Combining biorelevant *in vitro* and *in silico* tools to simulate and better understand the *in vivo* performance of a nano-sized formulation of aprepitant in the fasted and fed states.

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Running Title: PBPK modeling of aprepitant in the fasted and fed state

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ABSTRACT

Introduction: When developing bio-enabling formulations, innovative tools are required to understand and predict *in vivo* performance and may facilitate approval by regulatory authorities. EMEND® is an example of such a formulation, in which the active pharmaceutical ingredient, aprepitant, is nano-sized. The aims of this study were 1) to characterize the 80 mg and 125 mg EMEND® capsules *in vitro* using biorelevant tools, 2) to develop and parameterize a physiologically based pharmacokinetic (PBPK) model to simulate and better understand the *in vivo* performance of EMEND® capsules and 3) to assess which parameters primarily influence the *in vivo* performance of this formulation across the therapeutic dose range.

Methods: Solubility, dissolution and transfer experiments were performed in various biorelevant media simulating the fasted and fed state environment in the gastrointestinal tract. An *in silico* PBPK model for healthy volunteers was developed in the Simcyp Simulator, informed by the *in vitro* results and data available from the literature.

Results: *In vitro* experiments indicated a large effect of native surfactants on the solubility of aprepitant. Coupling the *in vitro* results with the PBPK model led to an appropriate simulation of aprepitant plasma concentrations after administration of 80 mg and 125 mg EMEND® capsules in both the fasted and fed states. Parameter Sensitivity Analysis (PSA) was conducted to investigate the effect of several parameters on the *in vivo* performance of EMEND®. While nano-sizing aprepitant improves its *in vivo* performance, intestinal solubility remains a barrier to its bioavailability and thus aprepitant should be classified as DCS IIb.

Conclusions: The present study underlines the importance of combining *in vitro* and *in silico* biopharmaceutical tools to understand and predict the absorption of this poorly soluble compound from an enabling formulation. The approach can be applied to other poorly soluble compounds to support

41 rational formulation design and to facilitate regulatory assessment of the bio-performance of enabling
42 formulations.

KEYWORDS

PBPK, modeling and simulation, nano-sized drugs, bio-enabling formulations, aprepitant

1. Introduction

In recent years there has been increasing interest from various regulatory authorities in facilitating earlier access to innovative medicines, without compromising their safety and/or efficacy. Indeed, EMA and FDA have taken initiatives to accelerate the approval of innovative medicines which address unmet medical needs.[1–3] On the other hand, the development of new drug products has become more demanding due to the implementation of stricter safety and quality requirements.[4] Contributing further to long development times are the increasingly challenging properties of new active pharmaceutical ingredients (APIs), which make formulation development more difficult and pose a significant barrier to drug absorption and clinical efficacy. Indeed, although about 40% of APIs in marketed drug products exhibit poor solubility and/or permeability, almost 90% of APIs in early drug development stages are saddled with these undesirable characteristics.[5,6] In response to these issues, the European Research Program "PEARRL" (www.pearrl.eu) aims to 1) develop creative bio-enabling formulations, 2) establish, validate and optimize innovative biopharmaceutical *in vitro* tools and 3) understand and predict the *in vivo* behavior of various drug products with physiologically-based pharmacokinetic (PBPK) modeling and simulation.

As part of the PEARRL consortium, the present research aims to link the results obtained using biorelevant *in vitro* tools with *in silico* PBPK models to simulate and better understand the *in vivo* performance of the marketed formulation of aprepitant in the fasted and fed states. Aprepitant is a selective substance P neurokinin (NK1) receptor antagonist which, in combination with other antiemetic agents, is indicated for the prevention of both acute and delayed nausea and vomiting associated with emetogenic cancer chemotherapy.[7,8] It is available as oral capsules (40, 80 and 125 mg), under the brand name EMEND® (reference listed product) and as a water soluble prodrug form, fosaprepitant dimeglumine, for intravenous administration (EMEND® for injection).[7] For ambulant therapy, EMEND® is administered for

three days with a recommended dosing regimen of 125 mg orally once on Day 1 and 80 mg orally once daily on Days 2 and 3.[7]

Aprepitant has both very weak acidic and very weak basic properties and possesses a logD of 4.8 at pH 7.0.[9–13] According to Wu et al.,[11] it exhibits very low aqueous solubility (3-7 µg/mL over the pH range 2-10), although solubilities of just 0.37 µg/mL and 0.8 µg/mL in phosphate buffers at pH 6.5 were reported by Söderlind et al. and Takano et al., respectively.[14,15] In line with the expected protonation behavior, Niederquell and Kuentz recently concluded that aprepitant acts like a neutral compound at small intestinal pH.[16] Concurrently, solubility values of 13 µg/mL in Human Intestinal Fluids (HIF)[14] and of 21 µg/mL in media simulating the canine fasted small intestine [$FaSSIF_{dog}$, 5mM sodium taurocholate (NaTc), 1.25 mM lecithin][15] have been reported, suggesting a pronounced effect of native surfactants on the solubility of aprepitant.

With regard to the permeability of aprepitant, a wide range of permeability values from Caco-2 assays has been reported in the open literature, including a P_{app} of 7.8 x 10^{-6} cm/s (no reference substance value provided),[10,11] a P_{app} of 170 x 10^{-6} cm/s (no reference substance value provided),[13] or a P_{app} of 21 x 10^{-6} cm/s with metoprolol as a reference compound (P_{app} = 5 x 10^{-6} cm/s)[15]. Due to its permeability and solubility properties, aprepitant has been classified as a borderline BCS II/IV compound.[9,10]

The aprepitant tablet formulations used in the early clinical phases exhibited high variability and a large food effect on absorption. Considering the target patient group addressed by aprepitant (cancer patients suffering from nausea and vomiting), administration with food was deemed unacceptable and, therefore, the next formulation efforts were focused on attenuating the food effect and improving dissolution characteristics. This was accomplished by decreasing the particle size to the nanoscale range (approx. 200 nm).[7,17] As illustrated e.g. by the study of Shono et al.,[9] nano-sizing aprepitant proved to be a successful strategy for reducing the food effect over the clinical dose range.[17] After administration of the EMEND® 80 mg and 125 mg capsules (the currently marketed formulation), the absolute bioavailability under fasting conditions is 67% (62-73%) and 59% (53-65%), respectively. In the

93 therapeutic dose range a standard breakfast results in a mild increase in bioavailability (the geometric
94 means AUC_{fed}/ AUC_{fasted} for the 125 mg and 80 mg dose are reported to be 1.20 and 1.09, respectively),
95 but this is not considered clinically relevant.[7,17,18]
96 The aims of this study were threefold: 1) to investigate the advantages of using biorelevant media vs.
97 simple buffers in simulating the *in vivo* performance of aprepitant, 2) to build a PBPK model following the
98 "middle-out" approach, combining experimental data and information available in literature with the
99 commercially available *in silico* software Simcyp Simulator V16.1 (Certara UK Ltd.) and 3) to
100 mechanistically understand the *in vivo* behavior of aprepitant in both the fasted and fed states.

2. Materials and Methods

2.1 Chemicals and reagents

Aprepitant was obtained as the European Pharmacopeia reference standard (code: Y0001825). Acetonitrile and water of HPLC-grade were from Merck KGaA (Darmstadt, Germany). Sodium dihydrogen phosphate dihydrate of analytical grade was from Merck KGaA (Darmstadt, Germany). Phosphoric acid, sodium chloride and sodium hydroxide, also of analytical grade, were purchased from VWR chemicals (Leuven, Belgium). Pepsin was from Sigma-Aldrich (Lot # SLBQ2263V). EMEND® capsules were purchased from MSD SHARP & DOHME GMBH (Lot # MO 49340 and MO 45740 for the 80 mg and 125 mg, respectively, Haar, Germany). Lipofundin® MCT/LCT 20% was purchased from B. Braun (B. Braun Melsungen AG, Melsungen, Germany). FaSSGF/FaSSIF/FeSSIF, FeSSIF V2 and FaSSIF V3 powders were kindly donated by biorelevant.com (London, England).

2.2 Experimental Methods

2.2.1 Solubility experiments

The solubility of pure aprepitant powder was investigated using the method of Andreas et al.[19] in Level I and Level II biorelevant media,[20] utilizing the Uniprep™ system (Whatman®, Piscataway, NJ, USA). Briefly, an excess amount of aprepitant is added to a 3 mL aliquot of the medium and the samples are shaken for 1, 2, 4, 24 or 48 h at 37 °C on an orbital mixer. After shaking, the samples are immediately filtered through pre-warmed 0.45 µm PTFE filters and analyzed by HPLC. Solubility measurements were carried out at least in triplicate (n=3).

2.2.2 Dissolution experiments

Dissolution experiments of the EMEND® capsules were performed using the paddle (USP II) apparatus (Erweka DT 600, Heusenstamm, Germany). Each vessel contained 250 mL or 500 mL, respectively, for media simulating gastric fluids or intestinal fluids. The rotating speed of the paddle was set at 75 rpm.

The temperature in the vessels was maintained at 37±0.5 °C throughout the experiment. All dissolution experiments were performed in triplicate (n=3).

Samples were withdrawn at 1, 2.5, 5, 7.5, 10, 20, 30, 40, 60, 90 and 120 min with glass syringes and filtered through a cylindrical polyethylene filter stick with a pore size of 4 µm attached to the end of the sampling tubes. Immediately thereafter, the samples were filtered through 0.1 µm Anotop 25 filters (Whatman GmBH, Dassel, Germany). After discarding the first 1 mL, the filtrate was diluted with mobile phase and analyzed by HPLC.

The efficiency of filtration was confirmed with Nanoparticle Tracking Analysis (NTA) on a Malvern Nanosight NS300 (Malvern Instruments, Malvern, UK) instrument that was equipped with a green laser (excitation at 468 nm, emission at 508 nm).

2.2.3 Transfer experiments

Transfer experiments were performed for both the 80 mg and 125 mg EMEND® capsules utilizing the USP II apparatus, as described previously by Berlin et al.[21] Briefly, 250 mL of Level III FaSSGF pH 2.0 and 350 mL of Level II FaSSIF V1 or FaSSIF V3 were used as the dissolution media in the gastric and duodenal compartment, respectively. The rotating speed of the paddles was set at 75 rpm. The temperature in the vessels was maintained at 37±0.5 °C throughout the experiment. A peristaltic pump set to first order kinetics ($t_{1/2}$ = 9 min) was used to transfer the dissolved drug from the gastric to the duodenal compartment, from which samples were withdrawn at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 180 and 240 min. Sample handling and analysis were as described for the dissolution experiments.

