The AAPS Journal (2018) 20:57
DOI: 10.1208/s12248-018-0217-6

Research Article

Measuring the Impact of Gastrointestinal Variables on the Systemic Outcome of Two Suspensions of Posaconazole by a PBPK Model

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Received 11 January 2018; accepted 7 March 2018

Abstract. For the last two decades, the application of physiologically based pharmacokinetic (PBPK) models has grown exponentially in the field of oral absorption and in a regulatory context. Although these models are widely used, their predictive power should be validated and optimized in order to rely on these models and to know exactly what is going on "under the hood". In this study, an automated sensitivity analysis (ASA) was performed for 11 gastrointestinal (GI) variables that are integrated into the PBPK software program Simcyp®. The model of interest was a previously validated workspace that was able to predict the intraluminal and systemic behavior of two different suspensions of posaconazole in the Simcyp® Simulator. The sensitivity of the following GI parameters was evaluated in this model: gastric and duodenal pH, gastric and duodenal bicarbonate concentrations (reflecting buffer capacity), duodenal bile salts concentration, gastric emptying, the interdigestive migrating motor complex (IMMC), small intestinal transit time (SITT), gastric and jejunal volumes, and permeability. The most sensitive parameters were gastric/duodenal pH and gastric emptying, for both suspensions. The outcome of the sensitivity analyses highlights the important GI variables that must be integrated into an *in vivo* predictive dissolution test to help and create a rational and scientific framework/design for product development of novel and generic drug products.

KEY WORDS: bioequivalence; dissolution; modeling; oral absorption; pharmacometrics; physiologically based pharmacokinetics; precipitation; simulations; supersaturation.

INTRODUCTION

Regarding the biowaiver concept, *in vivo* bioequivalence studies can be replaced by USP 1 and 2 dissolution experiments in the case of immediate-release (IR) dosage forms of BCS classes 1 and 3 drugs in pharmacopeial buffers, ranging from pH 1.2 to 6.8 (1–4). Predicting the *in vivo* performance of biopharmaceutics classification system (BCS) class 2/4 compounds still remains a challenge: not only because of the properties of the drug but even more because of the drug product. The formulations for these compounds are designed with the aim to resolve their poorly soluble properties through solubilization and/or supersaturation (5). As the underlying gastrointestinal (GI) physiology may have a significant impact on the release of the drug and thus on the systemic exposure, it is needless to say that these GI variables should be implemented into an *in vivo* predictive dissolution (iPD) device (6). Ongoing projects in Europe (IMI/EFPIA-funded program; OrBiTo) and USA (FDA-funded project; Advancing twenty-first century Bioequivalence Science) are pursuing goals to optimize the predictive power of biopharmaceutical *in vitro* tools in R&D of novel and generic drug products (7,8).

Besides *in vitro* tools, a lot of attention goes out to physiologically based pharmacokinetic (PBPK) models (9). To date, many R&D companies focus on applying an accurate PBPK model that predicts the *in vivo* outcome of new drug candidates in an early stage of drug discovery and development. These models will allow the prediction of human plasma concentration-time profiles based on physicochemical and *in vitro* drug and formulation data (e.g., dissolution profiles), prior to *in vivo* testing in clinical trials, thereby

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Published online: 29 March 2018

preventing clinical failures at an early stage. However, their predictive power should be validated and optimized in order to rely on these models. The increasing importance of PBPK modeling in drug development has been rising in an almost exponential manner, as depicted in Fig. 1.

PBPK modeling has been frequently applied in a regulatory context. The European Medicines Agency (EMA) has recently released a guideline that describes the expected content of PBPK modeling and simulation reports included in the regulatory submission (10): "A growing number of regulatory submissions include physiologically-based pharmacokinetic (PBPK) models that require the use of specialized software platforms. If PBPK modeling is intended to support a regulatory decision, the PBPK platform needs to be qualified for the intended use and the predictive performance of the specific drug models needs to be evaluated. It is expected that the extent of use of PBPK modeling will expand as additional system knowledge is gained and confidence increases."

In this way, PBPK modeling opens perspectives as a potential application for requesting biowaivers from regulatory agencies (8). This was already successfully demonstrated for etoricoxib (weak base; pK_a 4.6), where two different batches showed to be bioequivalent after input of both dissolution profiles into an *in silico* absorption model (11). This also implies that a reliable and validated PBPK model will help us to reveal which underlying physiological variables can be highly responsible for potential failures in *in vivo* BE studies by looking at the sensitivity of the different implemented GI variables on systemic exposure of the drug. Recently, the PBPK modeling and simulation tool Simcyp® was used to simulate GI and systemic concentration—time profiles of posaconazole (BCS class 2b drug (12)), which were directly compared with intraluminal and systemic data measured in humans (13). This model was able to evaluate GI dissolution, supersaturation, and precipitation of posaconazole, formulated as an acidified (pH 1.6) and neutral (pH 7.1) suspension. Based on this evaluation, we would like to highlight in this manuscript which GI variables are highly sensitive towards the systemic exposure of posaconazole and are thus indispensable to adequately implement into an *in vivo* predictive dissolution device to generate reliable dissolution profiles as input for PBPK software programs. The sensitivity of the following 11 GI variables was explored in the Simcyp® model: gastric and duodenal pH, gastric and duodenal bicarbonate concentrations (reflecting buffer capacity), duodenal bile salts concentration, gastric emptying, the interdigestive migrating motor complex (IMMC), small intestinal transit time (SITT), gastric and jejunal volumes, and permeability.

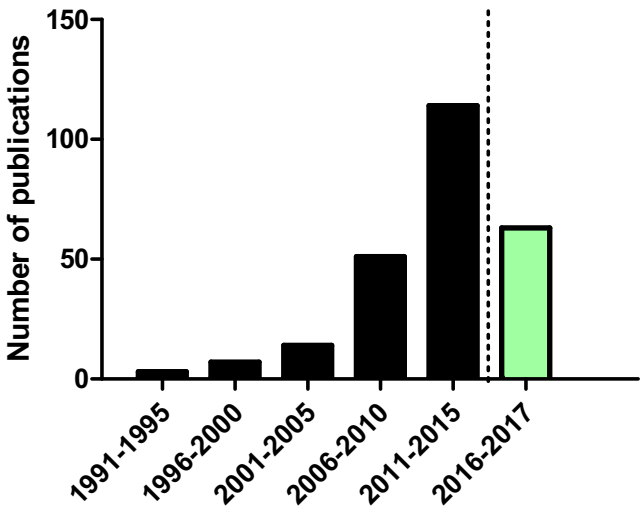

Fig. 1. Number of publications per 5 years in the ISI Web of Knowledge® database mapping to the topics "PBPK modeling" and "oral Absorption." The green bar represents the number of publication since 2016 until now (last accessed on 10 Oct 2017)

MATERIALS AND METHODS

Simcyp® Population-Based PBPK ADME Simulator

The PBPK modeling tool Simcyp® was recently explored by Hens *et al.* to simulate intraluminal and systemic concentrations of posaconazole, formulated as an acidified (pH 1.6; 40 mg dose) and neutral (pH 7.1; 40 mg dose) suspension (13). The predictive performance was judged based on the observed *in vivo* data for both suspensions of posaconazole, as examined in healthy volunteers in a previous cross-over study. The Advanced Dissolution Absorption and Metabolism (ADAM) model, implemented within the Simcyp® population-based simulator, was applied using physicochemical and disposition parameters of posaconazole (derived from literature data) as well as population and trial design properties matching the *in vivo* study (derived from Hens *et al.*) (13). A virtual population of five healthy volunteers was selected in the Simcyp® Simulator to reflect the clinical study. Twenty virtual trials were performed with five healthy volunteers each to reassemble the estimates of variability under standard fasting state conditions. For more details, authors would like to refer the reader to the relevant manuscript. Sensitivity analyses were performed in the Simcyp® Simulator (version 16, Simcyp Ltd., Sheffield, UK—licensed to the University of Michigan) for 11 GI variables that are integrated into the model: an automated ten-step sensitivity analysis (ASA) was performed for each variable keeping all other variables constant in the Simcyp® Simulator. The 11 GI variables of interest were gastric and duodenal pH, gastric and duodenal bicarbonate concentrations (reflecting buffer capacity), duodenal bile salts concentration, gastric emptying, the IMMC, SITT, gastric and jejunal volumes, and permeability. The standard values as applied in the simulator for both formulations are shown in Table I.

