



Physiologically Based Biopharmaceutics Modeling for Gefapixant IR Formulation Development and Defining the Bioequivalence Dissolution Safe Space

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Abstract

Gefapixant is a weakly basic drug which has been formulated as an immediate release tablet for oral administration. A physiologically based biopharmaceutics model (PBBM) was developed based on gefapixant physicochemical properties and clinical pharmacokinetics to aid formulation selection, bioequivalence safe space assessment and dissolution specification settings. *In vitro* dissolution profiles of different free base and citrate salt formulations were used as an input to the model. The model was validated against the results of independent studies, which included a bioequivalence and a relative bioavailability study, as well as a human ADME study, all meeting acceptance criteria of prediction errors $\leq 20\%$ for both C_{max} and AUC. PBBM was also applied to evaluate gastric pH-mediated drug-drug-interaction potential with co-administration of a proton pump inhibitor (PPI), omeprazole. Model results showed good agreement with clinical data in which omeprazole lowered gefapixant exposure for the free base formulation but did not significantly alter gefapixant pharmacokinetics for the citrate based commercial drug product. An extended virtual dissolution bioequivalence safe space was established. Gefapixant drug product batches are anticipated to be bioequivalent with the clinical reference batch when their dissolution is $> 80\%$ in 60 minutes. PBBM established a wide dissolution bioequivalence space as part of assuring product quality.

Keywords bioequivalence safe space · biopharmaceutics · dissolution specification · GastroPlus · gefapixant · IR formulation selection · pharmacokinetics · physiologically based biopharmaceutics model(s)(ing) (PBBM)

Introduction

Gefapixant (MK-7264, AF-219) has been developed as an immediate release (IR) drug product for the treatment of chronic cough. It is a weak base with pH-dependent solubility; and based on the equilibrium solubility and moderate permeability Caco-2 data gefapixant would strictly be categorized as a BCS 4 drug. Gefapixant free base exhibited a pH-mediated drug-drug interaction (DDI) in the clinic when co-dosed with

a proton-pump inhibitor (PPI) drug, omeprazole (1), resulting in exposure reduction by nearly 50%. Hence, mitigation of the PPI – DDI effect using a formulation strategy was explored. A successful gefapixant formulation containing its citrate salt, which exhibited high solubility across physiological pH conditions, was used in support of late phase clinical studies without PPI-DDI. Gefapixant citrate-based formulation was bridged to the early free base formulation via a relative bioavailability (rBA) clinical study and showed comparable PK performance under normal fasted dosing conditions.

Over the last decade, PBBM has been used to prospectively predict oral formulation bioperformance by integrating *in vitro* dissolution and drug properties by both pharmaceutical industries and regulatory agencies (2–8). PBBM has been used widely in drug development applications such as drug absorption evaluation, biopharmaceutics risk assessment, food effect, PPI-DDI prediction (9, 10) and *in vitro-in vivo* correlations. PBBM has been used to set clinically relevant specifications for *in vitro* dissolution by defining the bioequivalence safe-space for the dissolution failure edge.

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Case examples of PBBM applications are reported in many publications (11–16).

The goal of this work was to develop a PBBM using multiple independent clinical studies (1) to (a) simulate and assess gefapixant oral absorption and bioavailability, (b) assess pH-mediated DDI risks for the commercial product and (c) develop an *in vitro* bioequivalent safe space to ensure drug product quality. *In vitro* dissolution was incorporated using an apparent Product Particle Size (PPS) model for fitting *in vitro* dissolution profile data and simulations as input to PBBM. This PPS model was validated against multiple independent clinical studies with both early prototype free base and late phase citrate salt formulations. Although gefapixant could be classified as a BCS IV drug based on equilibrium solubility and Caco-2 permeability data, PBBM in combination with clinical PK and ADME data provides additional insights to gefapixant citrate *in vivo* behavior which is overall more consistent with that of a BCS I compound.

