also observed by other authors [37–39]. Moreover, intraduodenal infusions (2 kcal/min for 90 min) of glucose and fructose have similar inhibitory effects on gastric motor functions (stimulation of pyloric and suppression of antral pressure waves) [40]. After a quiescent period of no contractions (MMC phase 1), the onset of MMC phase 2 contractions will facilitate the mixing of gastric content (*i.e.,* drug and co-administered beverage), promoting drug release and dissolution in the stomach. The impact of MMC phase 2 contractions on appropriate mixing has recently been explored by Van Den Abeele *et al.* showing that a more homogeneous

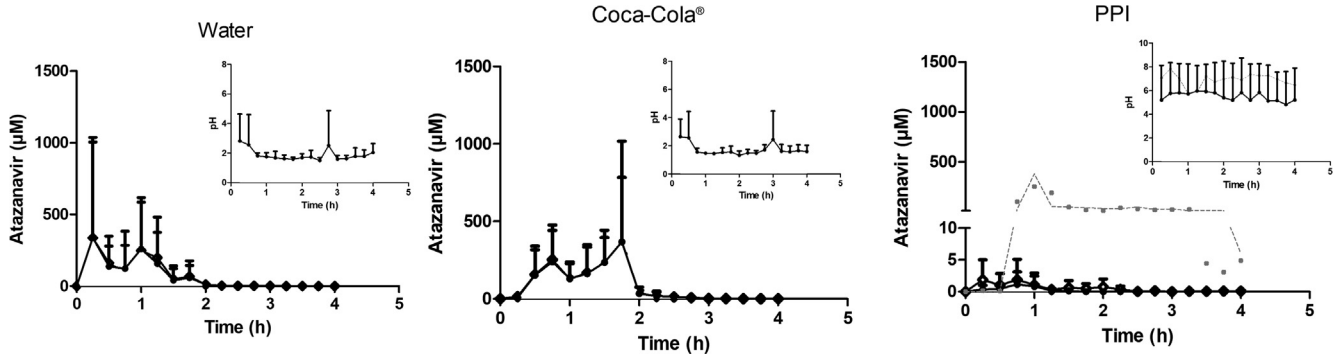

Fig. 4. Mean gastric concentration-time profiles for dissolved (●) and total (◊) concentrations of atazanavir after oral administration of one capsule of Reyataz® (mean + SD, n = 5). Mean gastric pH profiles as a function of time are depicted as an insert in each graph. With respect to the PPI condition, the obtained solution (—) and total (×) concentration-time profiles of HV 1 could be considered as outlying results and are depicted by the gray line.

Table 2
Descriptive parameters of the gastric solution concentration-time profiles for each subject in each test condition. The final row depicts the average values and standard deviations. *Results of HV 1 in the PPI condition were considered as outlying results.

	Water			Coca-Cola®			PPI		
	Gastric C_{max} (mM)	Gastric T_{max} (h)	Gastric $AUC_{0\text{-}4h}$ (mM min)	Gastric C_{max} (mM)	Gastric T_{max} (h)	Gastric $AUC_{0\text{-}4h}$ (mM min)	Gastric C_{max} (mM)	Gastric T_{max} (h)	Gastric $AUC_{0\text{-}4h}$ (mM min)
HV 1	0.59	0.75	36	0.43	0.75	37	0.38*	1*	11*
HV 2	0.16	0.50	2.7	1.3	1.8	30	4.0	0.75	0.15
HV 3	0.004	0.50	0.12	0.032	2.0	0.91	0.060	0.75	1.0
HV 4	0.72	1.0	24	0.40	1.2	24	0	0.50	0
HV 5	1.6	0.25	15	0.40	0.75	13	0.61	0.75	25
Average ± SD	**0.61 ± 0.62**	**0.60 ± 0.28**	**16 ± 15**	**0.51 ± 0.47**	**1.3 ± 0.58**	**21 ± 14**	**1.0 ± 1.7**	**0.75 ± 0.18**	**7.4 ± 11**