In studies to determine BE after a single dose, the parameters to be analyzed are AUC_{0-t} and C_{max} (1). For these parameters, the 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80.00–125.00%. Therefore, the sensitivity of the model parameters was expressed towards the plasma C_{max}, T_{max}, and $AUC_{0-8\ h}$. A sensitivity factor was calculated for each systemic parameter by the following equation:

$$Sensitivity\ factor = \frac{(Maximum\ value - Minimum\ value)}{Maximum\ value} \quad (1)$$

Table I. Overview of the Selected GI Variables to Explore for Their Sensitivity in this Study

GI variable	Standard value		Reference
	Acidic suspension	Neutral suspension	
Gastric pH	2.34	3.28	Arithmetic averages across all 5 individuals of gastric pH values measured at different times over the duration of the clinical studies (14).
Duodenal pH	5.97	6.37	Arithmetic averages across all 5 individuals of duodenal pH values measured at different times over the duration of the clinical studies (14).
Gastric bicarbonate (mM)	7.3	7.3	Standard values as applied in the Simcyp® Simulator.
Duodenal bicarbonate (mM)	6.5	6.5	Standard values as applied in the Simcyp® Simulator.
Duodenal bile salts concentration (mM)	3.3	3.3	Standard values as applied in the Simcyp® Simulator.
Gastric emptying (h)	0.175	0.175	(15)
IMMC (h)	1.55	1.55	Standard values as applied in the Simcyp® Simulator.
SITT (h)	3.6	3.6	Standard values as applied in the Simcyp® Simulator.
Gastric volume (mL)	50	50	Standard values as applied in the Simcyp® Simulator.
Jejunal volume (mL)	21.1	21.1	Standard values as applied in the Simcyp® Simulator.
Intestinal permeability ($\times 10^{-4}$ cm/s)	6.4	6.4	Predicted using built-in P_{app} to P_{eff} correlations (Simcyp® Simulator, version 15, release 1); calibration compounds were not available.

Standard values as applied in the simulation to predict the plasma profiles for both suspensions are demonstrated (13)

where the maximum value refers to the highest predicted plasma $AUC_{0-8\ h}$ or C_{max} or T_{max} after performing a sensitivity analysis for a certain GI parameter. The minimum value refers to the lowest predicted plasma $AUC_{0-8\ h}$ or C_{max} or T_{max} after performing a sensitivity analysis for a certain GI parameter. These maximum and minimum values of the predicted systemic parameters (plasma $AUC_{0-8\ h}$, C_{max}, or T_{max}) were only extracted from a physiological range, based on literature data, as the ten-step ASA may predict the systemic exposure of posaconazole for an irrelevant value (e.g., gastric pH value of 15). The physiological range will be indicated on the graph by a green box. A sensitivity value of "0" means that no sensitivity was observed for the selected GI variable whereas a value of "1" means that a maximum sensitivity is observed. The higher the value, the more sensitive. Afterwards, a comparison can be put forward to assess the sensitivity of each underlying physiological parameter on the systemic outcome of posaconazole, for each suspension.

Assessment of the Solubility of Posaconazole

The solubility of posaconazole was determined as a function of bile salt and lecithin concentrations (SIF powder; Biorelevant, Croydon, UK). Briefly, three different fasted state simulated intestinal fluids (FaSSIF) were prepared by adding different amounts of SIF powder to FaSSIF blank buffer (pH 6.5). Final concentrations of taurocholate in the media were 6, 9, and 12 mM, respectively. An excess of posaconazole (Noxafil®; Merck Sharp & Dohme Corp., Kenilworth, NJ, USA) was added to these media, and samples were incubated for 24 h at a temperature of 37 °C. Subsequently, samples were centrifuged for 15 min at a speed of 17,000×*g* (AccuSpin Micro 17, Fisher Scientific, Pittsburgh, PA). The supernatant was 1:1 diluted with methanol and ready to be analyzed by HPLC. Samples were analyzed for posaconazole by HPLC-UV (Hewlett Packard series 1100 HPLC Pump combined with Agilent Technologies 1200 Series Autosampler). A volume of 100 μL was injected into the HPLC system connected to a UV lamp that was able to detect posaconazole at a wavelength of 254 nm (Agilent 1100 Series UV lamp). A gradient run with a mixture of acetonitrile and purified water (both containing 0.1% TFA) was used to detect posaconazole at a retention time of 8.9 min using a C-18 column (Kinetex C18 HPLC column, 250 × 4.60 mm − 5 μm, Phenomenex, Golden, CO) and a 1-mL/min flow rate. Calibration curves were made in mobile phase based on a stock solution of posaconazole in methanol (7 mM). Linearity was observed between 50 μM and 39 nM. The observed peaks were integrated using ChemStation software (Agilent Technologies). The developed analytical method met the FDA requirements for bioanalytical method validation (16). Solubility experiments were performed in triplicate.

RESULTS AND DISCUSSION

Mean Plasma Concentration-Time Profile and 95th and 5th Percentiles

Figure 2a, b depicts the simulated mean plasma concentration-time profile after administration of both suspensions, respectively, and the 95th and 5th percentiles of a virtual population of 100 healthy volunteers using the standard fasting state conditions in Simcyp®. Below these values of percentiles, 95 and 5% of the observations can be found. From this point on, it is interesting to explore which underlying GI variable may explain the upper/lower limit values in systemic exposure which will be widely discussed below. The simulated profiles slightly overpredicted the plasma concentrations at early time points. It would have been interesting to collect blood samples in shorter time

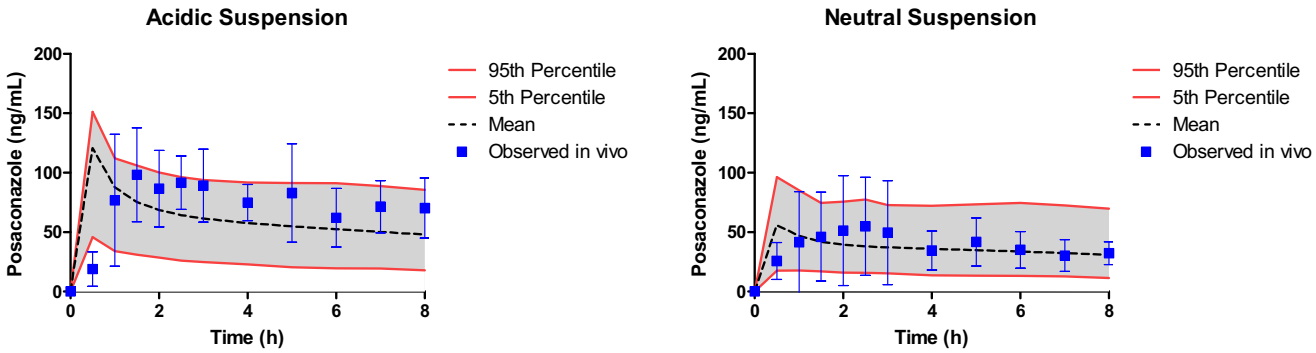

Fig. 2. Plot of the simulated mean plasma concentration-time profile and 95th and 5th percentiles of the total population for the acidic (**a**) and neutral suspension (**b**) after administration to a virtual population of 100 healthy volunteers. The blue squares represent the mean systemic concentrations (mean ± SD; $n = 5$)

periods (e.g., every 15 min) especially in the first 3 h of the study to have a better view on the initial absorption phase.

ASA of Gastric and Duodenal pH

Figure 3 depicts the ASA that was performed for the gastric and duodenal pH for both formulations. The green box represents the physiological range of pH values as observed during the clinical aspiration studies of posaconazole (14,17).