Materials and Methods

Drug Substance: Gefapixant is a weak base and two basic pKa values of 6.4, 10.2 and a logD of 0.72 at pH 7.4 were measured. Gefapixant was synthesized by Merck & Co., Inc. (Rahway, New Jersey, USA). Two forms of gefapixant, the free base and citrate salt, were used over the course of the clinical formulation development. The equilibrium solubility of the free base is high (5.5 mg/mL) at normal stomach pH (target pH 1.8; final pH 3.7) but low at intestinal pH (0.02 mg/mL at target pH 6.8 (final pH 6.7)). Gefapixant citrate has a higher solubility than the free base form with 11 mg/mL at target pH 1.8 (final pH 2.8) after 24 h. However, the citrate salt quickly converts to the free base at higher pH conditions e.g. pH 6.8 in a few minutes and starts precipitation, which makes it challenging to measure the equilibrium solubility at higher pH conditions. To accurately measure the kinetic solubility of the citrate salt and free base during the dissolution and absorption timeframe, intrinsic dissolution rates (IDR) were measured and used for solubility calculation for higher pH later on. The IDR experiment was carried out in triplicate using a Pion surface imaging SDi2 system (Pion Inc. Ltd) (17). Gefapixant free base and citrate salt powder ~ 10 mg was compacted using a 100 kg load and placed into a flow cell. Then pH 6.8 potassium phosphate buffer was introduced into the cell at a flow rate of 1.0 mL/min. The SDi2 flow cell was illuminated using alternate LED 1s pulsing at 255 nm and 280 nm wavelengths to measure the solubility of gefapixant citrate as well as that of the free base.

IDR measurements were used to inform the solubility settings in GastroPlus PBBM. The apparent solubility for both

gefapixant citrate salt and free base were measured as 10 mg/mL at pH 1.2. The solubilities of the citrate salt and free base at pH 4.5 and pH 6.8 were calculated using their respective corresponding IDR values against that of the pH 1.2 assuming proportionality. The final apparent pH-solubility profiles of this IDR measurements were entered as inputs to the PBBM in GastroPlus™ and the full experimental results can be found in Appendix Table S1.

Drug Product

Gefapixant was originally isolated as a crystalline free base anhydrate for early formulation development. The first-in-human formulation, F01, at 50 mg potency is an IR tablet containing free base. The free base F01 formulation exhibited suboptimal bioperformance as it showed a reduced bioavailability by more than 50% in AUC and C_{max} when co-dosed with a PPI in Phase 1 clinical studies comparing to normal stomach pH conditions (1). A F02 formulation with added acidulant, citric acid, was developed and no PPI-DDI was observed. However, high PK variability was observed, likely due to variable amount of *in-situ* salt formation from the free base and citric acid excipient. Gefapixant free base formulations F01 and F02 were tested at a 50 mg strength.

The pH-mediated omeprazole DDI liability with the free base led to the selection of gefapixant citrate. For late phase development, citrate salt based formulations -film-coated tablets (FCT) (F04, F04A tablets) and commercial drug product tablets (F04B) were chosen. Those citrate salt formulations are highly similar in formulation composition with minor differences in excipients e.g. lubricant or disintegrant for optimized process robustness. The citrate salt formulations successfully maintained solubilized drug in higher pH conditions and negated the pH-mediated DDI effect from omeprazole of the free base formulation (1). Citrate salt formulations F04, F04A and commercial drug product F04B were developed as 15 mg and 45 mg tablets. As only the 45 mg strength was registered, filed and commercialized for appropriate markets, PBBM are primarily focused on the PK of the 45 mg dose.

In Vitro Dissolution

Multi-media dissolution profiles were generated using USP Apparatus I (40-mesh baskets) at 100 rpm in 900mL of pH 1.2, 4.5, 6.8 dissolution media for similarity comparison per ICH M9 guidance (18). Gefapixant F04A and F04B FCTs exhibited very rapid dissolution profiles with $\geq 85\%$ dissolved in 15 min (min) for the 15 mg and 45 mg strengths (Fig. 1, F04A, F04B 45 mg potency). Similar dissolution behavior was obtained for the F04 formulation which is discussed in later sections.

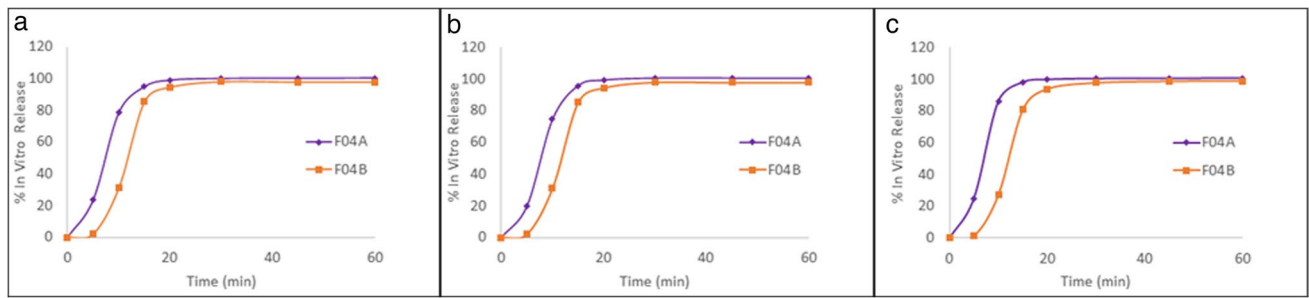


Fig. 1 Dissolution profiles of 45 mg F04A and F04B film-coated tablets, in 900 mL of **a** pH 1.2, **b** pH 4.5, **c** pH 6.8 media via apparatus I (40 mesh basket) at 100 RPM