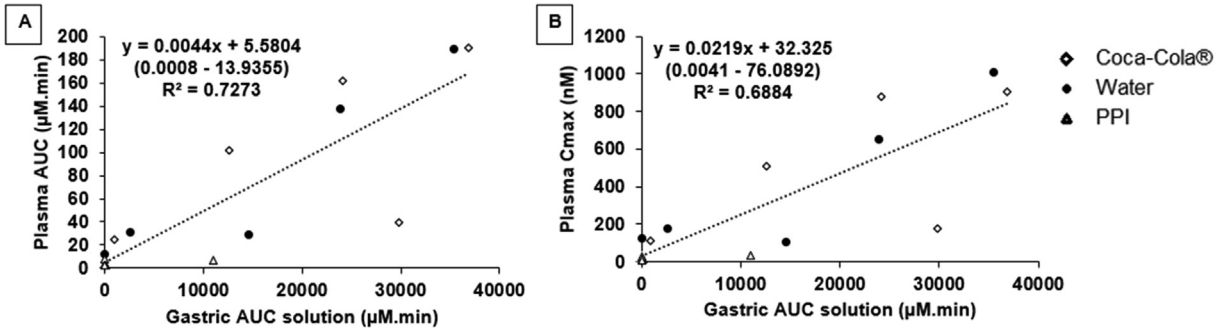

Fig. 5. (A) Plot of plasma AUC as a function of gastric AUC. (B) Plot of plasma C_{max} as a function of gastric AUC. Linear trendlines for both graphs are given by the black dotted line and expressed by the slope and intercept. The Pearson coefficient of determination is expressed as R^2. Standard errors of slope and intercept, respectively, of the linear regression equation are indicated in parentheses. Regression for both plots was significant with $p < 0.05$ (ANOVA).

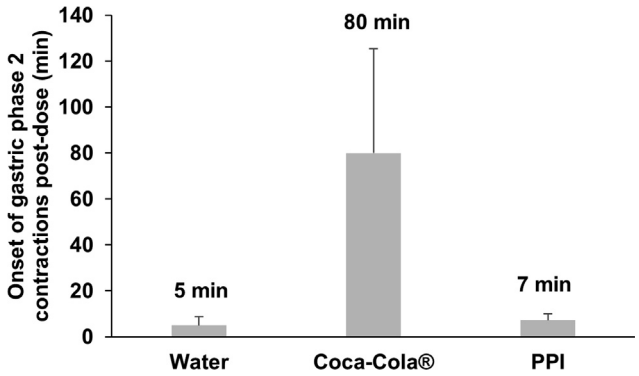

Fig. 6. The onset of gastric MMC phase 2 contractions post-dose for all three test conditions (mean + SD).

drug distribution throughout the stomach can be observed when an immediate-drug product is administered during a period of contractions (MMC phase 2) instead of administering the drug product during a period when no contractions occur (MMC phase 1) [41]. The postponed appearance of these gastric MMC phase 2 contractions after taking atazanavir with a glass of Coca-Cola® supports the longer residence time of atazanavir in the stomach, thereby regulating the gastric emptying process differently compared to the water condition. The length of each contractile phase and the frequency of occurrence for each of the different beverages administered are summarized in Table 3. This table demonstrates the impact of the co-administered beverage on gastric motility, as the co-administered beverage was the only factor that changed among each arm of the study. Due to the delay in the onset of the MMC phase 2 contractile time period, the total duration was considerably shorter after administration of Coca-Cola® compared to the two other test conditions. Exploring the impact of the different durations for each MMC phase may have important consequences on the formed supersaturated concentrations and the precipitation kinetics upon transfer into the upper small intestine.

Measuring of gastric emptying times, based on the obtained concentration-time profiles, for the different test conditions remains extremely challenging in this study as the underlying fluid volumes in the different parts of the GI tract are not known. Simultaneous imaging of the fluid volumes by magnetic resonance imaging (MRI) can serve as a valuable option but remains extremely challenging. A more convenient approach would be to make use of a highly soluble and non-absorbable marker (BCS class 3 compound) that can easily be dissolved in the co-administered beverage, as was done for paromomycin and phenol red in previous aspiration studies [6,42]. After measuring the luminal concentrations of this marker in the aspirated GI fluids, simulations can be performed in physiologically-based pharmacokinetic (PBPK) software packages to isolate the gastric emptying rate that was needed to adequately reflect the gastric and intestinal concentration-time profiles, making use of a relevant residual fluid volumes throughout the different regions of the GI tract. However, since atazanavir is classified as a BCS class 2b compound and administered as a solid dosage form (*i.e.,* hard gelatin capsule), some assumptions need to be made if someone wants to determine the gastric emptying rate of the drug based on the obtained luminal concentration-time profiles. Future experiments should be focusing on biopredictive dissolution experiments to explore the differences in formulation behavior in presence of the different co-administered beverages. In addition to solubility and permeability experiments, the gathered information can serve as valuable data to be used as input for PBPK platforms to simulate the systemic profiles of atazanavir under the different arms of this study. In these workspaces, a parameter sensitivity analysis (PSA) can be performed to evaluate the impact of gastric emptying time on the systemic exposure of atazanavir.'