2.2.4 Chromatographic assays

For the quantitative analysis of samples, a HPLC-UV system was used (Hitachi Chromaster; Hitachi Ltd., Tokyo, Japan or Spectra System HPLC, ThermoQuest Inc., San Jose, USA). The analytical column was a BDS Hypersil C18, 3µm, 150 x 3mm (Thermo Scientific) combined with a pre-column (BDS Hypersil C-18, 3µm, 10 x 4mm). The mobile phase consisted of 50:50 % v/v buffer (NaH_2PO_4, 10mM, pH=2.5) :

acetonitrile. The detection wavelength was set at 220 nm, the injection volume at 50 µL and the flow rate at 1 mL/min. The LOD (limit of detection) and LOQ (limit of quantification) were 0.02 µg/mL and 0.07 µg/mL, respectively.

2.3 Pharmacokinetic data and methods

2.3.1 Literature pharmacokinetic data

In order to build the PBPK model for aprepitant following the "middle-out" approach,[22,23] plasma data for both dose strengths for fasted and fed state were derived from the study of Majumdar et al. and digitalized with WebPlotDigitizer v. 4.0, Texas, USA.[18] The study reported by Majumdar et al. consisted of two parts. The first part was a single-period, double blind study, in which 2 mg of aprepitant were intravenously administered to nine healthy volunteers (mean age was 31 years with a range of 24-40). The second part was a randomized, four period, cross-over study with the aim of investigating the absolute bioavailability of the 80 mg and 125 mg EMEND® capsules under fasted and fed state conditions. In this part of the study, twenty-five healthy volunteers (mean age was 28 years with a range of 18-43) were administered: 1) one aprepitant 80 mg capsule orally following a standard breakfast, 2) one aprepitant 125 mg capsule orally following a standard breakfast, 3) one aprepitant 80 mg capsule orally with 8 oz. of water, along with 2 mg intravenous, isotope-labeled aprepitant, and 4) one aprepitant 125 mg capsule orally with 8 oz. of water, along with 2 mg intravenous, isotope-labeled aprepitant. The authors commented that the co-administration of the 2 mg intravenous isotope-labeled aprepitant had little effect on the disposition of the drug relative to the 80 mg and 125 mg capsules. Since there were earlier data demonstrating non-linearity of the pharmacokinetics of aprepitant with increasing dose, the bioavailability of the capsule formulations was determined by comparing the dose-standardized AUC values following the capsule dose to the dose-standardized AUC values following the capsule dose simultaneously administered with 2mg intravenous, isotope-labeled aprepitant.[17,18]

2.3.2 Modeling methods and strategies

The *in vivo* performance of aprepitant capsules was modeled with the Simcyp Simulator V16.1 (Certara UK Ltd., Sheffield, UK). The substrate parameters for building the i.v. and/ or oral PBPK model are presented in Table 1.

Disposition parameters were calculated from the available i.v. data and the resulting fit of the model to the observed data is shown in Figure 1.[18] The distribution of aprepitant was described using a minimal PBPK model with a Single Adjusting Compartment (SAC). SAC is a non-physiological compartment that represents a cluster of tissues (excluding the liver and portal vein). It is used to extend the use of minimal PBPK models to APIs with high volumes of distribution, i.e. where the tissue concentration exceeds the blood concentration. The Q_{SAC} (inter-compartment clearance), V_{SAC} (apparent volume associated with the SAC) and V_{ss} (steady state volume of distribution) were estimated using the Parameter Estimation Tool and simultaneous fit of the three intravenous PK profiles available in the open literature.[18]

To model the clearance, the "Enzyme Kinetics" option in Simcyp was chosen since aprepitant exhibits saturable metabolism and non-linear pharmacokinetics. As mentioned in the Public Assessment Report of the EMA and FDA of EMEND® capsules, as well as the research article of Sanchez et al., aprepitant is mainly metabolized by CYP3A4,[8,24,25] although CYP2C19 and CYP1A2 may also be involved to some extent. The V_{max} (*in vitro* maximum velocity for metabolism of the compound by the given isoform of enzyme) and K_m (*in vitro* Michaelis-Menten constant for metabolism of the compound by the given isoform of enzyme) for CYP3A4 were derived by Sanchez et al.[24] The f_{umic} (fraction of compound unbound in an *in vitro* microsomal preparation) was predicted using the Simcyp Prediction Toolbox.[26] Furthermore, as indicated in the EMA scientific discussion document for the approval of EMEND® capsules, after intravenous administration of the radio-labelled prodrug of aprepitant (which is rapidly and completely converted to aprepitant) no unchanged drug is recovered in the urine.[25,27] Therefore, the renal clearance for aprepitant was set at a minimum value corresponding to the product of plasma f_u (fraction unbound) and urine flow.[28] This approach is further supported by the fact that impaired renal

function does not result in a clinically significant difference in the PK of aprepitant when compared to healthy control subjects and no dose adjustment is required for patients with renal insufficiency, end-stage renal disease or those undergoing hemodialysis.[8,25,29]

To model the absorption process, the Advanced Dissolution, Absorption and Metabolism (ADAM) model was utilized.[30] The segmental (total) solubility input option was used, based on the maximum concentration measured in the dissolution experiments in each biorelevant medium (Section 3.1). Permeability was estimated by using the Parameter Estimation Tool by fitting the *in vivo* PK data following oral administration in the fasted state (simultaneous fit of PK profiles after administration of 80 mg and 125 mg) and was in line with the P_{app} values reported in literature by Shono et al.,[9] Wu et al.[11] and Takano et al.[15]

All physiological parameters of the gastrointestinal (GI) tract were maintained at the default values for the healthy volunteer population in the Simcyp Simulator for both the fasted and fed state simulations.

2.4 Statistics

To assess the prediction accuracy, average fold error (AFE) and absolute average fold error (AAFE) were calculated according to the equations published by Obach et al., as also described by Andreas et al.[31,32] The 95% confidence intervals were calculated with the Simcyp Simulator V16.1

3. Results

3.1 Experimental part

Solubility experiments

Mean solubility values (± SD) of pure aprepitant (Form I, most thermodynamically stable polymorph) at 24 h in Level I and Level III FaSSGF, Level I and Level II FaSSIF V1 and FaSSIF V3, Level I and Level II FeSSIF V1 and FeSSIF V2 are presented in Table 2, together with the pH value at the end of the solubility experiment (pH_{final}). In every case the pH_{final} was only slightly or not at all different from the initial pH value of the medium.

The solubility values obtained in Level II compared with Level I biorelevant media indicate a major impact of native surfactants on the solubility of aprepitant. Similar observations have been reported by Zhou et al. and Niederquell and Kuentz.[16,33]

Dissolution and transfer experiments

Dissolution and transfer experiments were performed, as described in sections 2.2.2 and 2.2.3, for EMEND® capsules at both dose strengths in biorelevant media simulating the contents of the fasted stomach (Level III FaSSGF), fasted upper small intestine (Level II FaSSIF V1, Level II FaSSIF V3), fed stomach (Level II $FeSSGF_{middle}$) and fed upper small intestine (Level II FeSSIF V1, Level II FeSSIF V2).[20] The mean values of % dissolved with time in fasted and fed state biorelevant media for the 80 mg and 125 mg dose are presented in Figures 2 and 3, respectively.

In the case of the USP II dissolution experiments the dissolution was fast, incomplete and reached a plateau value within approximately the first 10 min. Interestingly, the concentrations of dissolved drug in the dissolution vessels exceeded the thermodynamic solubility observed for the pure API in the respective media. In particular, the mean maximum concentrations of dissolved drug in Level III FaSSGF, Level II FaSSIF V1, Level II FaSSIF V3, Level II $FeSSGF_{middle}$, Level II FeSSIF V1 and Level II FeSSIF V2 were approx. 15 µg/mL, 27 µg/mL and 26 µg/mL, 75 µg/mL, 120 µg/mL and 150 µg/mL respectively, at both doses. These results are consistent with those of Shono et al. and Takano et al.[9,15]

Decreasing the particle size of aprepitant to the nanoscale results in a much higher dissolution rate, which can be attributed to the large increase in surface area and surface energy. As reported by Kesisoglou and Mitra, apart from the increase in the dissolution rate, nano-sizing can also lead to some increase in the apparent solubility of the API, according to the Freundlich-Ostwald equation.[10] However, the quantitative effect of nano-sizing on saturation solubility remains unclear,[9,34,35] and it can be assumed that the increase in surface area plays the predominant role in the increase in dissolution rate for

nanosized formulations. Additionally, some authors have suggested that apparent increases in solubility that have been reported may be largely due to the use of stabilizers (e.g. surfactants).[36]

With regard to the transfer experiments, no precipitation of aprepitant was observed over the four hour experimental duration. The maximum dissolved concentration was achieved more slowly than in the dissolution experiments, since the appearance of the drug in the intestinal compartment is limited by the rate of transfer from the compartment representing the stomach to the one representing the small intestine. Additionally, the maximum dissolved concentration and plateau values achieved in the transfer experiments were somewhat lower than those of the dissolution experiments in media simulating the fasted upper small intestine. This is attributable to the dilution of the intestinal compartment by fluid transferred from the gastric compartment. The results of the transfer experiments with the 80 mg and 125 mg doses are presented in Figure 4.

3.2 PBPK model and simulations

Initially, modeling and simulation of the *in vivo* intravenous (i.v.) data was performed to estimate the post-absorptive parameters, as described in 2.3.2. The PBPK model for oral administration of aprepitant was then built using the "middle-out" approach. This entailed implementation of (i) the calculated post-absorptive parameters from the i.v. data together with (ii) results from the *in vitro* dissolution experiments to simulate the plasma profiles. These simulated plasma profiles were then compared with data obtained by Majumdar et al. after oral administration of 80 mg or 125 mg EMEND® capsules in healthy volunteers in the fasted and fed states.[18] Since the results from the transfer experiments indicated no precipitation over a four hour period, there was no need to invoke precipitation in the PBPK simulations.

The simulated plasma profiles after i.v. administration of radio-labelled aprepitant, as well as after oral administration of capsules at both dose strengths in fasted and fed states vs. the observed plasma

concentrations, are presented in Figures 1, 5 and 6, respectively. The AAFE and AFE for each simulation are presented in Table 3.

4. Discussion

Fasted state

The *in vitro* solubility and dissolution experiments suggest that the solubilization of aprepitant by native surfactants is likely one of the major properties affecting the *in vivo* dissolution of aprepitant from the marketed formulation. The solubility experiments performed with the pure API powder exhibited great differences between the solubility values measured in Level I (simple buffers) and Level II (addition of bile salts and lecithin) biorelevant media. For example the solubility in Level I FaSSIF V1 was below the limit of detection (0.02 μg/mL), but in Level II FaSSIF V1 it was 9.87 ± 2.40 μg/mL. From the dissolution experiments performed with the formulated (nano-sized) drug, the importance of surfactants on the plateau value attained is also evident. Illustratively, the plateau value reached in Level II FaSSIF V1, containing 3 mM NaTc, was approximately double the plateau value reached in Level III FaSSGF, which contains only 0.08 mM NaTc. In agreement with these results, Roos et al. recently demonstrated the importance of colloidal structures in increasing the bioavailability of aprepitant from various nano-suspensions in rat intestinal perfusion experiments.[37]

In the present study, dissolution experiments in the Level II biorelevant media proved more useful than the equilibrium solubility experiments for identifying the relevant apparent solubility of the marketed aprepitant formulation. Thus, the experimental results demonstrated not only that the final concentration of aprepitant in the dissolution experiments was well above the equilibrium solubility, but also that application of the plateau values from the dissolution experiments led to a more accurate simulation of the plasma profiles.