The dissolution of F04A and F04B biobatches was also conducted with a proposed release quality control (QC) method for the BE study using Apparatus I (40-mesh baskets) at 100 rpm in 900mL of 10 mM pH 6.8 potassium phosphate buffer dissolution media. Similarly, gefapixant F04A and F04B FCT exhibited very rapid dissolution profiles for both potencies with more than 85% dissolved in 15 min, except for F04B FCT of 45 mg strength which showed a slightly slower dissolution (81% dissolved in 15 min).

As a part of early formulation development, *in vitro* dissolution was also tested for the free base formulation F01 and F02 in pH 6.8 buffer as the worst scenario for assessment of elevated pH impact on gefapixant release from acid reducing agents including PPI and H2 blockers. While full drug release in low pH 1.2 conditions for both formulations was observed, the higher pH 6.8 medium significantly decreased gefapixant F01 release by ~30%, suggesting a possible pH-mediated DDI risk for the prototype F01 formulation under elevated stomach pH conditions with acid reducing agents.

Clinical Pharmacokinetic Studies

All clinical studies were funded by Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA and conducted in conformance with applicable country

or local requirements regarding ethical committee review, informed consent, and other statutes or regulations for the protection of the rights and welfare of human participants in biomedical research and Good Clinical Practice. Respective study protocols were reviewed and approved by Independent Ethics Committee.

- (1) *A Phase I single-ascending-dose (SAD) study* in which gefapixant exposure (AUC and C_{max}) increased in a dose proportional manner through the range of 10~450 mg. The SAD studies were supported with gefapixant suspension in an acidic vehicle.
- (2) *A Study in Healthy Subjects to Assess the Pharmacokinetics of gefapixant F01 formulation* as described by Gupta (1). This study was a part of a series of multi-day, non-crossover, sequential dosing clinical studies with no wash-out, combining fasted, fed and pH-mediated DDI studies for assessment of food, PPI medicines on the F01 PK performance, respectively. The effect of co-administration of a PPI – omeprazole was evaluated on the PK of the F01 formulation. Comparing to the control fasted state pharmacokinetics, co-administration of omeprazole with the free base F01 formulation decreased the AUC and C_{max} of gefapixant by more than 50% (1) (Table I).

Table I Gefapixant Plasma Pharmacokinetics following the Administration of Single Oral Doses of Gefapixant Free Base F01 Formulation, Free Base F02 and Citrate Salt F04 Formulations (rBA) (1),

and Citrate Salt F04A/F04B Formulation in BE (1) in Healthy Participants under Fasted Conditions

PK parameter	Relative Bioavailability	Relative Bioavailability		Bioequivalence	
	F01 Free Base + Omeprazole/F01 (fasted, 50 mg)	F04/F02 Citrate (fasted, 50 mg)	F04 Citrate + Omeprazole/F04 (fasted, 50 mg)	F04B/F04A Citrate (fasted, 15 mg)	F04B/F04A Citrate (fasted, 45 mg)
GMR* (90% CI)		GMR (90% CI)	GMR (90% CI)	GMR (90% CI)	GMR (90% CI)
AUC _{0–inf} or 0–t	0.41 (0.35–0.47)	1.16 (0.99–1.35)	0.97 (0.90–1.05)	1.01 (0.98–1.06)	0.97 (0.93–1.01)
C _{max}	0.33 (0.28–0.38)	1.32 (1.00–1.76)	0.91 (0.82–1.02)	1.02 (0.96–1.08)	0.95 (0.86–1.04)