For ionizable compounds, the present pH along the GI tract will determine the fraction dissolved of the drug and thus the fraction available for intestinal absorption. The ASA that was performed for posaconazole demonstrates the enormous impact of pH on systemic exposure. Walravens *et al.* explored the intraluminal and systemic behavior of posaconazole after oral

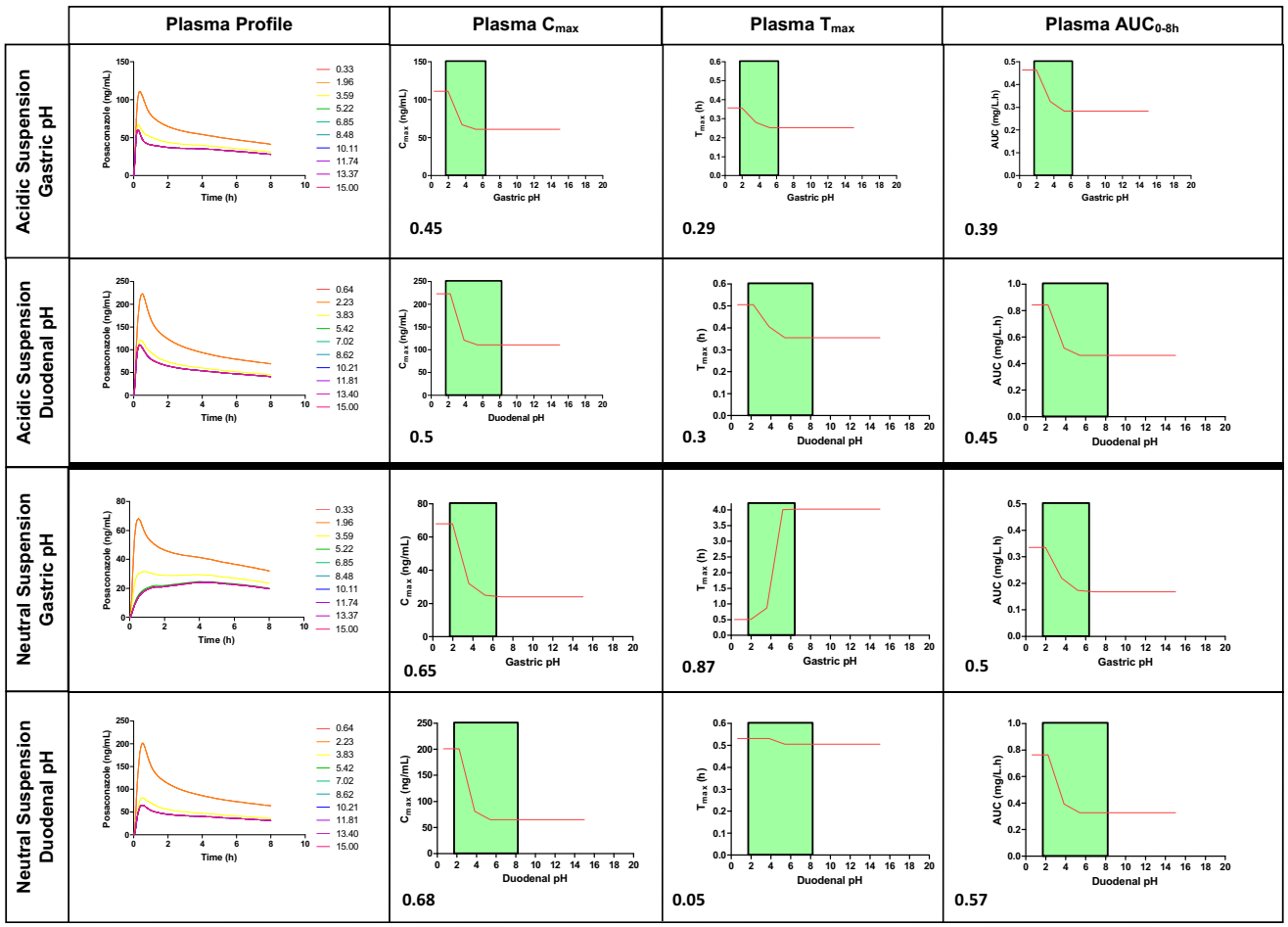

Fig. 3. ASA performed for gastric and duodenal pH in the Simcyp Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of gastric and duodenal pH based on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

administration of a 400-mg therapeutic dose to healthy volunteers in different gastric scenarios by modifying the gastric pH with the help of co-medication (proton pump inhibitors) and beverages (Coca-Cola®) (18). Authors found a clear correlation (Spearman $r = 0.82$; $p < 0.0001$) between the measured gastric concentrations the systemic concentrations of posaconazole, showing that the amount of dissolved posaconazole in the stomach is crucial for the amount of posaconazole that will be absorbed. It was hypothesized that, after intake of Coca-Cola® (pH 2.5), the combination of a favorable gastric pH and prolonged gastric residence time due to the caloric content of the beverage (139 kcal) resulted in elevated gastric and duodenal concentrations, enhancing the intestinal absorption of posaconazole. Another independent study of Cristofoletti *et al.* confirmed the impact of gastric pH on the fraction absorbed of posaconazole and the other weakly basic drug ketoconazole by performing a sensitivity analysis (19). The formulation dependent effect of achlorhydria was also demonstrated by Doki and colleagues for the drug compounds nifedipine and levothyroxine in a virtual BE study (20). It should be noted that the pH of the GI tract is tremendously variable and dynamic due to the low buffer capacity of the fluids (21,22). This was observed for fluids that were aspirated after oral intake of an 800-mg dose of ibuprofen but also observed for fluids that were aspirated in aspiration studies of posaconazole after single-dose administration of the weak base posaconazole as a solution, an acidic/neutral suspension and a solid dispersion tablet (14,17,21). Although the intestinal pH can be extremely variable, the pH along the intestinal tracts remains, in general, neutral (pH 6–7). It has been demonstrated in the 1970s that intestinal secretions are maximal during phase III contractions, the so-called motility-induced intestinal secretions or "the secretomotor complex," in order to sufficiently titrate/neutralize the incoming gastric fluids with bicarbonate (23–25). Figure 4 depicts the impact of gastric pH on the solubility of posaconazole as observed for five healthy volunteers, after oral administration of the posaconazole solid dispersion tablet in fed state conditions (14,17).

The solubility of posaconazole is initially low, due to elevated pH in the stomach after oral administration of the liquid meal (Ensure Plus®; pH 6.6). After digestion and gastric emptying of the meal, pH values return to the initial and typical fasted state values which are more acidic (pH 1–2). Based on the sensitivity factors that are depicted in the left corner below each graph in Fig. 3, the impact of pH is more important for the neutral suspension compared with the acidic suspension. The reason why the acidic suspension is less affected by pH compared with the neutral suspension is because of the predissolved amount of posaconazole initially present in the formulation: the neutral suspension is more sensitive to changes in solution concentrations because of its lower predissolved state (2.3%) whereas the predissolved amount of posaconazole in the acidic suspension is already 70% and is, therefore, less sensitive to a dynamic drop in pH.

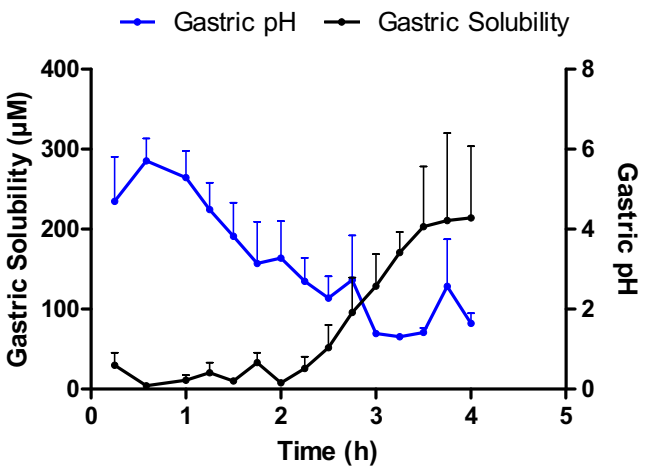

Fig. 4. Thermodynamic solubility of posaconazole measured in the aspirated fluids of the stomach after oral administration of the tablet in fed state conditions. Data are presented as mean + SEM ($n = 4$) and obtained from Hens *et al.* (17)

Due to pH difference between stomach and small intestine, precipitation will logically occur. Extensive precipitation upon entry the upper small intestine was observed for posaconazole after administration of solution/suspensions in humans and was also reflected in the simulated duodenal profiles by Simcyp®. In general, precipitation behavior requires further characterization, particularly in relation to their possible time dependence (e.g., pH, bile salt concentrations). Integration of such information into PBPK models is expected to improve simulations. Therefore, in these simulations, redissolution of posaconazole was not considered (Fig. 5).

The negative dissolution rate in the first time period reflects the ongoing precipitation process that decreases after approximately 5 min and stops around 1.25 h. Although redissolution was not taken into account, the simulated intraluminal and systemic profiles were in line with the observed *in vivo* data. Additional characterization of the solid state of a precipitate should be considered in order to give any definite answer about redissolution of posaconazole happening or not (26).

ASA of Gastric and Duodenal Bicarbonate

The buffer present in the GI tract to regulate pH is bicarbonate. The dynamic change in buffer capacity along the intestinal tract is due to the available CO_2 concentrations in the GI fluids, both in solution ($CO_{2(aq)}$) and the luminal gas phase ($CO_{2(g)}$) as these concentrations directly determine the formation of bicarbonate (27,28). Reported values in the literature of bicarbonate concentrations measured in the upper small intestine of humans ranged from 4 to 21 mM with an average of 15 mM (29). Values measured in the stomach range from 9 to 20 mM (29). The standard values in the Simcyp® Simulator for gastric and duodenal bicarbonate concentrations are 7.3 and 6.5 mM, respectively. A sensitivity analysis was performed on both parameters for the two suspensions. Results are depicted in Fig. 6. The green box represents the physiological range of bicarbonate concentrations as observed in healthy humans using an intubation technique (29,30).