Table 3
The total length and frequency of contractile MMC phases during each test condition. These calculations are based on a time period of 4 h (equal to 240 min), *i.e.,* the total length of the study starting at the time 0 min when the drug product was administered.

	Water			Coca-Cola®			PPI		
	Total length of phase period (min) – Frequency (#)			Total length of phase period (min) – Frequency (#)			Total length of phase period (min) – Frequency (#)		
	MMC phase 1	MMC phase 2	MMC phase 3	MMC phase 1	MMC phase 2	MMC phase 3	MMC phase 1	MMC phase 2	MMC phase 3
HV 1	18–1	233–2	3–1	52–2	196–3	5–2	23–2	205–3	10–2
HV 2	67–1	173–2	3–1	93–1	62–2	3–1	103–2	135–3	10–2
HV 3	42–2	195–3	6–2*	70–1	167–2	3–1	26–2	203–2	9–2*1
HV 4	27–1	181–1	3–1	186–1	73–1	3–1	34–2	200–3	6–2*1
HV 5	/	240–1	/	154–2	32–2	3–1	139–3	92–3	12–3
Average ± SD	**39–1 (21–1)**	**204–2 (30–1)**	**4–1 (2–1)**	**111–1 (57–1)**	**106–2 (71–1)**	**3–1 (1 ± 0)**	**65–2 (53 ± 0)**	**167–3 (51–0)**	**9–2 (2–0)**

* both MMC phase 3 contractions originated from the upper small intestine.
*1 one of the two MMC phase 3 contractions originated from the upper small intestine.

3.4. Intestinal concentration-time profiles of atazanavir

Upon GI transfer, the dynamic change in pH, fluid composition and volume may affect the intraluminal behavior of a drug tremendously. Especially in the case of weakly basic compounds, this transfer is accompanied by a drop in solubility and possibly the creation of supersaturated concentrations. This supersaturated state can be maintained for a certain period of time, all depending on the physiological circumstances (*e.g.,* pH, secretions and transit) and drug characteristics (*i.e.,* fast versus slow precipitating drugs). Fig. 7 depicts the mean duodenal solution concentrations of atazanavir. The grey background area represents the mean solubility values of atazanavir as measured in the aspirated fluids. When comparing the solution concentrations of atazanavir to the measured solubility values, the degree of supersaturation can be expressed (insert). It should be noted that no statistical difference was observed between the measured solubilities in aspirated duodenal fluids when water or Coca-Cola® was used during dosing, suggesting that the ingredients present in Coca-Cola® having neither a positive nor a negative impact on the solubility of atazanavir in the aspirated duodenal fluids. Nevertheless, prolonged supersaturation was observed for atazanavir after administration of the drug with Coca-Cola® compared to the water condition (3.25 h versus 1.25 h, respectively), which could be explained by the aforementioned differences in gastric emptying. Also, a higher maximal DS was observed for the Coca-Cola® condition compared to the water condition (13 versus 9, respectively). Supersaturated concentrations of atazanavir were negligible under PPI conditions with a maximum DS of 3 for a duration of 15 min. The slower rate of gastric emptying during the Coca-Cola® condition may be beneficial further down in the intestinal tract to promote a more sustained degree of supersaturation. A faster gastric emptying process may indeed lead to an initial higher degree of supersaturation (see Fig. 8).