*GMR geometric mean ratio

- (3) *A relative BA study for pharmacokinetic comparison of the free base formulation F02 vs. citrate formulation F04.* This was an open label, single-dose relative bioavailability study that was conducted to evaluate the PK and bioperformance of gefapixant F04 formulation relative to the F02 formulation. The F04 was also administered in the fasted state with co-administration of 40 mg of omeprazole for assessment of potential DDI effect with PPIs. The result indicated a comparable PK performance for the F04 to the F02 formulations. Under fasted conditions, gefapixant exposure following administration of F04 was slightly higher than the F02 with geometric least-squares mean ratio (GMR) (F04 fasted/F02 fasted) at 1.16 and 1.32 for $AUC_{0-\infty}$ and C_{max} , respectively (Table I). The GMRs of AUC or C_{max} with co-administration of omeprazole to the fasted state are 0.97 or 0.91, respectively.
- (4) *A BE study (1) between the Phase 3 F04A and commercial drug product F04B at two clinical doses 15 mg and 45 mg.* This is an open-label, two-part, two-period crossover study to determine bioequivalence between gefapixant F04A and F04B formulations at 45 mg and 15 mg single doses in healthy participants. The BE study results showed that the GMRs and 90% CI of gefapixant AUC and C_{max} for the F04B vs. F04A formulations fell within the [0.8, 1.25] prespecified bound (Table I), supporting bioequivalence between the commercial drug product F04B and phase 3 F04A formulations.
- (5) *Human ADME (Absorption, Distribution, Metabolism, Excretion) study (19).* Gefapixant absorption was estimated to be at least 78% based on human ADME study result.

In Silico PBBM

Gefapixant PBBM was built using the advanced compartmental absorption and transit model (ACAT) which models the human gut physiology (GastroPlus™ software, v.9.7, Simulation Plus, Inc., Lancaster, CA, USA). A scheme illustrating the development of gefapixant PBBM is shown in Fig. 2. Gefapixant pharmacokinetics used in this PBBM were determined in several clinical studies as described below and summarized in Table II along with drug substance physicochemical properties.

Physiology

For human physiology settings in GastroPlus™, gastric pH was set at 1.3 (using the default Opt logD Model SA/V 6.1) except for simulations of PPI co-dosing condition in

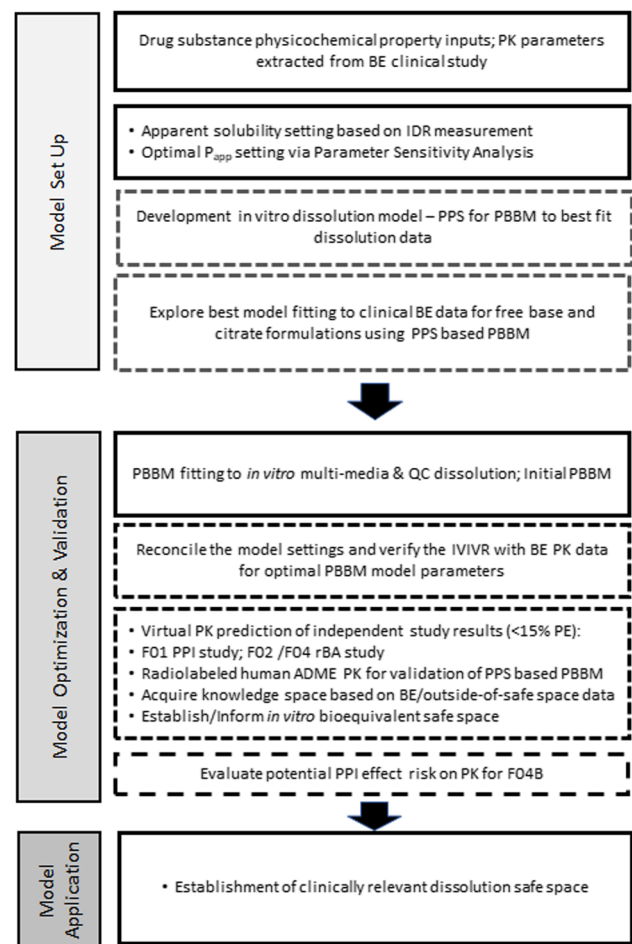


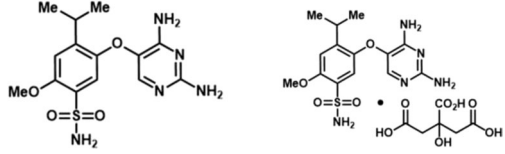
Fig. 2 Gefapixant PBBM development scheme

which the gastric pH was set at 6.8 as the most conservative estimate. Bile micelle solubilization was not included in the model as gefapixant does not exhibit food effect (20). Given the low logD value and high apparent solubility of gefapixant citrate in intestinal range, the impact of drug partitioning into bile micelles on solubilization was considered negligible.