The role of bile salts in the dissolution and hence bioavailability of EMEND® in the fasted state was further investigated with the Parameter Sensitivity Analysis (PSA) tool. It should be noted that, due to the way

290 the PBPK model was developed for aprepitant, it was not able to account for inter-individual variations
291 in bile salt concentrations, because the "segmental" (total) solubility input option was used (in this case
292 the plateau value from the dissolution experiment). An alternative way to simulate potential effects of
293 inter-subject solubility differences on *in vivo* performance would be to use the estimated micelle-water
294 partition co-efficient (Km:w) in the Simcyp Simulator. This would require a precise value of the intrinsic
295 solubility of aprepitant as an input parameter. However, the solubility of aprepitant is a) very small, b) is
296 associated with a relatively large coefficient of variation and c) is not representative of the concentrations
297 achieved in dissolution experiments.

298 Using PSA, the duodenal total solubility value was allowed to range from 10 µg/mL to 90 µg/mL, i.e. three
299 times lower and higher than the value experimentally derived from the dissolution experiments and used
300 in the PBPK model for aprepitant in the fasted state (30 µg/ml). This range of values for the total solubility
301 reflects the range of bile salt concentrations that has been observed *in vivo* (approx. 2-6 mM in fasted
302 state duodenum and jejunum).[38,39] The PSA is presented in Figure 7. The results suggest that variations
303 in intestinal bile concentration among individuals would mainly affect C_{max} rather than AUC values.
304 Furthermore, according to the PSA for both the 80 mg dose and 125 mg dose, variations in the observed
305 C_{max} can be explained by differences in bile component concentrations among subjects.

306 The PSA is largely in agreement with conclusions drawn by Shono et al., who identified intestinal solubility
307 as the most important parameter driving the predicted C_{max} in the fasted state.[9] The results are also in
308 general agreement with the observations of Takano et al., who investigated the rate-limiting step for
309 absorption of various poorly soluble drugs, including aprepitant, in dogs.[15] In that study it was shown
310 that reducing the particle size of aprepitant below 2 µm produces no further increase in the bioavailability
311 of aprepitant in dogs, even though the dissolution rate continued to increase with particle size reduction.
312 Takano et al.'s study underlined the fact that, for poorly soluble drugs, the rate limiting step to absorption
313 can shift from dissolution to solubility, depending on the formulation strategy adopted.[15] Taking all of

these points into consideration, it seems that aprepitant should be classified as a DCS IIb compound[40] and that, for fast-dissolving formulations, the *in vivo* solubility is likely to remain a limitation to its *in vivo* performance.

Fed state

The *in vitro* experiments conducted in biorelevant media simulating the fed state also highlight the importance of surfactants on the apparent solubility of nano-sized aprepitant. For example, the maximum concentration achieved in FeSSIF V1 was more than 4-fold greater than that achieved in FaSSIF V1 (similar to the ratio of NaTc in FeSSIF V1 to FaSSIF V1, which is 15:3). Comparing the plateau values reached in the dissolution experiments in FeSSIF V1 and FeSSIF V2, a slightly higher value (approximately 120 µg/mL vs. 150 µg/mL, respectively) was observed in FeSSIF V2. This increase might be due to the additional presence of glyceryl monooleate in FeSSIF V2, which has been found to have a positive solubilization effect on aprepitant powder.[33]

When the PBPK model was adapted to simulate the plasma profile of aprepitant in the fed state, the fits to the observed data were generally very good (AFE and AAFE both less than 1.5). However, the predicted profiles exhibited an earlier t_{max} of about 2 h compared to the clinically observed mean value, i.e. 4 h. We note that the default value for mean gastric residence time in Simcyp is set at one hour, which seems rather short for the fed state. It is believed that in the fed state, liquids and smaller particles (<3–4 mm) such as disintegrated tablets and capsules often empty with food over a time-span which depends largely on the caloric value of the meal.[41] Thus, the gastric emptying time in the fed state can vary and high caloric meals can result in long gastric emptying times.[41–45] In the study of Majumdar et al., the EMEND® capsules were administered to the volunteers 15 minutes following a “standard light breakfast”, although no specific information, e.g. the caloric value of the meal, was provided with regard to the nature of the breakfast. According to the information provided in the FDA Public Assessment Report, the effect of food on the performance of EMEND® capsules was investigated after the administration of a meal that “is

similar to FDA recommended high-fat, high-calorie breakfast".[8] We therefore used the PSA Tool to explore the sensitivity of the simulated pharmacokinetic profiles of the EMEND® capsules in the fed state to the mean gastric residence time (MGRT). Sensitivity analysis was performed by floating the MGRT values over the range of 1 h (default value in Simcyp Simulator) to 4 h (maximum value allowed in Simcyp Simulator). The results of this analysis are presented in Figure 8. Figure 8 indicates that a MGRT greater or equal to 2.5 h would improve the goodness of fit of the simulated profiles. For example, for a MGRT equal to 3 h, the AFE and AAFE for the 80 mg and 125 mg doses would be 1.26 and 1.32, and 1.09 and 1.34, respectively.

Since aprepitant is characterized as borderline BCS Class II/IV and various permeability values have been reported in the literature, a further PSA was conducted in which the MGRT values were allowed to float over the range 1 h to 4 h and the P_{eff} values were simultaneously allowed to float over the range of 1.16-2.15 x 10^{-4} cm/s (based on the lowest value reported in literature and the value used in the PBPK model developed, respectively). The results from this additional sensitivity analysis (shown in Figure 9) indicate that, for a mean gastric residence time equal to or greater than 2 h, a relatively small decrease in the permeability would result in a significant prolongation of the predicted t_{max}, without a profound effect on the AUC or C_{max}. For example, if a MGRT of 2.5 h and a P_{eff} of 1.4 x 10^{-6} cm/s (i.e. 35% smaller value than the one used in the currently developed model) are implemented, the predicted t_{max} increases by 30%, whereas the C_{max} and AUC decrease only by 8.7% and 2.2%, respectively, compared to simulation with a MGRT of 2.5 h and no change in permeability. For this particular example, the AFE and AAFE for the 80 mg and 125 mg dose are 1.22 and 1.29, and 1.04 and 1.29, respectively. Therefore, it seems that inter-individual differences in permeability may also affect the absorption of EMEND® in the fed state.

Verification of the model and clinical implications

Ideally, an appropriate validation of a compound-specific PBPK model would require access to individual *in vivo* data from all clinical trials conducted for the compound. However, in practice, academic scientists

usually have to rely on mean plasma profiles, along with their respective variability (if available), or even on single values of summary pharmacokinetic parameters (C_{max}, AUC) reported in the open literature. In order to validate the model presented in this study, simulated plasma profiles or single pharmacokinetic data (C_{max}, t_{max} and AUC values) were compared to information available in the literature. To this end, virtual trials (10 trials, each with 10 volunteers) were performed in the Simcyp Simulator by implementing the PBPK model described in sections 2.3.2 and 3.2. For the fed state model, based on the results of the PSA, a MGRT of 2.5 h was used (instead of 1 h, which is the default value in Simcyp for IR formulations administered in the fed state).

Plasma concentration profiles for aprepitant after administration of one 125 mg EMEND® capsule in the fed state in healthy volunteers were reported in the study of Gore et al., 2009.[46] In the same study, the $AUC_{0\text{-}24h}$ and C_{max} values were presented as geometric means together with their respective ranges; $AUC_{0\text{-}24h}$ 19456 (15251, 24817) ng·h/mL and C_{max}= 1539 (1229, 1927) ng/mL. Majumdar et al. conducted a second study with the aim of investigating the pharmacokinetic profile during a three-day 125 mg/80 mg aprepitant regimen.[18] In that study, the volunteers were administered a single oral dose of one 125 mg EMEND® capsule on Day 1 and one 80 mg EMEND® capsule on Days 2 and 3. The doses were administered 15 minutes following administration of a light standard breakfast. The mean plasma profile of aprepitant up to the 24^{th} hour (end of Day 1) was used for model evaluation purposes. The data from these studies were digitalized, as described earlier, and compared with the predicted plasma profiles for the 125 mg capsule in the fed state. The results are presented in Figure 10. For both cases the fit was acceptable, with AFE and AAFE less than 1.5.

It should be mentioned that a third study, published by Ridhurkar et al., was also identified. This was a bioequivalence study of 125 mg EMEND® capsules after administration of a high-caloric breakfast. [47] Since the reported pharmacokinetic parameters (mean C_{max} 2304 ng/mL, mean AUC 82997 ng·h/mL, mean t_{max} 7.7 h, $t_{1/2}$ of 25.4 h) were much higher than those of the FDA and EMA Public Assessment

Report of EMEND® capsules, or any other comparable clinical study, this study was not considered further for modeling.

With regard to cancer patients, who are the patients most often prescribed aprepitant, the EMA Public Assessment Report stated that, based on limited data, the pharmacokinetics of EMEND® capsules in healthy volunteers and patient populations seem to be similar.[25] That said, the pharmacokinetic studies in cancer populations that have been published to date are not completely consistent with this appraisal. Takahashi et al. investigated the pharmacokinetics of EMEND® as a 125 mg/80 mg three-day dose regimen in twenty Japanese cancer patients who were concomitantly receiving intravenous granisetron (40 μg/kg on Day 1) and dexamethasone (on Days 1-3).[48] In this study the reported C_{max} and AUC for the Day 1 of the 125 mg/80 mg dose regimen are 2210 ± 870 ng/mL and 30000 ± 8700 ng·h/mL, respectively, which are slightly higher than those reported for healthy volunteers in the fasted state (C_{max} = 1003 ng/mL, AUC = 21633 ng·h/mL).[18,48] In a later study, Bubalo et al. investigated the pharmacokinetics of aprepitant in cancer patients undergoing hematopoietic stem cell transplantation. In this study, fourteen Caucasian patients were administered one 125 mg EMEND® capsule one hour before the first chemotherapy or radiation dose. Ondansetron and dexamethasone (12 mg on Day 1 and 8 mg on the subsequent days) were co-administered prophylactically to all subjects. In this study greater overall variability, as well as the changes in the post-absorptive parameters of the pharmacokinetics of aprepitant were observed. The observed geometric means (range) of key pharmacokinetic parameters were: t_{max}= 7 h, C_{max}= 977 (741-1289) ng/mL, AUC 27190 (12878-53269) ng·h/mL, CL 0.93 (0.47-1.85) mL/min/kg and V_{ss} 1.54 (1.30-1.84) L/kg. The authors commented that in all subjects the observed V_{ss} was larger than that reported for healthy volunteers and that an altered fraction of unbound drug either in plasma or in tissues could have led to a greater V_{ss}.[27] The observed discrepancies between the pharmacokinetic parameters reported for healthy volunteers and those reported for cancer patients in the studies of Bubalo et al. and Takahashi et al. may be partly attributable to the co-administration of

dexamethasone, which is reported to result in a 30 % increase in the AUC of aprepitant on Day 1, when administered at higher doses,[7,17] or to the combined effect of all concomitantly administered compounds. Nonetheless, there may be yet other factors, which could also affect the pharmacokinetics of aprepitant in diseased populations. Further data in cancer populations would be needed to reach solid conclusions about the *in vivo* performance of EMEND® in these patients.