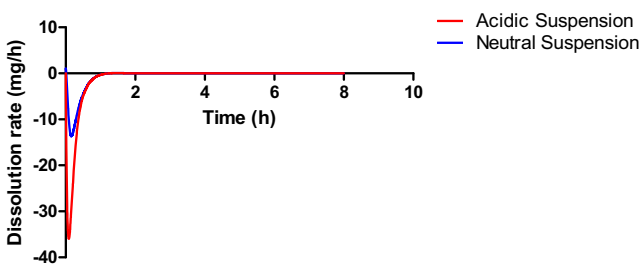

Fig. 5. Simulated dissolution rate for both suspensions as a function of time in the duodenal segment of the Simcyp® Simulator

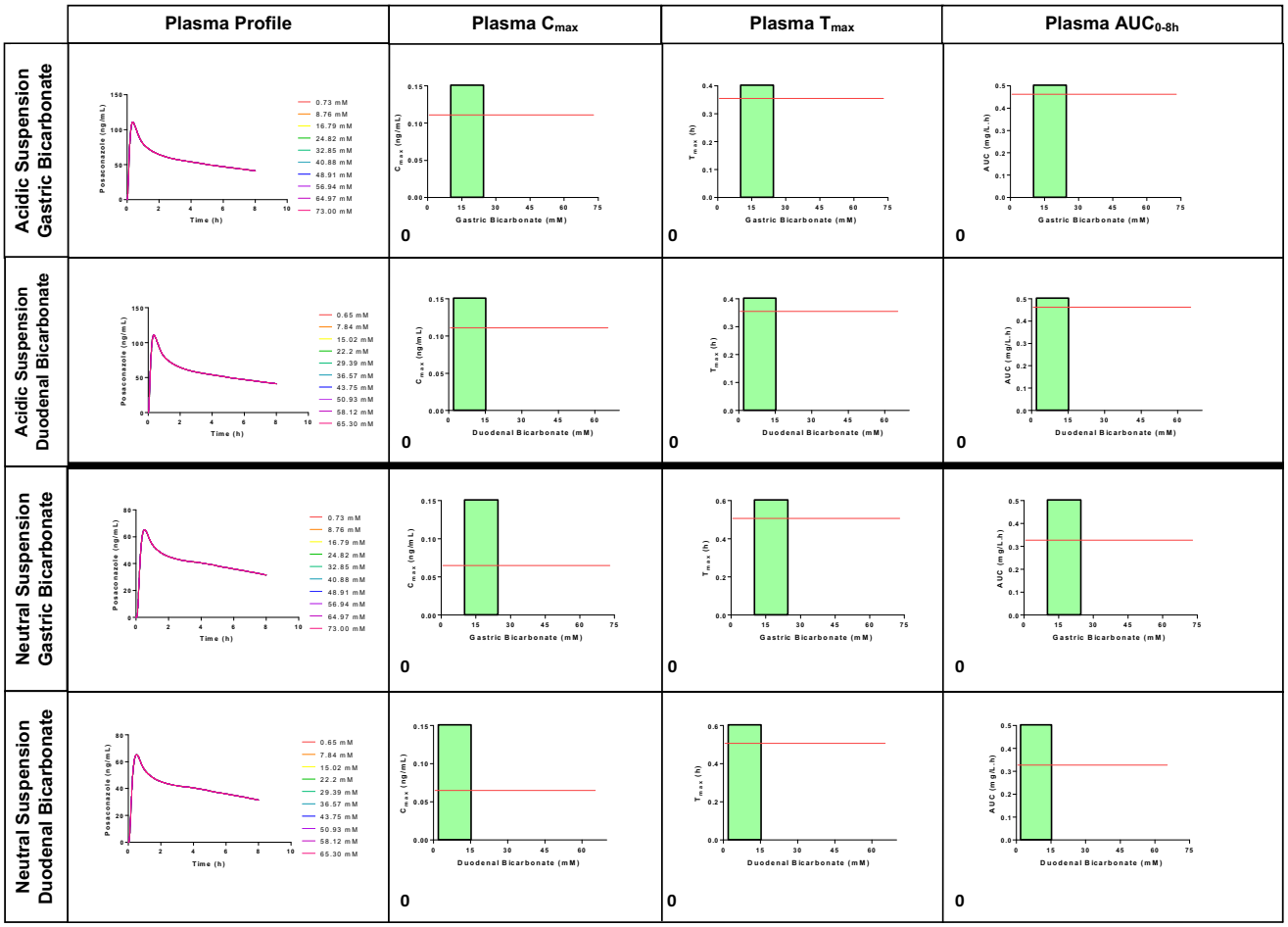

Fig. 6. ASA performed for gastric and duodenal bicarbonate concentrations in the Simcyp Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of gastric and duodenal bicarbonate based on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

Remarkably, there was no effect observed on the systemic outcome of posaconazole when the bicarbonate concentrations were varied over a well-defined physiological range. As recently demonstrated, the reason for the dynamic change in pH can be explained by the low buffer capacity of the GI fluids (21). In addition, one should not ignore the possibility of a change of the pH in the small intestine during the dissolution of a drug molecule: if the drug compound is supported with acidic/basic properties, an extra drop or rise in pH can be observed, influencing the drug available for intestinal absorption (21,22). This will be definitely more relevant in the case of high administered doses, but in this case, related to the low administered dose of posaconazole in this study (40 mg), this effect will be negligible. Regarding the low intrinsic solubility of posaconazole (0.000981 mg/mL), the dissolution of the drug is regulated by the bulk pH (pH 6–7) (13). Whenever the buffer capacity of the intestinal fluids would drop, the free base is hardly present and the dissolution rate will significantly increase due to the presence of the ionized form. Obtained information regarding intrinsic solubility, pK_a value(s), and diffusion coefficients, can accurately describe the dissolution rate as a function of the bulk solution pH, as demonstrated by Mooney and colleagues in 1981 for a series of ionized drug compounds (31). Related to *in vitro* dissolution studies, different research groups demonstrated the added value of using bicarbonate buffers instead of phosphate buffers (29,32). For example, physiological bicarbonate buffers proved to be more discriminative of the drug release behavior of enteric-coated formulations for ileocolonic delivery, resulting in better reflections of *in vivo* disintegration-dissolution times than observed for conventional phosphate buffers (33,34). To conclude, although the impact of pH on systemic outcome was successfully captured by the PBPK software, the interplay/connection with the buffer capacity remains unanswered as no sensitivity was observed for this parameter. Therefore, the interplay between pH and buffer capacity should be revised and taken into account whenever a sensitivity analysis is carried out. The report of an FDA public workshop resulted in a couple of panel questions that suggested that the interplay or correlation between parameters should be considered/revised (35).

ASA of Duodenal Bile Salts Concentration

The observed range of bile salts concentrations in the duodenum can be highly variable (0.03–36.18 mM) in the fasted state (36). The standard value of duodenal bile salts concentration in the Simcyp® simulator is 3.3 mM, in line with the bile salts concentration as used for biorelevant dissolution media (37). Whenever the bile salts concentration would be extremely high *in vivo* (based on the observed range), more unionized drug will be solubilized, resulting in an increased driving force for intestinal absorption of posaconazole (Fig. 7).

Especially for posaconazole (cLogP 4.6), an enormous amount of precipitated drug could be solubilized in the intestinal tract, leading to enhanced oral drug absorption and, thus, a higher systemic exposure. Based on the ASA that was performed in Simcyp® for duodenal bile salts, the same phenomenon was adequately reflected in the simulated outcomes for both suspensions (Fig. 8). The green box represents the physiological range of duodenal bile salts as observed in healthy humans (36,38).

The standard values in the Simcyp® Simulator that were implemented for solubility in fasted state simulated intestinal fluid (FaSSIF; pH 6.5) and pH 7.0 buffer media differed by a factor of 2.8 (0.0028 *versus* 0.00098 mg/mL, respectively). In this way, the effect of bile salts is taken into account by the simulator. This is likely the reason why it is advised to administer Noxafil® oral suspension to patients in fed state conditions as higher systemic concentrations will be observed (39,40). Solubility values that were measured in the aspirated jejunal samples of healthy volunteers after administration of the tablet and fasted and fed state conditions showed that solubility of posaconazole was higher in the fed state aspirated samples compared with the solubility measured in fasted state aspirated samples of the jejunum, most likely due to the higher abundance of bile salts that are present in postprandial conditions (Fig. 9).