Pharmacokinetic Parameters

Gefapixant PK parameters were derived using the PKPlus module in GastroPlus™ by fitting into individual gefapixant plasma concentration vs. time profiles of the BE PK data. A pooled PK data at 15 mg and 45 mg doses from both gefapixant F04A and F04B formulations were used and fitted to a 2-compartment pharmacokinetic model. Gefapixant pharmacokinetic parameters derived from the suitable fitting in PKPlus module were used in the PBBM simulation: $CL/F = 0.161$ L/h/kg, $Vc/F = 1.16$ L/kg, $K_{12} = 0.048$ h⁻¹, $K_{21} = 0.074$ h⁻¹ (Table II). For the

Table II Gefapixant PBBM Input Parameters

Gefapixant Parameter	Used value in PBBM
Molecular Structure (free base, citrate salt)	
Molecular Weight (g/mol)	353.4 (free base, molecular formula C ₁₄ H ₁₉ N ₅ O ₄ S) 545.52 (citrate salt, molecular formula C ₁₄ H ₁₉ N ₅ O ₄ S.C ₆ H ₈ O ₇)
logD (pH 7.4)	0.72 (measured)
pK _a	6.4, 10.2 (measured)
Apparent solubility (mg/mL)* for PBBM	Free base: 10.0 (pH 1.2), 0.82 (pH 4.5); 0.19 (pH 6.8) Citrate salt: 10.0 (pH 1.2); 9.5 (pH 4.5); 9.0 (pH 6.8)
Dosage form/Formulations	IR Tablet F01 (free base tablet) F02 (free base tablet containing acidulent – citric acid) F04, F04A, F04B (citrate salt based tablet batches)
Passive intestinal permeability	Caco-2 (A-B): P _{app} 0.634 × 10 ⁻⁶ cm/s Human effective permeability: P _{eff} : 2.5 × 10 ⁻⁴ cm/s. Optimized top down with PSA, consistent with human radiolabeled ADME data of at least 78% absorption (20).
B/P ratio, fu%	B/P ratio = 1.1, fu = 45.3%
Dose (mg)	15, 45, 50
Dosing volume (mL)	250
Dissolution models	Johnson, Product Particle Size (PPS) model, bile salt solubilization effect not included
D _{eff}	0.703 × 10 ⁻⁵ cm ² /s (calculated from MW on GastroPlus)
Precipitation time (s)	900 (default)
Drug particle density (g/ml)	1.2 (default)
CL/F (L/hr/kg)	0.161 (PKPlus)
Vc/F (L/kg)	1.16 (PKPlus)

* Calculated based on Intrinsic Dissolution Rate (IDR) experiment; Details in Tables S1

purposes of modeling, an average of 70 kg human body weight was used.

In Vitro Intestinal Permeability

Gefapixant permeability was measured as 0.634×10^{-6} cm/s in Caco-2 cell monolayers, which converts to human $P_{\text{eff}} < 1.0 \times 10^{-4}$ cm/s based on an internal correlation for initial modeling assessment. Gefapixant absolute bioavailability was assessed to be at least 78% in the human ADME study (19), indicating moderate to high absorption. In addition, a higher absorption rate constant ($k_a = 2.57 \text{ h}^{-1}$) derived from BE PK data using GastroPlus™ PKPlus support a higher permeability. Gefapixant human P_{eff} was optimized top down through Parameter Sensitivity Analyses (PSA) by optimized fitting to observed clinical BE PK data. The PBBM simulation showed better fitting

to the F04B PK profiles using 2.5×10^{-4} cm/s with prediction error of 6% or 4% for the AUC and C_{max} , respectively as compared to -68% and -83% with the Caco-2 based permeability setting of 1.0×10^{-4} cm/s. Therefore, a P_{eff} setting of 2.5×10^{-4} cm/s was applied to the PBBM. While there could be some uncertainty in the precision of the P_{eff} fitted against the clinically observed fraction absorbed ($\geq 78\%$), the increased permeability of 2.5×10^{-4} cm/s leads to more sensitivity of dissolution on PK as permeability is not rate controlling systemic exposure.