With respect to the water condition, a fast appearance of maximal concentrations was observed, followed by a gradual decrease in concentrations as a result of precipitation, transit, secretions and mucosal absorption. Based on the difference between total and solution concentrations, a maximum precipitated fraction (π) of 0.5 was observed on average (Fig. 9).

A more delayed appearance of duodenal concentrations was observed after intake of atazanavir with a glass of Coca-Cola® due to a slower gastric transfer resulting in a maximal concentration of 55 μM after 2.25 h. In this case, the maximal precipitated fraction of atazanavir was 0.43 on average. Surprisingly, a maximum solution concentration of 29 μM was observed after intake of atazanavir under PPI conditions, resulting in a limited timeframe of supersaturated concentrations. Nevertheless, the maximum fraction of solid amount (Ψ) of atazanavir that was found in the duodenal aspirates was 0.87 on average indicating that atazanavir was especially present as solid material in the duodenal aspirates. The descriptive parameters of atazanavir's disposition in the duodenum are depicted in Table 4.

Pursuing a fast and high DS has been debated as a non-ideal scenario for an improved systemic outcome [43]. This study confirmed that more sustained supersaturated concentrations of atazanavir for a longer time window (as observed with the Coca-Cola® condition) will, in the end, result in a better performance compared to a fast and high onset of supersaturation (as observed for the water condition). With

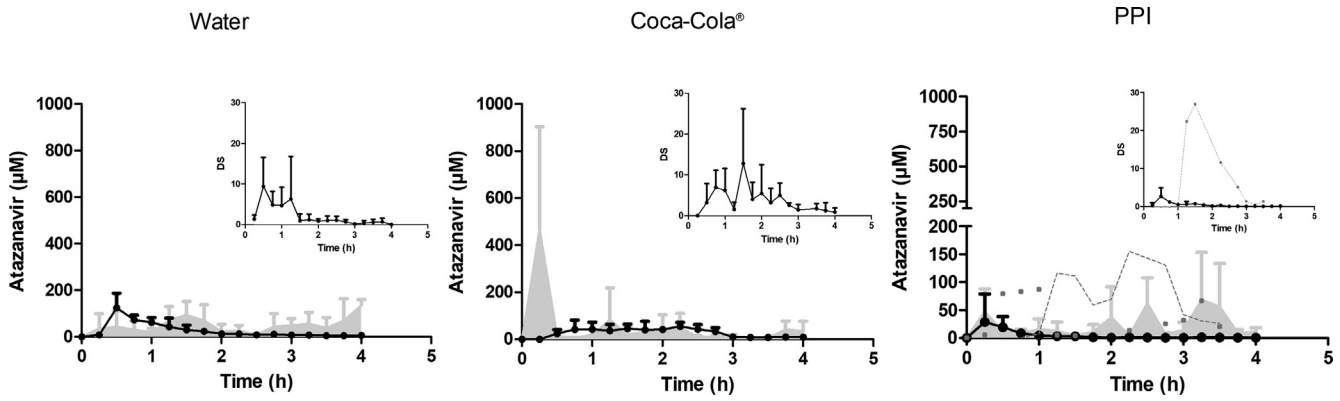

Fig. 7. Mean duodenal concentration-time profiles for dissolved (●) and solubility (grey area) concentrations of atazanavir after oral administration of one capsule of Reyataz® (mean + SEM, n = 4). Inserts represent the mean DS profiles (mean + SD, n = 4). With respect to the PPI condition, the obtained solution (—) and solubility (×) concentration-time profiles of HV 1 could be considered as outlying results and are depicted by the gray line.