ASA of Gastric Emptying and the Interdigestive Migrating Motor Complex

The rate of gastric emptying will determine how fast the gastric content will be transferred from stomach to small intestine. Gastric emptying is implemented in the simulator software as a first-order process. The gastric emptying time (GET) as described in the software code of the program can easily be converted to the gastric half-life ($t_{1/2,G}$; i.e., the time needed to remove half of the stomach content) by the following equation:

$$t_{1/2,G} = GET \times ln2 \quad (2)$$

The impact of different gastric emptying rates on precipitation kinetics of poorly soluble weak bases has already been investigated

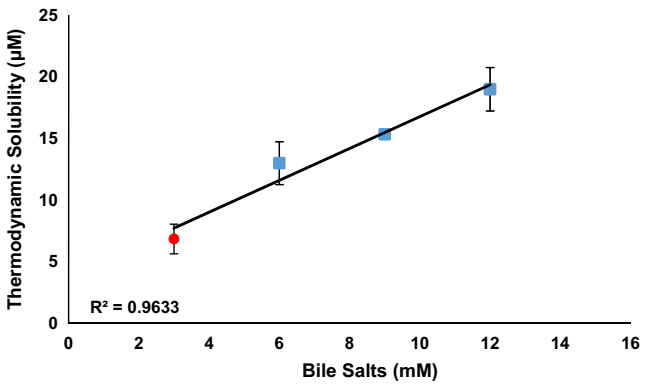

Fig. 7. Thermodynamic solubility of posaconazole (determined by the shake-flask method) as a function of bile salt concentration in three different FaSSIF media (blue squares; mean ± SD). To compare, thermodynamic solubility of posaconazole in "normal" FaSSIF was added to the figure presented by the red dot as mean ± SD. This value (red dot) was obtained from the literature (38)

by Kostewicz and co-workers (41). The authors concluded that a fast gastric emptying rate tends to lead not only to a higher maximum drug concentration in the intestine but also to an earlier onset of precipitation combined with a faster decrease in drug concentration. It was hypothesized that *in vivo* drug precipitation will be more pronounced when the drug is quickly delivered from the stomach into the intestine. Recent research work clearly demonstrated that, besides gastric emptying, also the physico-chemical properties of the drug compound are a major source of explaining delayed/accelerated precipitation behavior (14,42). Gastric emptying of liquids occurs to be extremely fast, ranging in a gastric half-life between 4 and 13 min (15). In contrast, the gastric residence time of solid formulations is prolonged as a disintegration of the formulation is required to easily pass the pylorus (< 1–2 mm) (43). The impact of different rates of gastric emptying on systemic exposure of posaconazole can be observed by the ASA as depicted in Fig. 10. The green box represents the physiological range of gastric emptying times (measured by an intubation technique) and duration times of the IMMC (measured by manometry) as observed in healthy humans (15,44). Gastric emptying times were based on values obtained from healthy volunteers after oral administration of a non-caloric, low-osmotic solution of the non-absorbable marker paromomycin.

These results suggest that rapid emptying from the stomach is favorable for the acidic suspension, as this will result in higher intestinal concentrations to promote faster intestinal absorption; the faster the drug will be transferred from the stomach to the small intestine, the faster the onset of intestinal absorption resulting in an increased systemic exposure. This clearly illustrates how the absorption phase will be tremendously influenced by how fast the drug will be emptied from the stomach. This was not seen for the neutral suspension. The reason for this phenomenon is presumably due to the higher predissolved state of the drug in the stomach for the acidified suspension compared with the lower predissolved state for the neutral suspension; for the neutral suspension, differences in gastric emptying rates seem to have no major impact on systemic exposure. Obviously, a delayed gastric emptying will result in a delayed plasma T_{max} as seen for many drugs in the fed state where the presence of a meal will delay gastric emptying in order to give the small intestine the chance to adequately digest the incoming food (43). Remarkably, no sensitivity was observed for the IMMC. In fasting state conditions, the GI muscular system contracts with a cyclical periodic IMMC, in which the phases of quiescence (phase I) are followed up by a phase of intermittent contractions (phase II) and a final phase of very strong contractions (phase III) resulting in a motility-varying gastric emptying rate (45,46). On average, the IMMC cycle lasts approximately 90–120 min but is highly variable, ranging between 15 min and 3 h (47–50). The mechanisms of motility-driven gastric emptying for liquids has been adequately evaluated in humans by Oberle *et al.* (44). Authors investigated the influence of the IMMC on gastric emptying of 50 and 200 mL volumes of phenol red solution. It was concluded that smaller volumes (50 mL) are more depending on motility to be emptied from the stomach than larger volumes (200 mL). However, for the 200-mL volume of phenol red solution, no difference in emptying rate was observed between phases I and II, but the emptying rate was significantly greater during late phase II/III, which will highly affect the intestinal absorption and onset of therapeutic response in humans. Based on this study, no differences in systemic outcome of posaconazole were observed for different duration times of the IMMC,

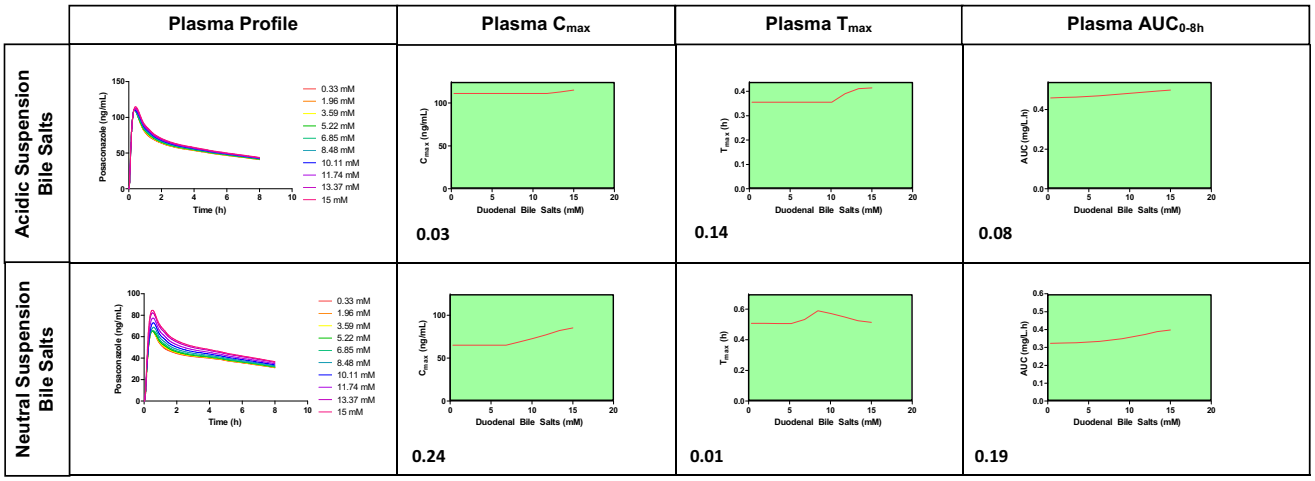

Fig. 8. ASA performed for duodenal bile salts concentration in the Simcyp® Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of duodenal bile salts concentration based on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

regardless of formulation. The impact of the IMMC may be even more highlighted after oral administration of a solid dosage form (Noxafil® delayed-release tablet). As observed for an immediate release dosage form of ibuprofen, a statistically significant ($p < 0.05$) linear regression was observed between plasma C_{max} and the time when phase III contractions appeared post-dose (21). This strong burst of phase III contractions, also known as "the house-keeper wave," will remove a majority of drug content to the small intestine, to be available for intestinal absorption. Besides the link between gastric emptying and the IMMC, a clear link has also been observed between the IMMC and duodenal secretions: pancreatic enzyme and bile secretion peak in the late phase II contractions (emptying of approximately 25% of gallbladder contents), whereas gastric acid and bicarbonate secretion into the duodenum peak during the initiation of phase III contractions (51–56). This interplay between phase II/III contractions and simultaneous ongoing duodenal secretions will increase duodenal pH and bile salts level, affecting the dissolution and absorption of a drug tremendously (21).

In summary, just as observed for pH and buffer capacity, an artifact can be noticed between gastric emptying time and IMMC, which should be revised. Generally speaking, future studies should focus on the impact of motility as this variable is still poorly explored in our field but definitely important and extremely crucial.