Dissolution Model for PBBM

Gefapixant dissolution data were fitted via a custom Product Particle Size (PPS) model. The PPS model with the Johnson dissolution set-up was used to describe the *in vitro* dissolution of gefapixant formulations. The Johnson model

calculates the *in vitro* dissolution based on a modified Noyes-Whitney equation taking the particle size into account. To achieve PPS value from dissolution profiles, *in vitro* dissolution is simulated in GastroPlus™ by adjusting the ACAT model with stomach transition time of 999,999 h (effectively keeping the drug inside stomach compartment). The stomach pH of 1.2, 4.5 or 6.8 is applied to reflect the dissolution of the drug product in specific media; 900 mL is to reflect the volume of the *in vitro* dissolution study. The dosage form is set as “IR Tablet” in GastroPlus™. For *in vivo* modeling using the PPS model, the dissolution model is set as Johnson Model. Illustration of the PPS model process is shown in Supplemental Figure S1. It is important to note here that the PPS value used by the PBBM is as a “drug substance” particle size input in GastroPlus™, however it represents the drug product behavior as a whole, comprises the effect of formulation wetting, disintegration, drug substance dispersion, eventually drug dissolution and release. Thus, the PPS input is a mathematical factor and should not be considered as the actual particle size of the API in the formulation.

Other Settings

Gastric emptying time for all PK simulations was 0.25 h. A Mixed Multiple Dose (.mdd) support file was created to introduce a disintegration lag time (T_{lag}) for both *in vitro* and *in vivo* simulations in the PPS model. Based on the observed dissolution data in multiple media pH 1.2, 4.5, 6.8 and pH 6.8 QC media, T_{lag} was set as 5 min and 8 min for F04A

and F04B formulation respectively in the PPS based PBBM. Default values from GastroPlus™ were used for the drug “Mean Precipitation Time” and “Particle Density”.

Formulation Selection for PBBM

The presented model focused on the intended commercial formulation (F04B) and the Phase 3 formulation (F04A) in the BE study. Ideally, PBBM would employ data from a non-bio-equivalent batch. Several alternate formulations like free base F01 and F02 were explored earlier in the product development. The IR F02 formulation containing an acidulent – citric acid was comparable in PK to the citrate formulation F04. The free base F01 formulation was explored as its exposure was significantly impacted by PPI co-administration and was decreased by more than half under elevated intragastric pH conditions.

PBBM Acceptance Criteria

Prediction error (PE) was calculated as : PE%

$$= (\text{simulated} - \text{observed}) / (\text{observed}) \times 100$$

The model acceptance criteria for PPS based PBBM simulations were defined as $PE \leq 20\%$ comparing to the observed PK results (21) such as AUC and C_{max} based on *in vitro* fitting to dissolution data as inputs via a PPS value. Different PPS values for different *in vitro* conditions are obtained by closely fitting to their drug release profiles, then used the same PPS values in PBBM for PK simulation and AUC/ C_{max} results as comparison to the observed PK data.