Last but not least, the relationship between aprepitant pharmacokinetics and its clinical effect should be considered. It has been reported that 80-90 % brain NK-1 receptor occupancy results in significant antiemetic effect, while maximum antiemetic effect is achieved with a greater than 95 % NK-1 receptor blockade.[17] Furthermore, it has been shown that plasma concentrations of approx. 100 ng/mL produce brain NK-1 receptor occupancy of approximately 90 %.[7] As previously discussed, the maximum plasma concentrations at the indicated doses (125 mg/ 80 mg dose regimen) can be as high as 1500 ng/mL and the trough levels on the third day of treatment are 600 ng/mL,[7,17] indicating high receptor occupancy throughout the whole treatment period. Provided there is no lag-phase for achieving the appropriate receptor concentrations, a three-day regimen of 80 mg/ 80 mg, or administration of another formulation that achieves the requisite plasma concentrations is expected to be clinically equivalent to the 125 mg/ 80 mg dose-regimen. The high dosing compared to the concentrations required to produce efficacy is likely the reason why the regulatory authorities do not consider the differences in the pharmacokinetic data of aprepitant between the fasted and fed state or between healthy volunteers and cancer patients to be clinically significant.

5. Conclusions

The *in vivo* performance of the aprepitant "enhanced" formulation (EMEND® capsules) in healthy volunteers, in both the fasted and fed state, was successfully predicted by coupling *in vitro* data acquired with biorelevant *in vitro* tools with a commercial PBPK modeling platform (the Simcyp Simulator). This study demonstrated the importance of evaluating the effect of gastric residence time as well as the

permeability-solubility interplay when predicting the absorption of a poorly soluble API under various dosing and prandial conditions. Using these *in vitro* and *in silico* biopharmaceutical tools, the performance of poorly soluble compounds can be characterized according to a mechanistically-based framework. This approach can support new and generic drug development by promoting rational formulation design and fewer and smaller, but equally robust clinical trials.

Acknowledgments

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References

1. European Medicines Agency - Research and development - PRIME: priority medicines. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000660.jsp. Accessed February 2, 2018.

2. Press Announcements - Statement from FDA Commissioner Scott Gottlieb, M.D. on new steps to facilitate efficient generic drug review to enhance competition, promote access and lower drug prices. Available at: https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm591184.htm. Accessed February 2, 2018.

3. Information for Consumers (Drugs) - FDA's Drug Review Process: Continued. Available at: https://www.fda.gov/Drugs/ResourcesForYou/Consumers/ucm289601.htm#review. Accessed February 2, 2018.

4. Parekh A *et al.* Catalyzing the critical path initiative: FDA's progress in drug development activities. *Clin Pharmacol Ther* 2015; 97(3): 221–233. doi:10.1002/cpt.42.

5. Kalepu S, Nekkanti V. Insoluble drug delivery strategies: review of recent advances and business prospects. *Acta Pharm Sin B* 2015; 5(5): 442–453. doi:10.1016/j.apsb.2015.07.003.

6. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods* 2000; 44(1): 235–49. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11274893. Accessed February 2, 2018.

7. EMA. EMEND: SUMMARY OF PRODUCT CHARACTERISTICS. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000527/WC500026537.pdf. Accessed October 25, 2017.

8. FDA-Emend Capsules Pharmacology Review Part 1.pdf. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-549_Emend.cfm.

9. Shono Y *et al.* Forecasting in vivo oral absorption and food effect of micronized and nanosized aprepitant formulations in humans. *Eur J Pharm Biopharm* 2010; 76(1): 95–104. doi:10.1016/j.ejpb.2010.05.009.

10. Kesisoglou F, Mitra A. Crystalline Nanosuspensions as Potential Toxicology and Clinical Oral Formulations for BCS II/IV Compounds. *AAPS J* 2012; 14(4): 677–687. doi:10.1208/s12248-012-9383-0.

11. Wu Y *et al.* The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: A Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. *Int J Pharm* 2004; 285(1–2): 135–146. doi:10.1016/j.ijpharm.2004.08.001.

12. Georgaka D *et al.* Evaluation of Dissolution in the Lower Intestine and Its Impact on the Absorption Process of High Dose Low Solubility Drugs. *Mol Pharm* 2017; 14(12): 4181–4191. doi:10.1021/acs.molpharmaceut.6b01129.

13. Sjögren E *et al. In Silico* Modeling of Gastrointestinal Drug Absorption: Predictive Performance of Three Physiologically Based Absorption Models. *Mol Pharm* 2016; 13(6): 1763–1778. doi:10.1021/acs.molpharmaceut.5b00861.

14. Söderlind E *et al.* Simulating Fasted Human Intestinal Fluids: Understanding the Roles of Lecithin and Bile Acids. *Mol Pharm* 2010; 7(5): 1498–1507. doi:10.1021/mp100144v.

15. Takano R *et al.* Rate-limiting steps of oral absorption for poorly water-soluble drugs in dogs; prediction from a miniscale dissolution test and a physiologically-based computer simulation. *Pharm Res* 2008; 25(10): 2334–2344. doi:10.1007/s11095-008-9637-9.

16. Niederquell A, Kuentz M. Biorelevant Drug Solubility Enhancement Modeled by a Linear Solvation Energy Relationship. *J Pharm Sci* 2018; 107(1): 503–506. doi:10.1016/j.xphs.2017.08.017.

17. EMEND® Clinical Pharmacology and Biopharmaceutics Review. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-549_Emend_biopharmr.pdf. Accessed August 31, 2017.

18. Majumdar AK *et al.* Pharmacokinetics of aprepitant after single and multiple oral doses in healthy volunteers. *J Clin Pharmacol* 2006; 46(3): 291–300. doi:10.1177/0091270005283467.

19. Andreas CJ *et al.* In vitro biorelevant models for evaluating modified release mesalamine products to forecast the effect of formulation and meal intake on drug release. *Eur J Pharm Biopharm* 2015; 97: 39–50. doi:10.1016/j.ejpb.2015.09.002.

20. Markopoulos C *et al.* In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: Choosing the appropriate test media. *Eur J Pharm Biopharm* 2015; 93: 173–182. doi:10.1016/j.ejpb.2015.03.009.

21. Berlin M *et al.* Prediction of oral absorption of cinnarizine – A highly supersaturating poorly soluble weak base with borderline permeability. *Eur J Pharm Biopharm* 2014; 88(3): 795–806. doi:10.1016/j.ejpb.2014.08.011.

22. Jamei M *et al.* A framework for assessing inter-individual variability in pharmacokinetics using virtual human populations and integrating general knowledge of physical chemistry, biology, anatomy, physiology and genetics: A tale of “bottom-up” vs “top-down” recognition of covariates. *Drug Metab Pharmacokinet* 2009; 24(1): 53–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19252336. Accessed April 17, 2019.

23. Cristofoletti R *et al.* Assessment of Bioequivalence of Weak Base Formulations Under Various Dosing Conditions Using Physiologically Based Pharmacokinetic Simulations in Virtual Populations. Case Examples: Ketoconazole and Posaconazole. *J Pharm Sci* 2017; 106(2): 560–569. doi:10.1016/j.xphs.2016.10.008.

24. Sanchez RI *et al.* Cytochrome P450 3A4 Is the Major Enzyme Involved in the Metabolism of the Substance P Receptor Antagonist Aprepitant Abstract : 2004; 32(11): 1287–1292. doi:10.1124/dmd.104.000216.given.

25. EMA. EMEND® - Scientific Discussion. 2004; (June): 1–30. Available at:

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000527/WC500026534.pdf.

26. Turner DB *et al.* Prediction of Non-specific Hepatic Microsomal Binding from Readily Available Physicochemical Properties. In: *9th European ISSX Meeting*. Manchester, UK. Available at: https://www.certara.com/wp-content/uploads/Resources/Posters/DavidISSX2006.pdf.

27. Bubalo JS *et al.* Aprepitant pharmacokinetics and assessing the impact of aprepitant on cyclophosphamide metabolism in cancer patients undergoing hematopoietic stem cell transplantation. *J Clin Pharmacol* 2012; 52(4): 586–594. doi:10.1177/0091270011398243.

28. Rowland M, Tozer TN. *Clinical pharmacokinetics : concepts and applications*. Williams & Wilkins, 1995. Available at: https://openlibrary.org/books/OL1101417M/Clinical_pharmacokinetics. Accessed August 7, 2018.

29. Bergman AJ *et al.* Effect of impaired renal function and haemodialysis on the pharmacokinetics of aprepitant. *Clin Pharmacokinet* 2005; 44(6): 637–647. doi:10.2165/00003088-200544060-00005.

30. Jamei M *et al.* Population-based mechanistic prediction of oral drug absorption. *AAPS J* 2009; 11(2): 225–37. doi:10.1208/s12248-009-9099-y.

31. Obach RS *et al.* The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J Pharmacol Exp Ther* 1997; 283(1): 46–58. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9336307. Accessed February 9, 2018.

32. Andreas CJ *et al.* Mechanistic investigation of the negative food effect of modified release zolpidem. *Eur J Pharm Sci* 2017; 102: 284–298. doi:10.1016/j.ejps.2017.03.011.

33. Zhou Z *et al.* Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs. *Eur J Pharm Sci* 2017; 99: 95–104. doi:10.1016/j.ejps.2016.12.008.

34. Kaptay G. On the size and shape dependence of the solubility of nano-particles in solutions. *Int J Pharm* 2012; 430(1–2): 253–257. doi:10.1016/j.ijpharm.2012.03.038.

35. Letellier P *et al.* Solubility of nanoparticles: nonextensive thermodynamics approach. *J Phys Condens Matter* 2007; 19(43): 436229. doi:10.1088/0953-8984/19/43/436229.

36. Tuomela A *et al.* Stabilizing Agents for Drug Nanocrystals: Effect on Bioavailability. *Pharmaceutics* 2016; 8(2). doi:10.3390/pharmaceutics8020016.

37. Roos C *et al.* In Vivo Mechanisms of Intestinal Drug Absorption from Aprepitant Nanoformulations. *Mol Pharm* 2017; 14(12): 4233–4242. doi:10.1021/acs.molpharmaceut.7b00294.