ASA of Small Intestinal Transit Time

The passage through and residence time in the intestinal tract plays a pivotal role in the oral drug absorption as this is the time window that a drug will be presented to the absorbing membrane of the intestinal tract. By making use of a wireless motility capsule or scintigraphy studies, an estimation of the SITT can be established (57). Measured small bowel transit times in healthy volunteers vary from 2 to 4 h, irrespective of prandial state (ranging from fasted state to heavy breakfast) and formulation type (solutions, small pellets, and single units (matrix tablets and osmotic pumps)). The standard implemented value for the net intestinal transit time in Simcyp® is 200 min (3.33 h). It should be noted that changes in transit as a result of diarrhea or constipation are not uncommon and, therefore, the sensitivity analysis is a valuable tool to demonstrate the impact of these scenarios on systemic drug exposure. The measured impact of SITT on systemic exposure of posaconazole in a plausible range is demonstrated in Fig. 11. The green box represents the physiological range of SITT as observed in healthy humans by gamma scintigraphy (58).

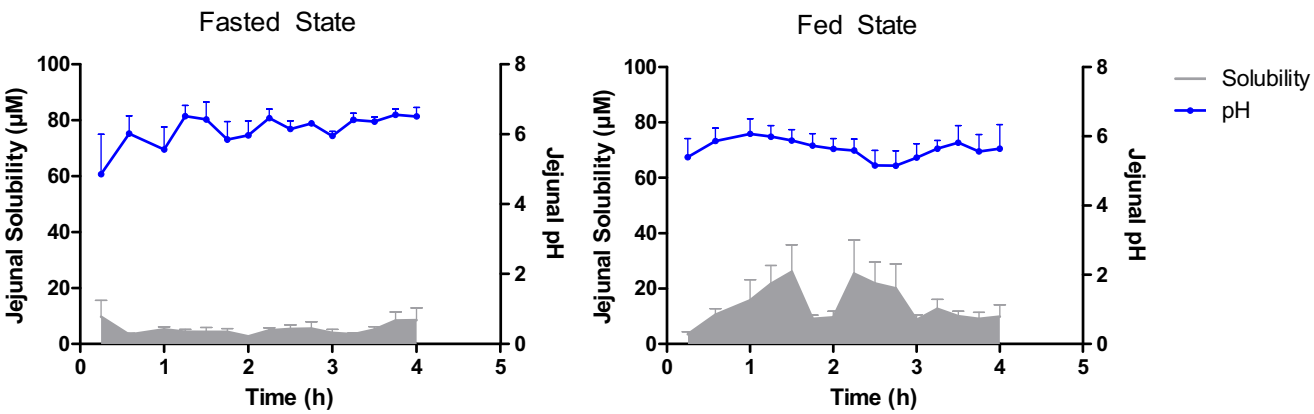

Fig. 9. Thermodynamic solubility of posaconazole (determined by the shake-flask method) in jejunal aspirated samples of healthy volunteers in fasted ($n = 5$) and fed state ($n = 4$) as a function of time (mean + SD). The blue line represents the average measured pH in these aspirated samples (mean + SD). Data obtained from the literature (17)

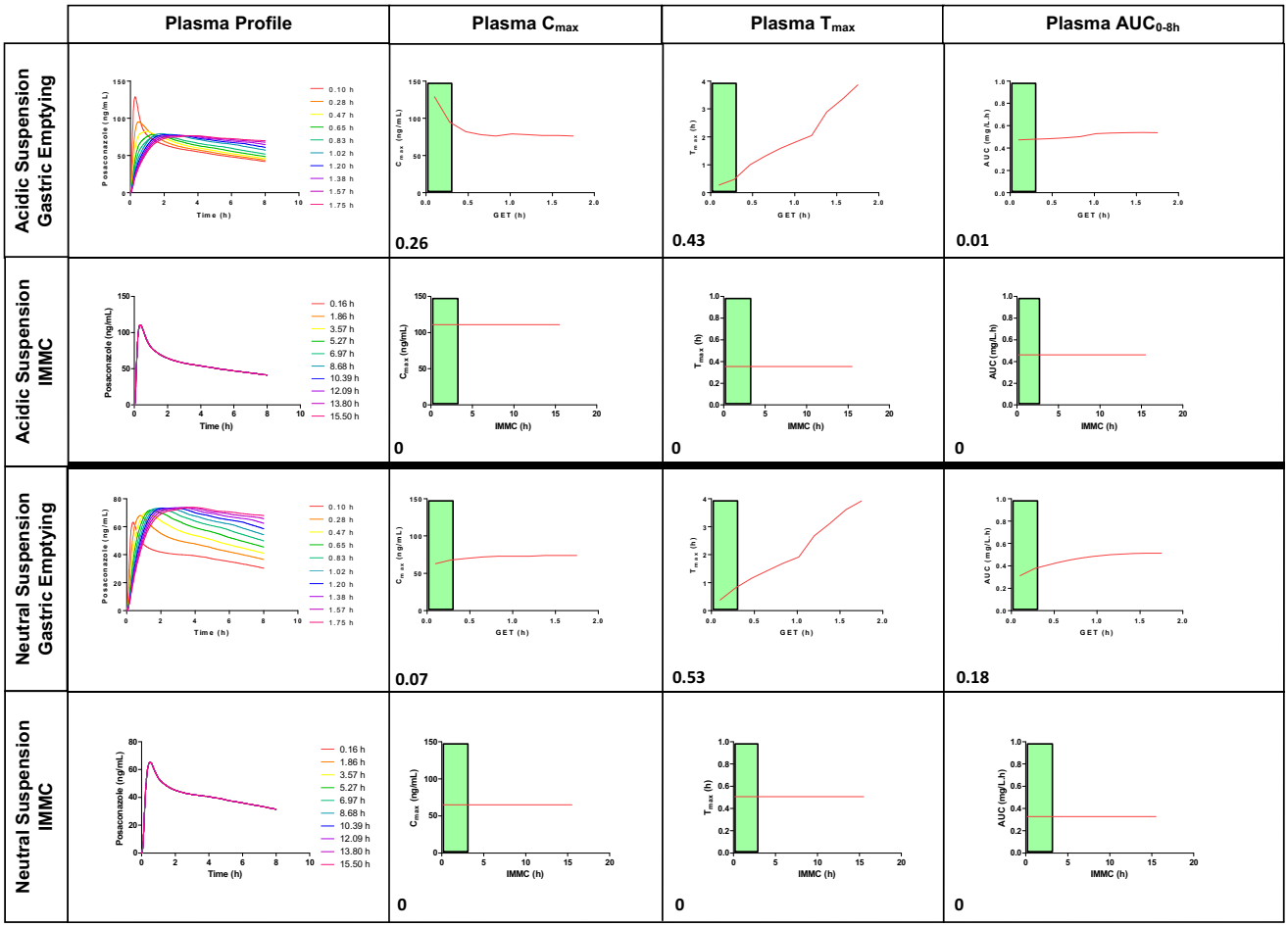

Fig. 10. ASA performed for gastric emptying and the interdigestive migrating motor complex (IMMC) in the Simcyp Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of gastric emptying and the IMMC based on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

As the fast gastric emptying rate (0.175 h) remained unchanged during these simulations, the effect of SITT on systemic drug exposure was more visible after the absorption phase (i.e., after the plasma T_{max}).

ASA of GI Volumes: Stomach and Jejunum

The GI tract (i.e., stomach and small intestine) is reflected in Simcyp® by eight different compartments: one for the stomach, one for the duodenum, two for the jejunum, and four for the ileum (59,60). Each compartment consists of a net fluid volume that is controlled by fluid intake, gastric emptying, SITT, water secretion, and intestinal absorption. The house keeping of fluid volume in each compartment as a function of time can be described by the following equation(s):

$$\frac{dV_{st}}{dt} = Q_{sec,s} - k_{t,st} V_{st} \tag{3}$$

$$\frac{dV_n}{dt} = Q_{sec,n} - k_{reabs,n} V_n + k_{t,n-1} V_{n-1} - k_{t,n} V_n \tag{4}$$

Equations 2 and 3 describe the volume change as a function of time in the stomach (V_{st}) and nth intestinal segment (V_n), respectively. The gastric fluid volume is depending on the fluid secretion rate ($Q_{sec,\ s}$) and gastric emptying rate constant ($k_{t,\ st}$), whereas the intestinal segment fluid is depending on the fluid secretion rate ($Q_{sec,\ n}$), the fluid reabsorption rate constant ($k_{reabs,\ n}$), and the transit rate constant ($k_{t,\ n-1}$). Relevant residual volumes were integrated into each segment based on work that was performed by Schiller *et al.* who explored the residual volumes in the GI tract with the help of magnetic resonance imaging (MRI) (61). The ASA that was performed on gastric and jejunal fluid volumes is depicted in Fig. 12. The green box represents the physiological range of residual gastric and jejunal volumes as observed in healthy humans by MRI (62).