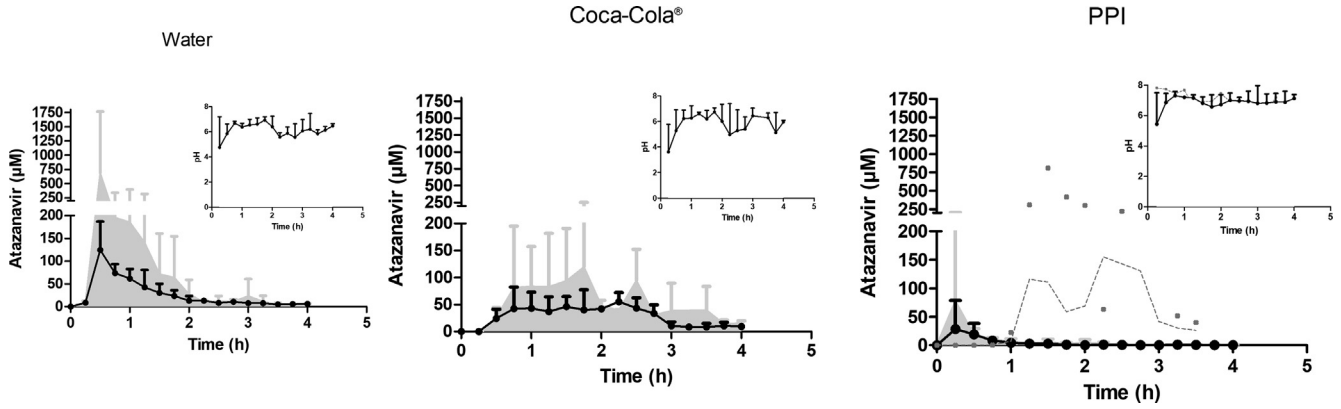

Fig. 8. Mean duodenal concentration-time profiles for dissolved (●) and total (grey area) concentrations of atazanavir after oral administration of one capsule of Reyataz® (mean + SD, n = 4). Mean pH profiles as a function of time are used as an insert in each graph. With respect to the PPI condition, the obtained solution (—) and total (×) concentration-time profiles of HV 1 could be considered as outlying results and are depicted by the gray line.

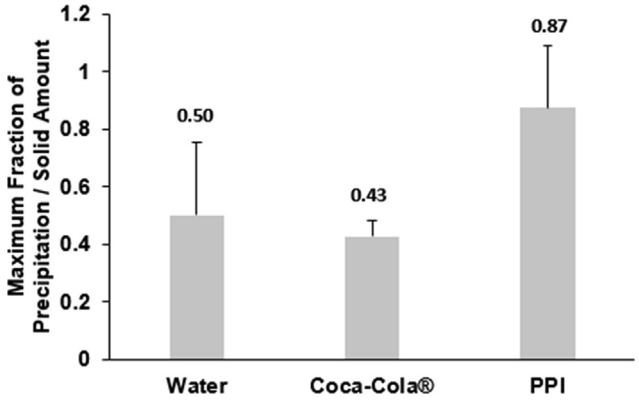

Fig. 9. The maximum precipitated fraction (for the water and Coca-Cola® condition) and the maximum fraction of solid amount (for the PPI condition) of atazanavir in the duodenum after administration of atazanavir under the different intake conditions (mean + SD, n = 4). In the case of the PPI condition, data of HV 1 were not considered due to outlying results.

respect to precipitation, it was observed that the maximum precipitated fraction for atazanavir (0.5) is lower than reported values for posaconazole (0.92) [17], yet higher than seen for ketoconazole (0.16) and dipyridamole (0.07) [23]. Although these compounds were intragastrically infused as solutions, it is justified to make the comparison with atazanavir as the drug was completely dissolved in the stomach after oral administration following a glass of water. However, caution should be taken when interpreting the precipitated fraction of atazanavir as the excipients present in the hard capsule (crospovidone, lactose monohydrate and magnesium stearate as stated in the summary of product characteristics (SmPC) of Reyataz® 150 mg hard capsule) may affect the precipitation of the drug (*i.e.,* accelerate or delay the precipitation kinetics). In the case of PVP, it has already been demonstrated that supersaturated concentrations of etravirine (weak base, pKa 4.5) were longer maintained compared to the control condition when no excipient was included in the dissolution medium (*i.e.,* FaSSIF) [44]. No additional experiments were carried out to investigate the solid-state characteristics of atazanavir being presented as a precipitate in the duodenal fluids. Indulkar *et al.* reported the complex solubilization behavior of atazanavir in the colloidal micelle structures throughout the intestinal tract. Depending on the obtained DS, these solubilization mechanisms are changing tremendously [45]. As this work reports clinically relevant levels of supersaturation for atazanavir in the human GI tract, it can hopefully serve as a reference/guide to further investigate the solid-state characteristics of the precipitate at these relevant luminal concentrations [46].