38. Fuchs A, Dressman JB. Composition and Physicochemical Properties of Fasted-State Human Duodenal and Jejunal Fluid: A Critical Evaluation of the Available Data. *J Pharm Sci* 2014; 103(11): 3398–3411. doi:10.1002/jps.24183.

39. Bergström CAS *et al.* Early pharmaceutical profiling to predict oral drug absorption: Current status and unmet needs. *Eur J Pharm Sci* 2014; 57(1): 173–199. doi:10.1016/j.ejps.2013.10.015.

40. Butler JM, Dressman JB. The Developability Classification System: Application of Biopharmaceutics Concepts to Formulation Development. *J Pharm Sci* 2010; 99(12): 4940–4954. doi:10.1002/JPS.22217.

41. O'Shea JP *et al.* Food for thought: formulating away the food effect - a PEARRL review. *J Pharm Pharmacol* 2018. doi:10.1111/jphp.12957.

42. Hunt JN, Stubbs DF. The volume and energy content of meals as determinants of gastric emptying. *J Physiol* 1975; 245(1): 209–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1127608. Accessed August 9, 2018.

43. Cassilly D *et al.* Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterol Motil* 2008; 20(4): 311–319. doi:10.1111/j.1365-2982.2007.01061.x.

44. Koziolek M *et al.* Intragastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. *J Control Release* 2015; 220: 71–78. doi:10.1016/j.jconrel.2015.10.022.

45. Koziolek M *et al.* Physiological Considerations and In Vitro Strategies for Evaluating the Influence of Food on Drug Release from Extended-Release Formulations. *AAPS PharmSciTech* 2018; 19(7): 2885–2897. doi:10.1208/s12249-018-1159-0.

46. Gore L *et al.* Aprepitant in adolescent patients for prevention of chemotherapy-induced nausea and vomiting: A randomized, double-blind, placebo-controlled study of efficacy and tolerability. *Pediatr Blood Cancer* 2009; 52(2): 242–247. doi:10.1002/pbc.21811.

47. Ridhurkar DN *et al.* Inclusion complex of aprepitant with cyclodextrin: Evaluation of physico-chemical and pharmacokinetic properties. *Drug Dev Ind Pharm* 2013; 39(11): 1783–1792. doi:10.3109/03639045.2012.737331.

48. Takahashi T *et al.* Pharmacokinetics of aprepitant and dexamethasone after administration of chemotherapeutic agents and effects of plasma substance P concentration on chemotherapy-induced nausea and vomiting in Japanese cancer patients. *Cancer Chemother Pharmacol* 2011; 68(3): 653–659. doi:10.1007/s00280-010-1519-2.

49. Liu J *et al.* Characterization and pharmacokinetic study of aprepitant solid dispersions with soluplus®. *Molecules* 2015; 20(6): 11345–11356. doi:10.3390/molecules200611345.

590 **Tables**

591 *Table 1: Parameter values used for the simulations of the in vivo performance of EMEND® in the fasted*
592 *and fed states*

Parameters		References and Comments
Dosage form	IR	
Molecular weight (g/mol)	534.44	
Log P	4.8	[9–11,49]
B/P (Blood/Plasma Coefficient)	1.20	Estimated using the PE Tool after fitting the available *in vivo* data (see 2.3.1)
f_u (unbound plasma)	0.02	[7,17,29]
	Absorption	
Absorption Model	ADAM	
Diffusion Layer Model		
Permeability Method		
$P_{eff,man}$ (x 10^{-4} cm/s)	2.15	Estimated using the PE Tool
Solubility fasted state (µg/mL)	13, 30	Stomach, Small Intestine
Solubility fed state (µg/mL)	75, 120	Stomach, Small Intestine
	Distribution	
Distribution Model	Minimal PBPK Model with SAC	
SAC Q (L/h)	21.92	Estimated after fitting the available intravenous *in vivo* data (see 2.3.1)
Volume [V_{SAC}] (L/kg)	0.7837	
V_{ss} (L/kg)	0.875	[7]
	Elimination	
Elimination Type	Enzyme Kinetics	
CYP3A4	V_{max}=120 pmol/mg/min	[24]
	K_m= 10.5 µM	[24]
	f_{umic}=0.143	Simcyp Prediction Toolbox
Renal clearance (L/h)	0.0024	[25,28]

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595 *Table 2: Mean (± SD) solubility of aprepitant in fasted and fed state biorelevant media at 24 h*

Biorelevant Medium	Solubility (µg/mL)	pH_{final}
Fasted state		
Level I FaSSGF	7.62 ± 0.64	1.6
Level III FaSSGF	5.76 ± 0.35	1.7
Level I FaSSIF V1	<LOD	6.6
Level II FaSSIF V1	9.87 ± 2.40	6.5
Level I FaSSIF V3	<LOD	6.8
Level II FaSSIF V3	3.03 ± 0.06	6.7
Fed state		
Level I FeSSIF V1	<LOD	5.0
Level II FeSSIF V1	53.89 ± 11.76	5.0
Level I FeSSIF V2	<LOD	5.8
Level II FeSSIF V2	68.58 ± 6.86	5.8

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597 *Table 3: Calculated average fold error (AFE) and absolute average fold error (AAFE) for the simulations*
598 *after oral administration of EMEND® capsules*

Dose	80 mg		125 mg	
Metrics	Fasted State	Fed State	Fasted State	Fed State
AFE	1.58	1.31	0.94	1.12
AAFE	1.58	1.44	1.14	1.45

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Figure Captions

Figure 1: Simulated (thick green line, population mean; dash grey lines, 5th and 95th percentile of population) and clinically reported plasma concentration-time profiles after i.v. administration of 2 mg radio-labelled aprepitant (diamonds), 2 mg radio-labelled aprepitant i.v. concurrently with one 80 mg EMEND® capsule (circles) and 2 mg radio-labelled aprepitant i.v. concurrently with one 125 mg EMEND® capsule (triangles).[18]

Figure 2: Mean (±SD) % aprepitant dissolved from 80 mg EMEND® capsules in: A) Level III FaSSGF (♦), Level II FaSSIF V1(■), Level II FaSSIF V3 (●) and B) Level II $FeSSGF_{middle}$ (x), Level II FeSSIF V1 (□) and Level II FeSSIF V2 (○).

Figure 3: Mean (±SD) % aprepitant dissolved from 125 mg EMEND® capsules in: A) Level III FaSSGF (♦), Level II FaSSIF V1 (■), Level II FaSSIF V3 (●) and B) Level II $FeSSGF_{middle}$ (x), Level II FeSSIF V1 (□) and Level II FeSSIF V2 (○).

Figure 4: Mean (±SD) % aprepitant dissolved from: A) 80 mg EMEND® capsules and B) 125 mg EMEND® capsules, during transfer experiments from Level III FaSSGF (pH =2) to Level II FaSSIF V1 (■) and to Level II FaSSIF V3 (●).

Figure 5: Simulated (thick green line, population mean; dash grey lines, 5th and 95th percentile of population) and clinically reported (circles) plasma concentration-time profiles after administration of an: A) 80 mg EMEND® capsule and B) 125 mg EMEND® capsule, in the fasted state.[18]

Figure 6: Simulated (thick green line, population mean; dash grey lines, 5th and 95th percentile of population) and clinically reported (circles) plasma concentration-time profiles after administration of an: A) 80 mg EMEND® capsule and B) 125 mg EMEND® capsule, in the fed state.[18]

Figure 7: The sensitivity of the simulated profiles after administration of an EMEND® 125 mg capsule in the fasted state to variations in the duodenal solubility (i.e. from 10 to 90 μg/mL). The thick line represents the profile using approximately the same solubility value as the one implemented in the currently developed PBPK model.

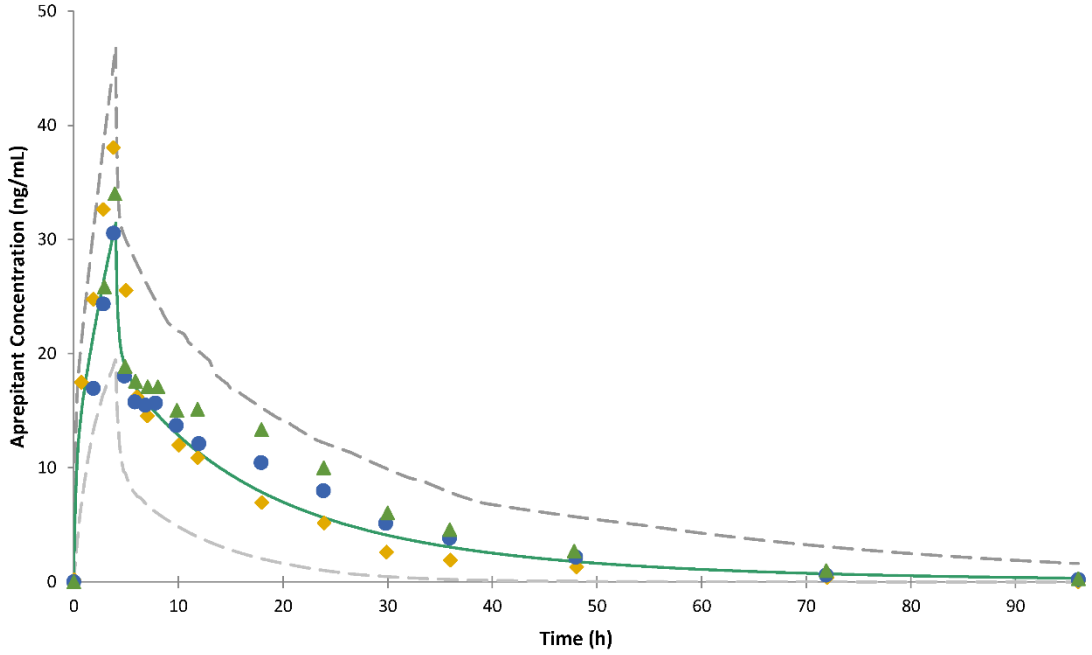
Figure 8: The sensitivity of the simulated profiles after administration of an: A) 80 mg EMEND® capsule and B) 125 mg EMEND® capsule, in the fed state to variations in mean gastric residence time (i.e. from 1 to 4 h).

Figure 9: The sensitivity of the simulated tmax after administration of an: A) 80 mg EMEND® capsule and B) 125 mg EMEND® capsule, in the fed state to variations in mean gastric residence time (i.e. from 1 to 4 h) and permeability values (i.e. P_{eff} from 1.16 to 2.15 x 10^{-6} cm/s).