All simulations demonstrate a higher systemic exposure (in terms of $AUC_{0-8\ h}$) whenever the fluid volumes were increased. The reason for that phenomenon can be explained by the fluid volumes present in the GI tract for the drug to dissolve. This effect was superior for the gastric compartment (both for plasma $AUC_{0-8\ h}$ but also for plasma C_{max}) as the predissolved amount of posaconazole will be increased, leading to a higher amount of drug that will enter the duodenum, enhancing intestinal absorption during the absorption phase (up until plasma T_{max}). Although relevant fluid volumes are integrated into the program based on the MRI study of Schiller *et al.* (61), a more recent MRI study

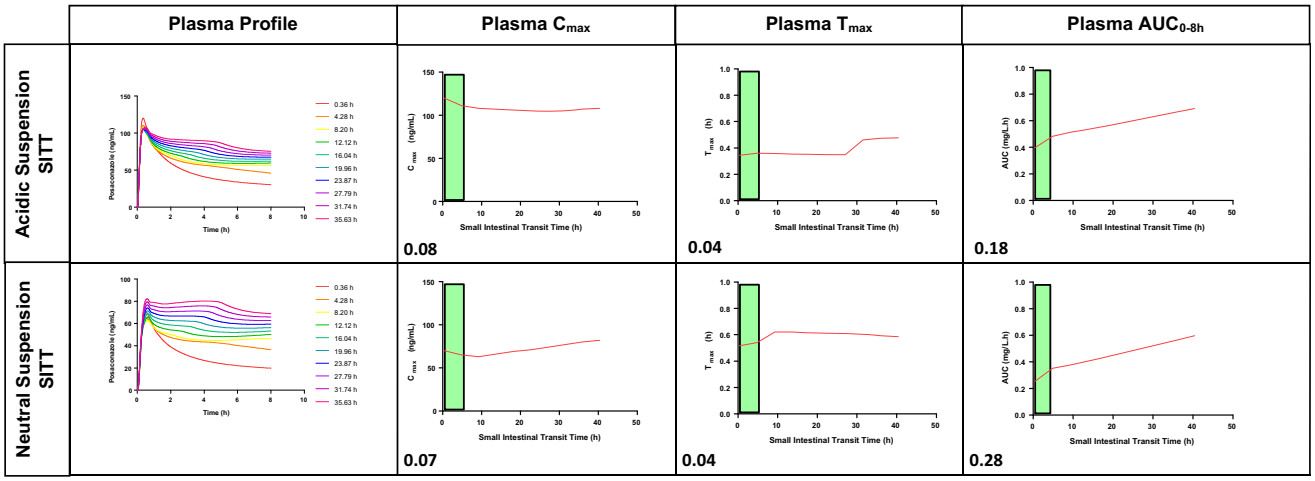

Fig. 11. ASA performed for small intestinal transit time (SITT) in the Simcyp® Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of SITT on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

sheds light on how the luminal water appears as a population of discontinuous liquid pockets of varying size rather than as a single, more static pocket (62). A dynamic fluid compartment absorption and transit (DFCAT) model has recently been proposed, taking into account the dynamic appearance and disappearance of fluids along the GI tract in presence of a 5-mL mucus layer volume in each compartment, though not in pockets as described by Mudie *et al.*

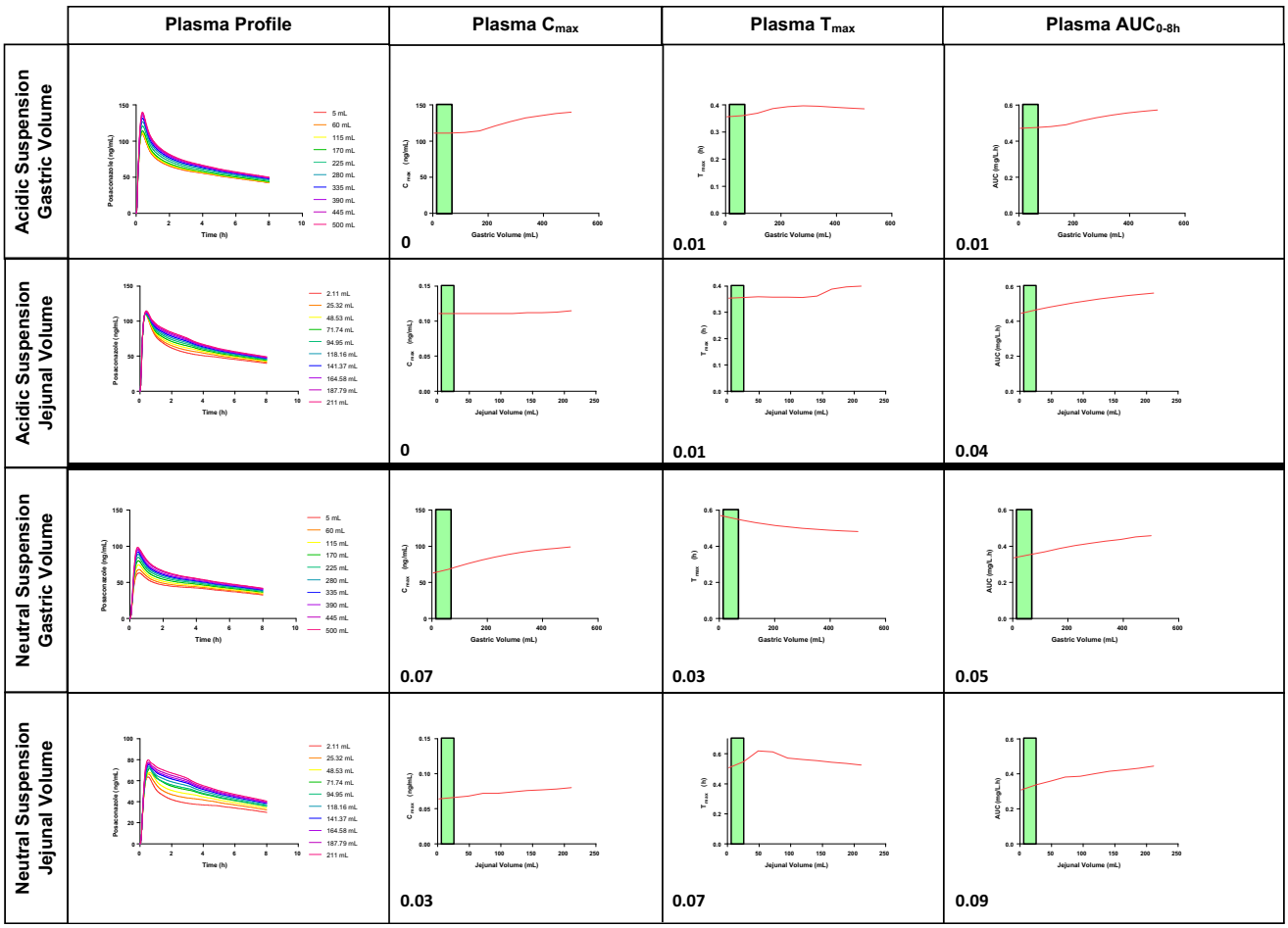

Fig. 12. ASA performed for gastric and jejunal residual volumes in the Simcyp® Simulator using the simulated plasma profiles as the end point. The green area demonstrates the physiological range of residual gastric and jejunal fluids measured in humans based on literature data. Numbers provided in the lower left corner of each graph represent the sensitivity factors

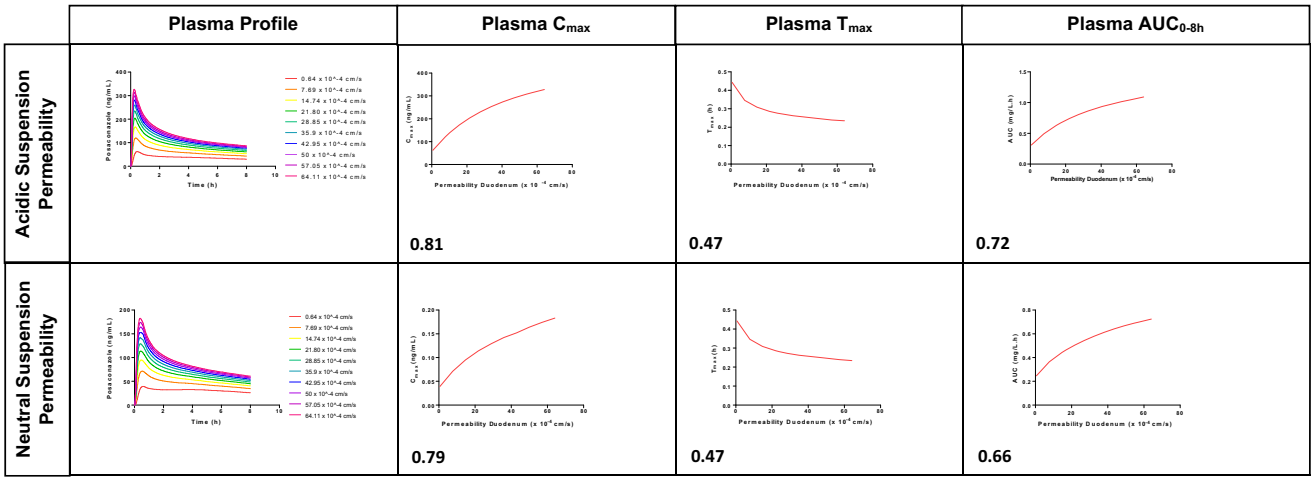

Fig. 13. ASA performed for duodenal permeability in the Simcyp® Simulator using the simulated plasma profiles as the end point. Numbers provided in the lower left corner of each graph represent the sensitivity factors

(62,63). However, to date, there is no clue about the impact of the present discontinuous pockets of water on intraluminal precipitation behavior of a weak base. Therefore, as a future direction in our field, MRI studies combined with profiling of the drug should help us to better understand the dynamic interplay between present volumes and oral drug behavior along the GI tract. These studies should reveal how motility along the GI tract will impact the formation of the formed liquid pockets that a drug needs in order to dissolve.