3.5. Conclusion & Future perspectives

This study aimed to describe the impact of GI physiology on the intraluminal behavior of a weak base under different 'real-life' intake conditions. In detail, the influence of pH and gastric motility was evaluated on the generated concentrations of atazanavir in the stomach and the upper small intestine when the drug was taken with (i) a glass of water, (ii) a glass of Coca-Cola® and (iii) a glass of water under hypochlorhydric conditions (caused by PPI intake). This cross-over study design demonstrated the statistically significant lower systemic exposure of atazanavir under hypochlorhydric conditions compared to the test conditions when the drug product was taken with a glass of water or Coca-Cola®. Intraluminal profiling and gastric motility recordings could contribute to a better understanding of the underlying mechanisms in the human GI tract to explain the observed differences in

Table 4
Descriptive parameters of the solution duodenal concentration-time profiles for each subject in each test condition. The final row depicts the average values and standard deviations. NA: not applicable. *Results of HV 1 in the PPI condition were considered as outlying results.

	Water			Coca-Cola®			PPI		
	Duodenal C_{max} (mM)	Duodenal T_{max} (h)	Duodenal $AUC_{0\text{-}4h}$ (mM min)	Duodenal C_{max} (mM)	Duodenal T_{max} (h)	Duodenal $AUC_{0\text{-}4h}$ (mM min)	Duodenal C_{max} (mM)	Duodenal T_{max} (h)	Duodenal $AUC_{0\text{-}4h}$ (mM min)
HV 1	0.12	0.50	9.0	0.083	0.75	7.8	0.16*	2.2*	13*
HV 2	0.21	0.50	7.2	0.094	1.8	7.0	0.004	1.5	0.28
HV 3	0.092	0.50	5.0	0.044	2.2	3.2	0.017	0.50	0.85
HV 4	NA	NA	NA	NA	NA	NA	NA	NA	NA
HV 5	0.081	0.75	4.4	0.071	1.0	6.8	0.086	1.5	1.6
Average ± SD	**0.12 ± 0.058**	**0.56 ± 0.12**	**6.4 ± 2.1**	**0.073 ± 0.021**	**1.4 ± 0.68**	**6.2 ± 2.0**	**0.067 ± 0.072**	**1.4 ± 0.70**	**3.9 ± 6.1**

systemic outcome. Firstly, the acidic gastric pH in combination with the gastric residence time played a pivotal role in the behavior of atazanavir along the GI tract. Secondly, the observed delay in gastric emptying (likely caused by the present calories in Coca-Cola®) resulted in more sustained supersaturated concentrations along the intestinal tract, which are more preferable and beneficial in order to increase the oral bioavailability. In contrast, a faster gastric emptying resulted in an initial higher DS, however, for a shorter period of time and not leading to an increase in systemic exposure compared to the Coca-Cola® condition. It was seen that a fast gastric emptying rate (coordinated by the MMC phase 2 contractions) tends to lead not only to a higher maximum drug concentration in the upper small intestine, but also to an earlier onset of precipitation combined with a faster decrease of drug concentration. Therefore, drug precipitation in the human GI tract is assumed to be more likely when the drug is rapidly emptied from the stomach. In contrast, slow gastric emptying rates lead to a slow drug arrival in the upper small intestine. Because of concurrent drug absorption across the intestinal membrane, precipitation may be slower in this case. Related to the PPI condition, it can be concluded that the gastric pH plays a pivotal role in the dissolution of a weak base in order to generate sufficiently high concentrations further on in the intestinal tract for a sufficient period of time. If these conditions are met, a beneficial driving force for intestinal absorption can be established. From a mechanistic point of view, it would be interesting to explore each ingredient of Coca-Cola® (*e.g.,* phosphoric acid, sugar, CO_2) separately in order to observe the specific impact of each ingredient on atazanavir's disposition in the GI tract and systemic circulation. To the best of our knowledge, only the impact of pH on drug and formulation behavior has been explored, so far, using posaconazole as a model drug [14,17,47]. The difference in pH has a significant impact on the obtained duodenal concentrations and, subsequently, on the systemic outcome of the drug. More clinical studies should be performed to evaluate the contribution of sugar and/or CO_2 on the disposition of a weakly basic drug to discriminate which factor has the most impact on the disposition of the drug.

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