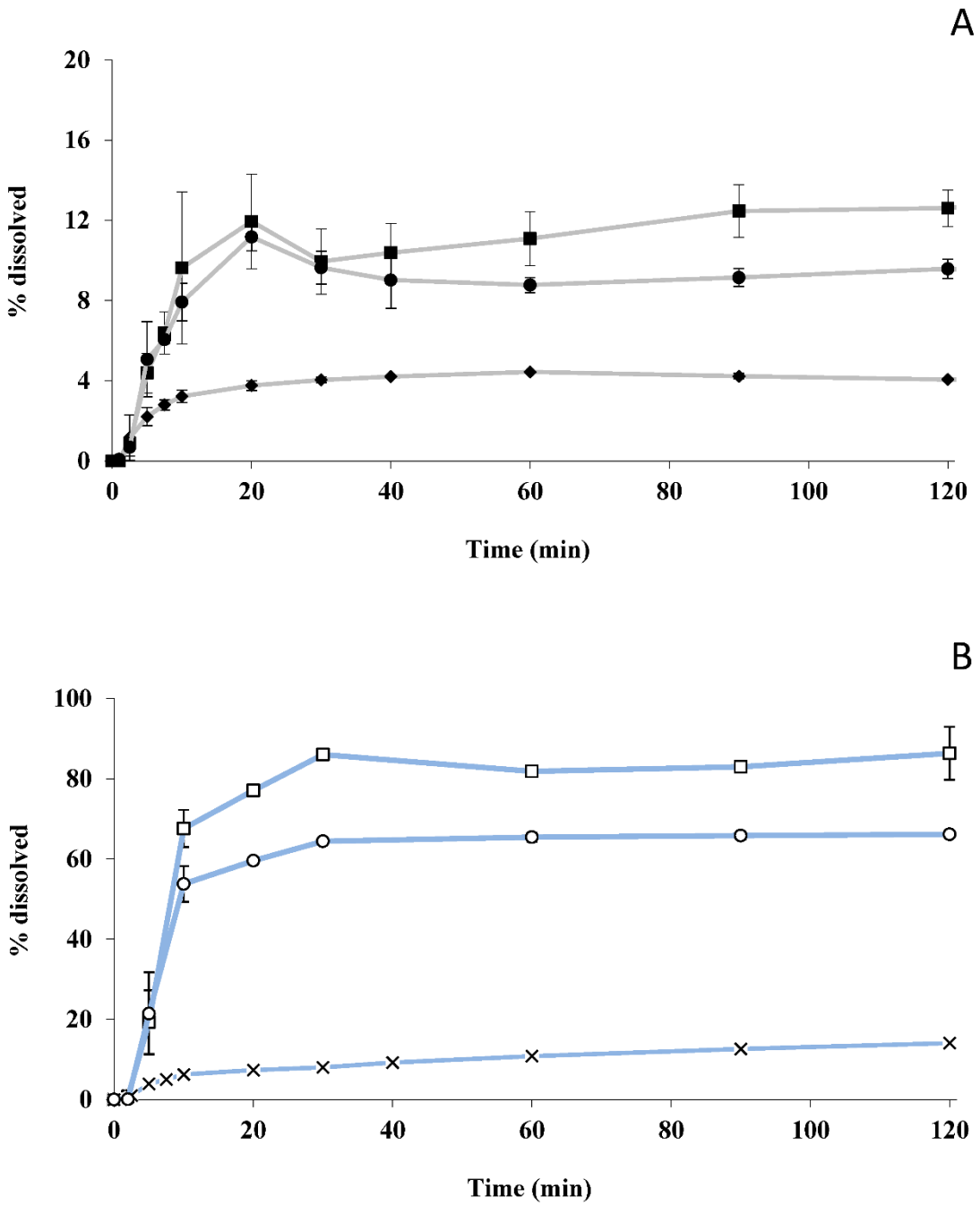
Figure 10: Simulated (thick green line, population mean; dash grey lines, 5th and 95th percentile of population) and clinically reported plasma concentration-time profiles after administration of 125 mg EMEND® capsules in fed state. Circles represent the data from Majumdar et al., upon which the PBPK model was based, squares represent the second clinical study conducted by Majumdar et al. to evaluate the pharmacokinetics of the 3-days aprepitant regimen and diamonds represent the study reported by Gore et al.[18,46]

Figures

Figure 1



652 **Figure 2**



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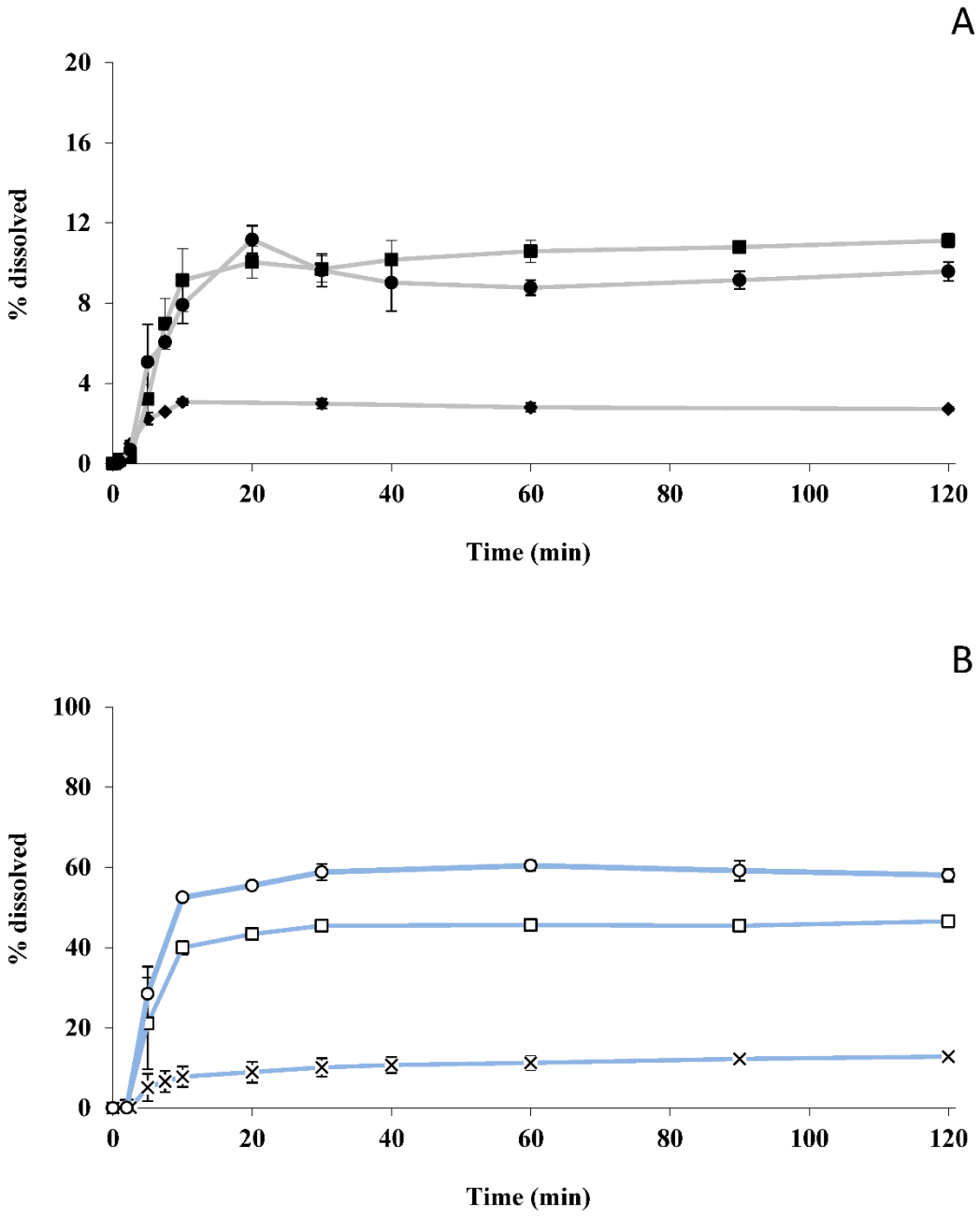
656 **Figure 3**