Table II. Ranking Order of the Selected GI Variables Starting with the Most Sensitive Value (Ranking No. 1)

Ranking	Plasma C_{max} GI variable	Sensitivity factor	Ranking	Plasma T_{max} GI variable	Sensitivity factor	Ranking	Plasma $AUC_{0–8\ h}$ GI variable	Sensitivity factor
Acidic suspension								
1	Duodenal pH	0.50	1	Gastric emptying	0.43	1	Duodenal pH	0.45
2	Gastric pH	0.45	2	Gastric pH	0.29	2	Gastric pH	0.39
3	Gastric emptying	0.26	3	Duodenal pH	0.30	3	SITT	0.18
4	SITT	0.08	4	Duodenal bile salts	0.14	4	Duodenal bile salts	0.08
5	Duodenal bile salts	0.03	5	SITT	0.04	5	Jejunal volume	0.04
6	Gastric volume	0	6	Gastric volume	0.01	6	Gastric volume	0.01
7	Jejunal volume	0	7	Jejunal volume	0.01	7	Gastric emptying	0.01
8	IMMC	0	8	IMMC	0	8	IMMC	0
9	Gastric bicarbonate	0	9	Gastric bicarbonate	0	9	Gastric bicarbonate	0
10	Duodenal bicarbonate	0	10	Duodenal bicarbonate	0	10	Duodenal bicarbonate	0
Neutral suspension								
1	Duodenal pH	0.68	1	Gastric pH	0.87	1	Duodenal pH	0.57
2	Gastric pH	0.65	2	Gastric emptying	0.53	2	Gastric pH	0.50
3	Duodenal bile salts	0.24	3	Jejunal volume	0.07	3	SITT	0.28
4	Gastric emptying	0.07	4	Duodenal pH	0.05	4	Duodenal bile salts	0.19
5	Gastric volume	0.07	5	Gastric volume	0.03	5	Gastric emptying	0.18
6	SITT	0.07	6	SITT	0.04	6	Jejunal volume	0.09
7	Jejunal volume	0.03	7	Duodenal bile salts	0.01	7	Gastric volume	0.05
8	IMMC	0	8	IMMC	0	8	IMMC	0
9	Gastric bicarbonate	0	9	Gastric bicarbonate	0	9	Gastric bicarbonate	0
10	Duodenal bicarbonate	0	10	Duodenal bicarbonate	0	10	Duodenal bicarbonate	0

Sensitivity was explored towards the systemic outcome parameters (plasma C_{max}, T_{max}, and $AUC_{0–8\ h}$) for the acidic suspension and the neutral suspension

ASA of Duodenal Permeability

Accurate assessment of effective intestinal permeability (P_{eff}) for drugs and nutrients are challenging to study *in vivo* in humans (64,65). However, several successful attempts have been accomplished. For instance, in 1985, Jobin and colleagues explored the fraction absorbed of metoprolol (BCS class 1) in the duodenal and jejunal segment (66). In 1988, regional differences in permeability have been explored for five different compounds in the human jejunum and ileum (67). All five compounds (hydrochlorothiazide, atenolol, salicylic acid, furosemide, cimetidine) demonstrated a higher permeability in the jejunum compared with the ileum. In the 1990s, a series of drug compounds were screened for their permeability with the introduction of the Loc-I-Gut® method to measure human P_{eff} after infusion of a drug solution in a demarcated segment of the GI tract (68). Recently, the same results were observed for three other compounds (metoprolol, atenolol, and ketoprofen) after administration of a solution in the jejunum, ileum and colon to 14 healthy volunteers (69). Nowadays, most permeability studies are carried out in a variety of animal models (e.g., *in situ* perfusion studies), cell-free-based or cell-based absorption models (64,70–74). The human P_{eff} that was applied in the Simcyp® Simulator was calculated based on the apparent permeability (P_{app}) derived from a Caco-2 model. The P_{eff} value was applied to all segments of the GI tract. Whether segmental differences in permeability for posaconazole are highly important remains a question. Because of the cross-over design of the study, differences between permeability are ruled out and will not be the responsible GI parameter that can explain the increased systemic exposure after administration of the acidic suspension. Figure 13 depicts the ASA of duodenal permeability for posaconazole. For both formulations, it can be observed that the higher the P_{eff} of the duodenum for posaconazole, the more posaconazole will appear systemically.

Overview and Ranking of the Sensitivity Factors

Table II depicts the ranking of the different discussed GI variables towards their sensitivity with respect to the systemic exposure of posaconazole. Table II (Acidic suspension) represents the ranking of the variables for the acidic suspension, and Table II (Neutral suspension) represents the ranking of the variables for the neutral suspension. It should be pointed out that duodenal permeability is not listed in both tables because of the lack of information regarding regional intestinal permeability.

In the case of the acidic suspension, it can be stated that gastric/duodenal pH and gastric emptying are the major important factors that have the most impact on the systemic outcome of posaconazole. The same has been observed for the neutral suspension; however, there is a higher sensitivity regarding pH which can be explained by the difference in predissolved state among both suspensions as earlier discussed.

CONCLUDING REMARKS AND FUTURE DIRECTIVES

The present study aimed to evaluate the impact of different GI variables, integrated into a PBPK model, on the systemic outcome of two different suspensions of a weakly basic drug. By performing sensitivity analyses in the Simcyp® Simulator, we evaluated the impact of these GI variables on the systemic outcome of a 40-mg acidic (pH 1.6; 70% predissolved) and a 40-mg neutral (pH 7.1; 2.3% predissolved) suspension. It was clearly observed that gastric/duodenal pH and gastric emptying are the most sensitive parameters that may cause variability in systemic outcome of the drug, for both suspensions. Related to the performed sensitivity analyses, cautiousness should be exercised regarding the interplay or correlation between parameters (e.g., pH *versus* buffer capacity and gastric emptying time *versus* IMMC): this interplay and connection between physiological parameters need some extra attention and should be revised/optimized based on clinical data, especially because of the fact that a previous study clearly demonstrated how motility/gastric emptying and pH/buffer capacity highly affected the systemic exposure of a weakly ionized drug compound (21). Differences in sensitivity of certain GI variables for both suspensions were related to the suspension pH and, thus, the predissolved fraction of posaconazole initially dissolved in the liquid formulations. The outcome of the sensitivity analysis highlights the pivotal role of certain GI variables that are indispensable to integrate into an *in vivo* predictive dissolution test that will help us in creating a rational and scientific framework/design towards product development for novel and generic drug products. More models in PBPK programs should be established for other weakly basic compounds, and the sensitivity of the GI variables on the systemic exposure of the drug should be tested, as well. Since gastrointestinal physiology is extremely dynamic and highly variable, it can be recommended to perform sensitivity analyses for multiple variables at the same time to evaluate their interplay. From a regulatory point of view and whenever a PBPK study report is submitted in the context of (novel) drug approval, results of sensitivity analyses for uncertain parameters should be discussed in the context of the simulation conditions and potential clinical consequences, as stated by the US FDA (75,76).

DISCLAIMER

This report represents the scientific views of the authors and not necessarily that of the FDA. Part of this work was presented at the Simcyp Virtual Webinar 2017—part II.

FUNDING INFORMATION

This work was supported by grant No. HHSF223201510157C and grant No. HHSF223201310144C by the US Food and Drug Administration (FDA). This work has received support from the internal funds of KU Leuven (PDM/17/164).

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