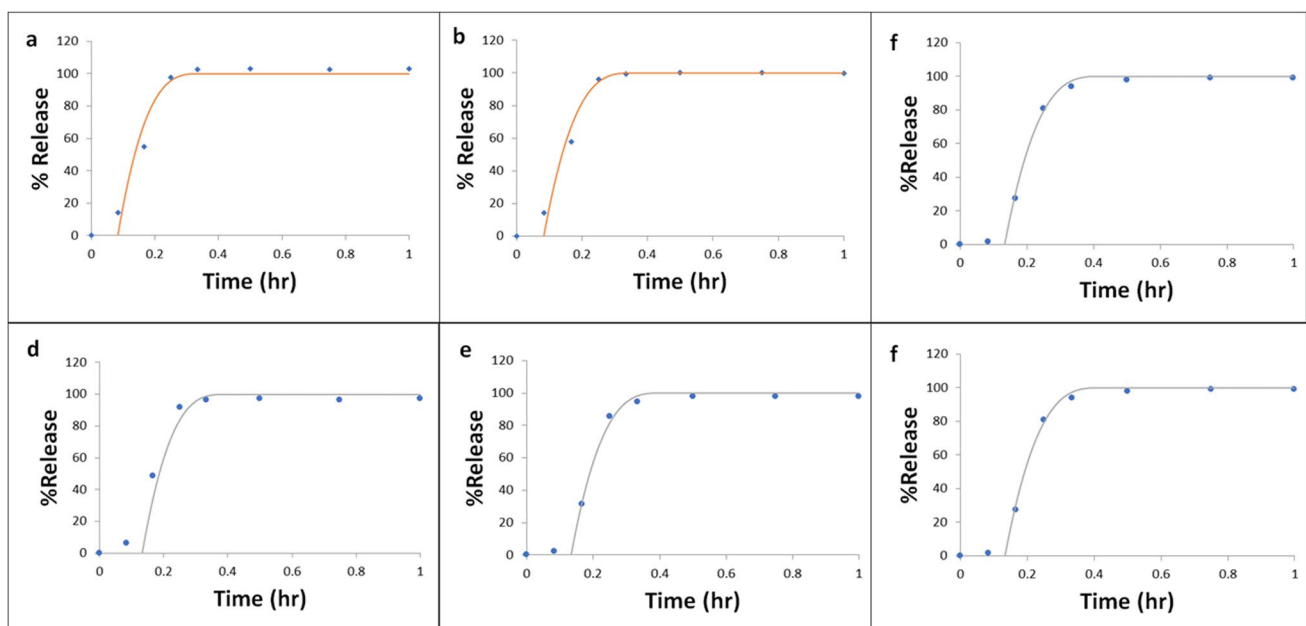


Fig. 3 The simulated vs observed dissolution profiles for the citrate formulation F04A (top row) and F04B (bottom row) in QC media; pH 1.2 (a and d), pH 4.5 (b and e) and pH 6.8 (c and f)

Once these criteria were fulfilled, model applications were conducted.

Results

Simulation of Multimedia and QC Dissolution Profiles in the PPS Model

Simulations of dissolution profiles in multimedia (Fig. 3. F04A and F04B 45 mg in 900 mL of pH 1.2, pH 4.5 buffer, and pH 6.8 QC media) were conducted using the methods

described in the PPS model. For the purpose of calculating dissolution kinetics, the fittings of *in vitro* dissolution profiles for F04A and F04B were shifted by applying a T_{lag} of 5 min and 8 min, respectively. For both formulations, an estimation of $PPS = 360 \mu\text{m}$ provided a good fit of *in vitro* dissolution profile in all three above dissolution media.

Based on the simulation results, a single PPS value of $360 \mu\text{m}$ was obtained in achieving satisfactory fits of the dissolution profiles for the F04A and F04B generated in all three pH 1.2, 4.5 and 6.8 QC media.