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659 **Figure 4**

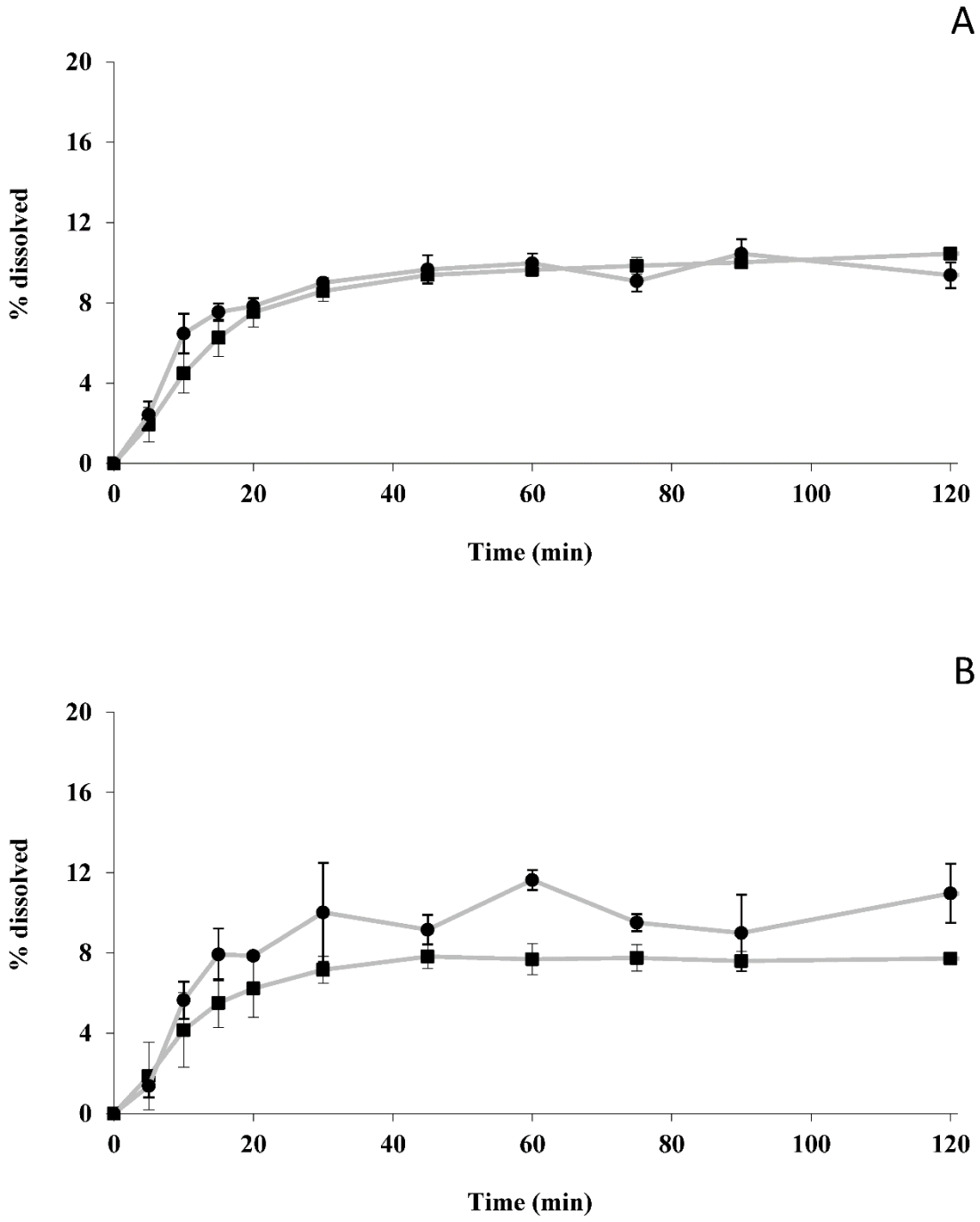

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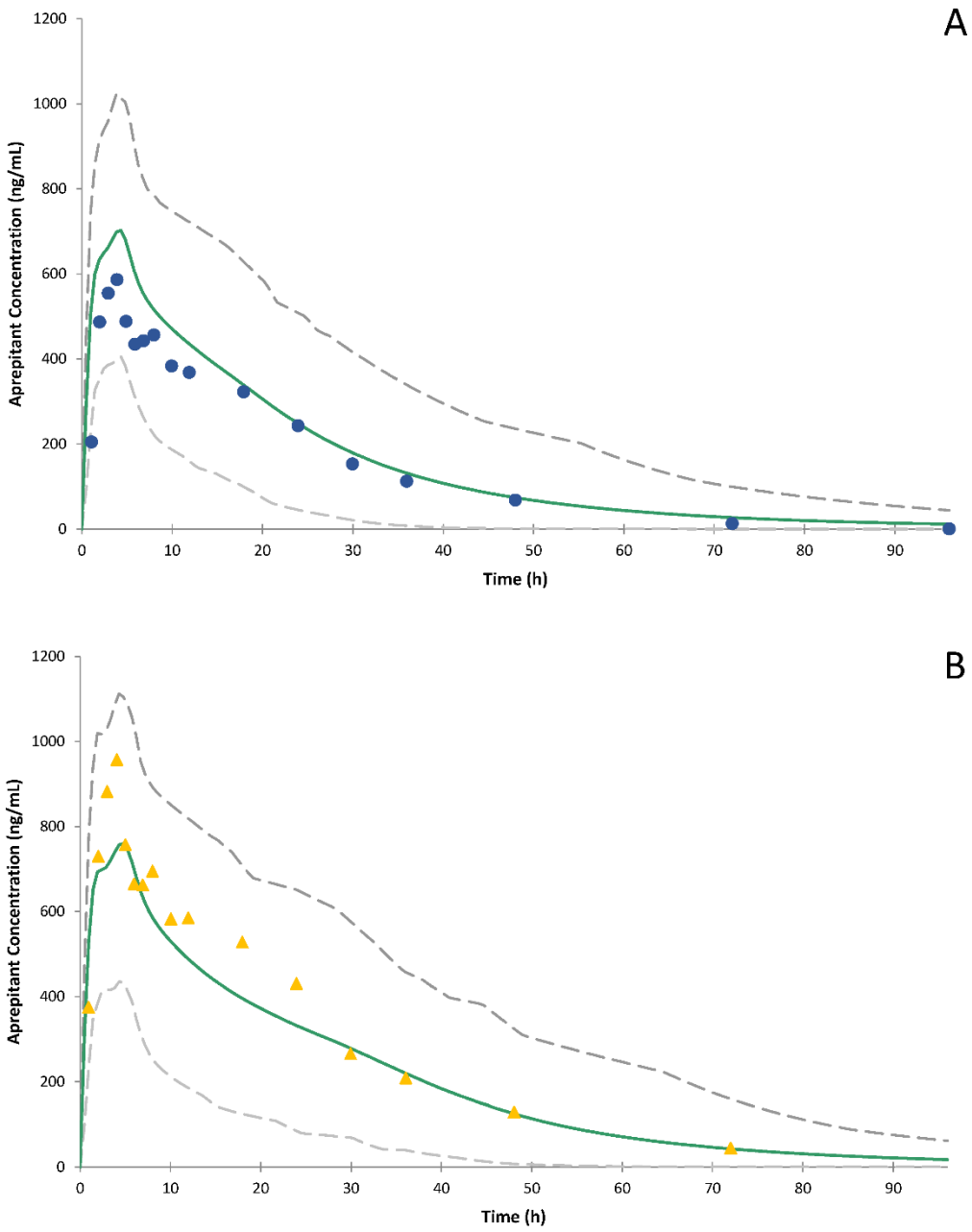
Figure 5



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667 **Figure 6**

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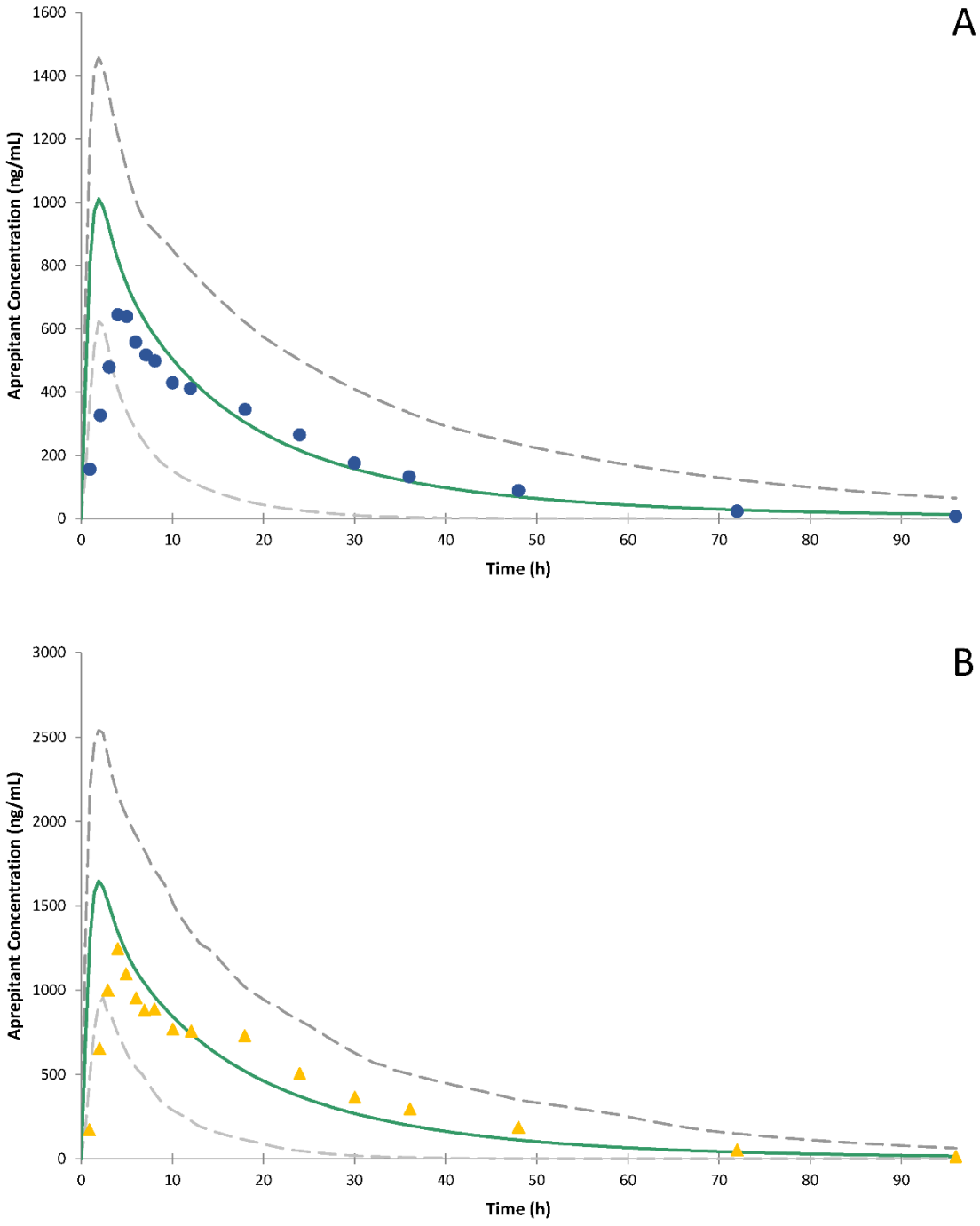

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671 **Figure 7**

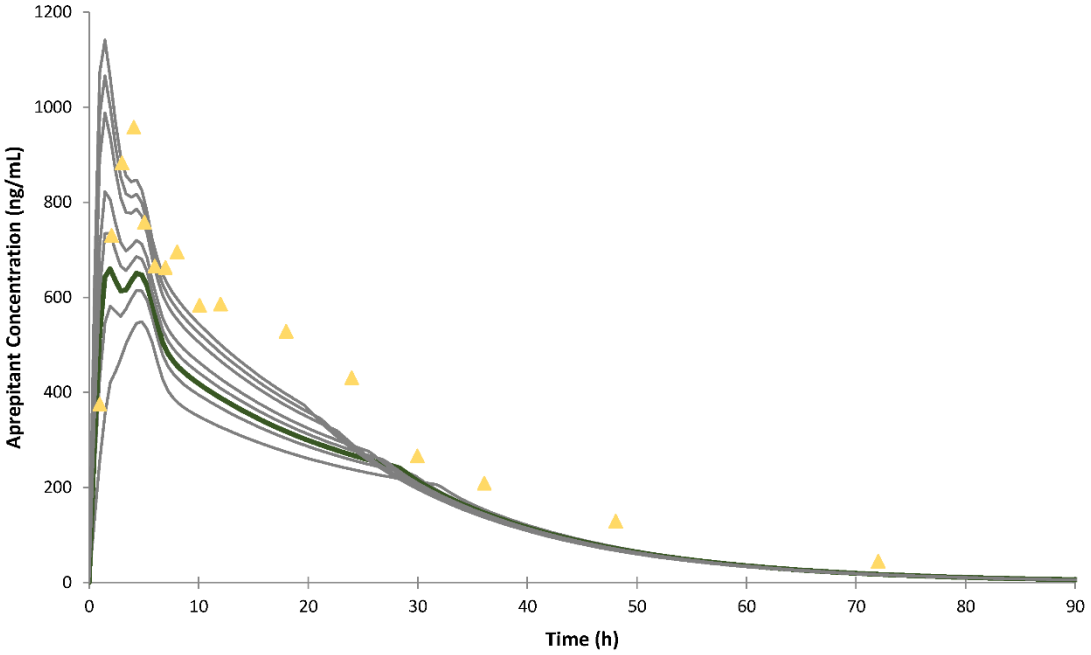

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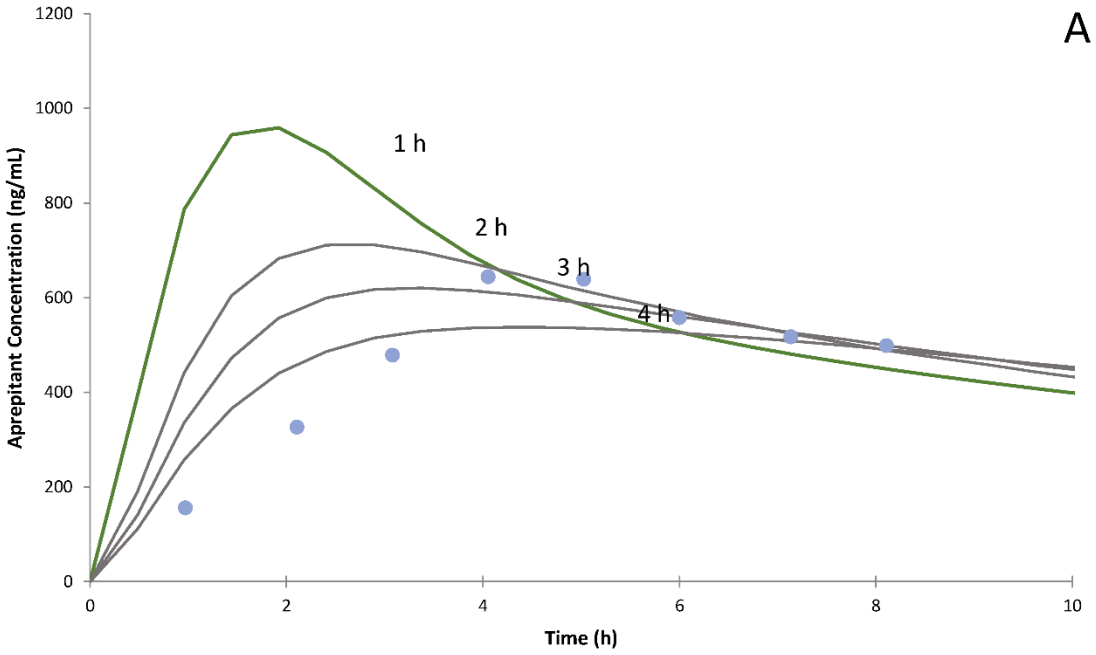
Figure 8



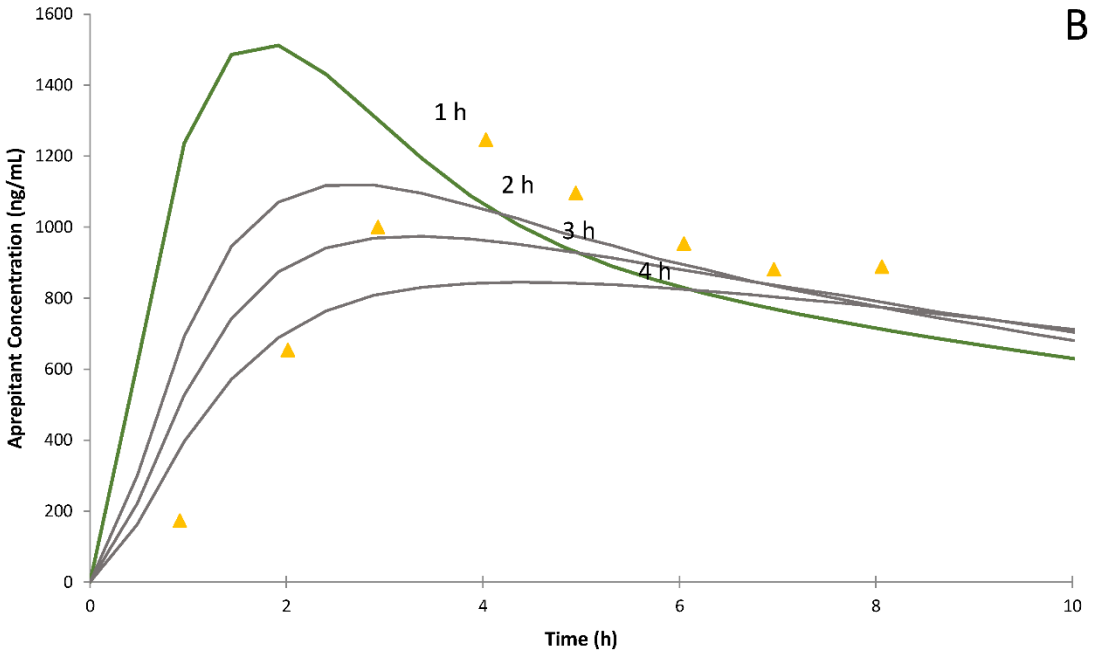


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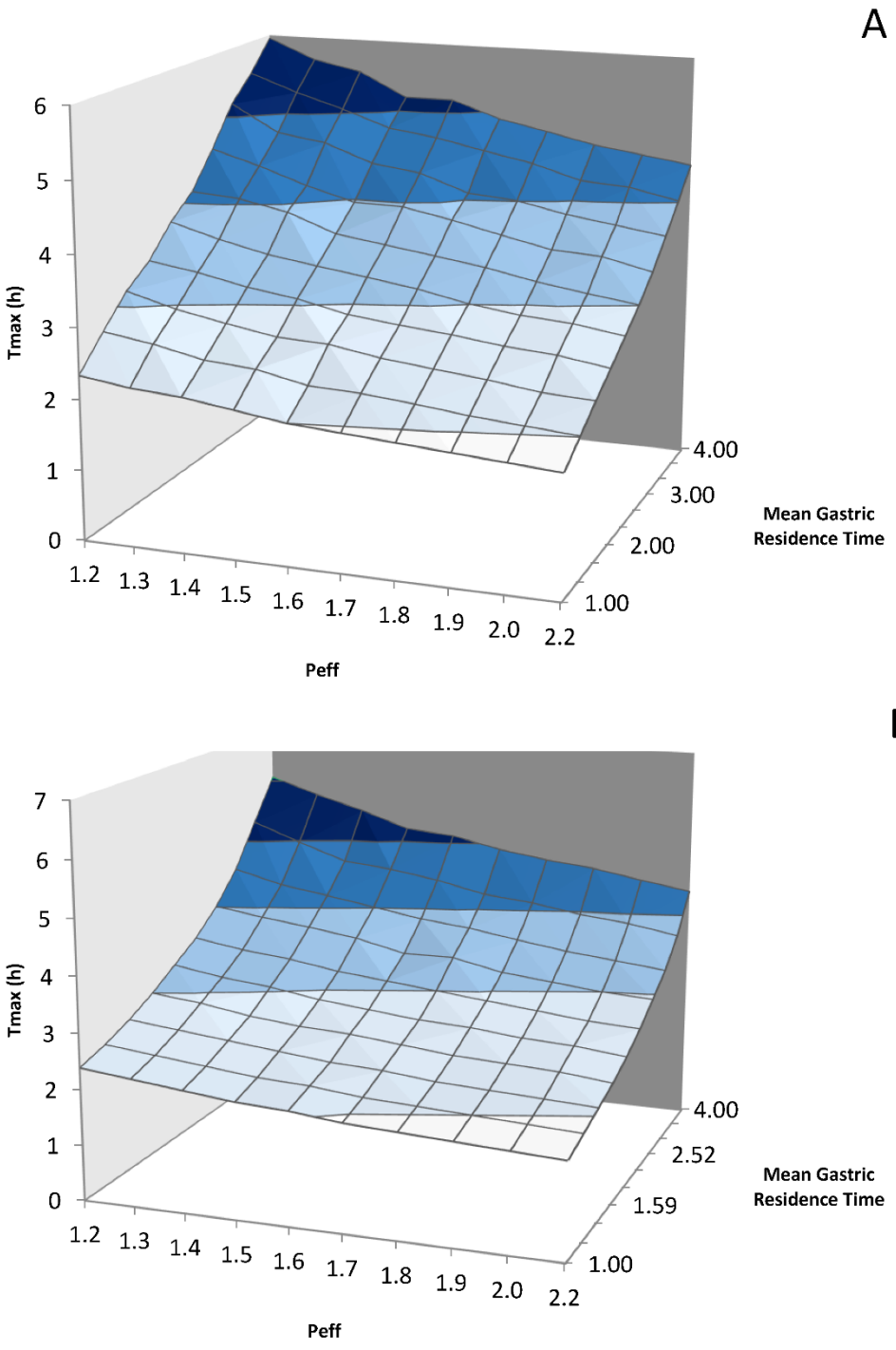
Figure 9



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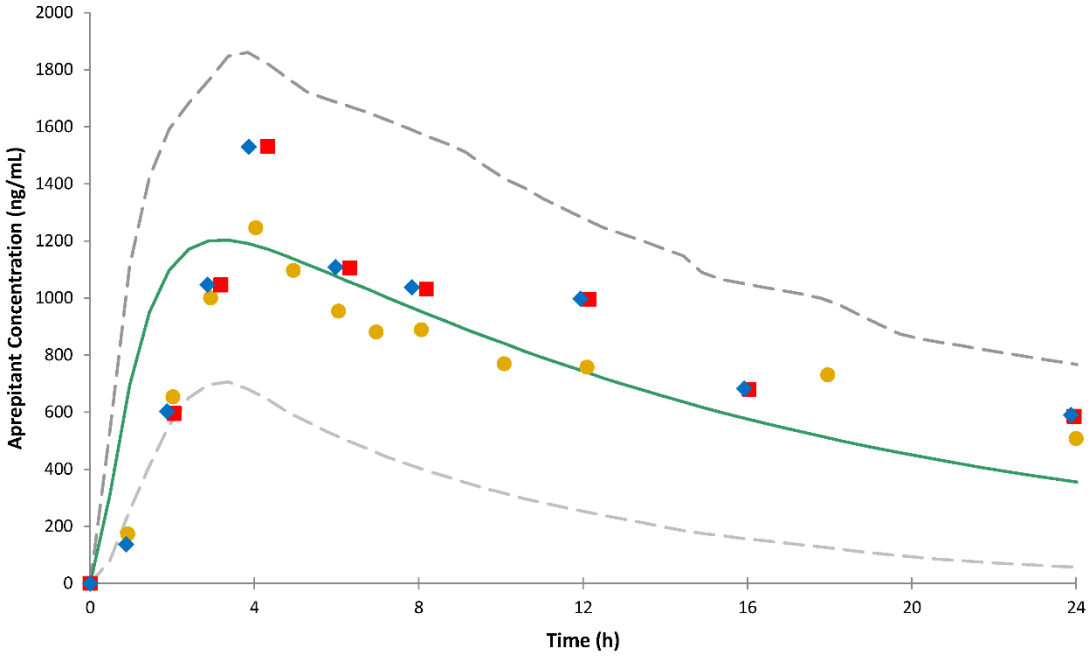
680 **Figure 10**



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