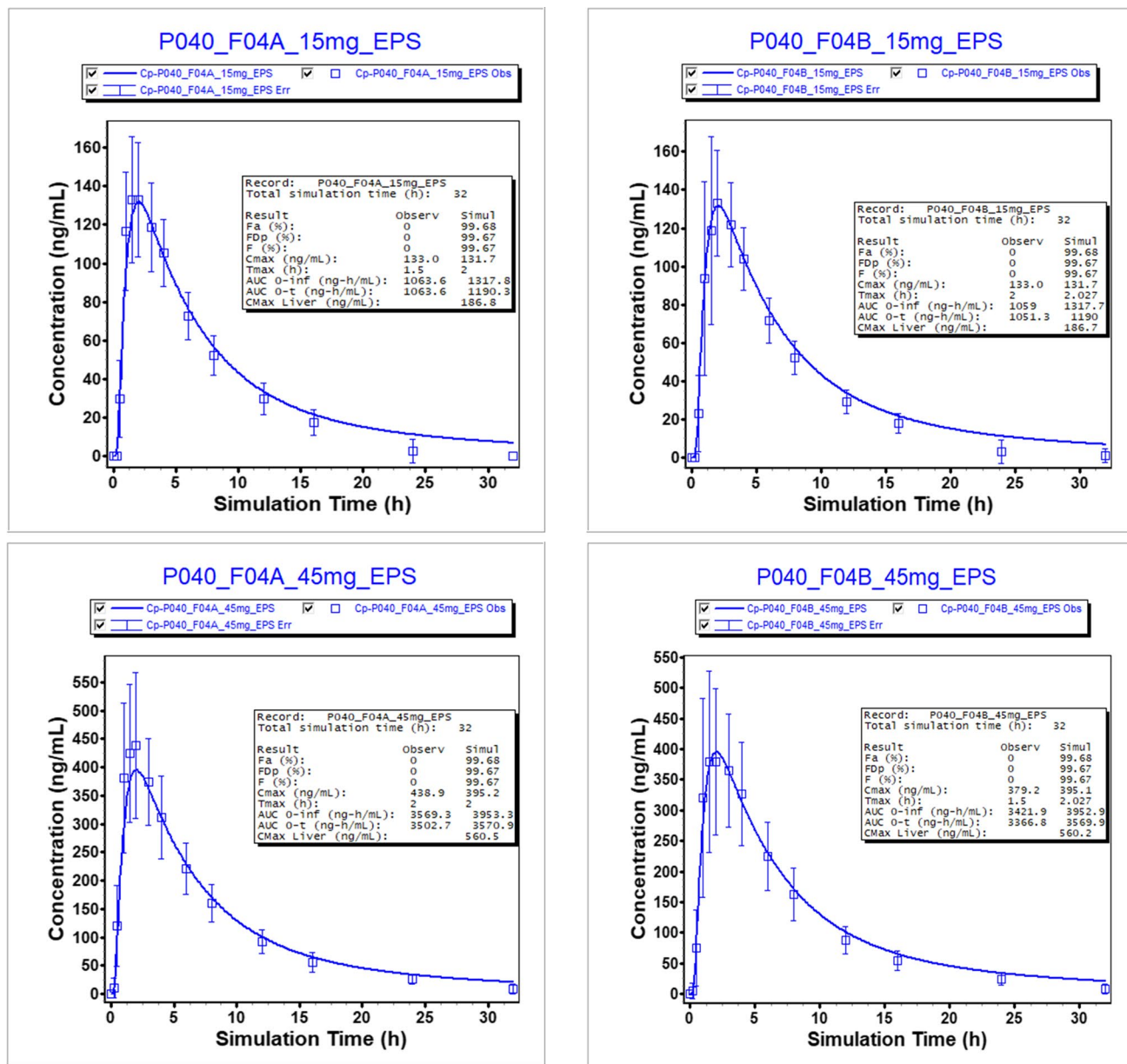


Fig. 4 Simulated versus observed PK profiles for citrate formulations F04A and F04B using the PPS-based PBBM

Table III Comparison of Simulated and Observed PK Parameters with Predictive Errors for the Citrate Formulations F04A and F04B using the PPS-based PBBM

Dose/PPS/T _{lag}	AUC _{0–32 h} (h*ng/mL)	C _{max} (ng/mL)	T _{max} (hr)	AUC_PE%	C _{max} _PE%
F04A					
15 mg /360 µm/5 min	1190	132	2.0	12	-0.8
*Observed 15 mg	1064	133	1.5	Reference	Reference
45 mg / 360 µm /5 min	3571	395	2.0	2.0	-10
*Observed 45 mg	3503	439	2.0	Reference	Reference
F04B					
15 mg /360 µm/8 min	1190	132	2.0	13	-0.8
*Observed 15 mg	1051	133	2.0	Reference	Reference
45 mg /360 µm /8 min	3570	395	2.0	6.0	4.2
*Observed 45 mg	3367	379	1.5	Reference	Reference

*Calculated using GastroPlus

PBBM Simulation of *In Vivo* PK Profiles of Citrate F04A and F04B Formulations with the PPS Model

Following *in vitro* dissolution simulation with PPS model, PBBM simulations were conducted in comparison to the observed plasma concentration profile for gefapixant F04A and F04B formulations at both 15 mg and 45 mg doses (Fig. 4). The comparison of modeling results *versus* observed data is shown in Table III, the %PEs are within 15% for either AUC or C_{max} ratios in all simulations.

Validation of PBBM via Simulation of Formulations F02 and F04 PK

As discussed earlier, gefapixant F02 formulation exhibited varying levels of *in-situ* salt formation across batches. Due to the presence of less soluble free base and more soluble *in-situ* citrate salt, the F02 formulation displayed slower sigmoidal-like dissolution profiles in pH 4.5 and 6.8 media that could not be closely simulated by this PPS model. Therefore, only the F02 dissolution data at pH 1.2 were used for the PPS modeling and compared to the F04 formulation.

Simulation of dissolution profiles for the F02 and F04 formulations in pH 1.2 medium was done using the PPS model as described in Fig. 3. For the purpose of calculating dissolution kinetics, the fittings of *in vitro* dissolution profiles were shifted by applying a T_{lag} of 2 min for the F02 or 5

min for the F04 formulations (Supplemental Figure S2). An estimation of PPS = 1100 µm for the F02 or 480 µm for the F04 provided a good fit of their *in vitro* dissolution profiles. The PPS value for the F04 is in line with the F04A and F04B formulations as expected given the composition similarity.

Using the estimated PPS settings, the model successfully simulated the clinical PK profiles of the F02 and F04 formulations as shown in Supplemental Figure S3. The PE% are within 15% for AUC and C_{max} ratios for both simulations (Table IV).

PBBM Validation via Human ADME PK Simulation

The dissolution profile of gefapixant free base oral suspension in pH 1.2 medium was simulated using the PPS model at a value of 280 µm. Based on the *in vitro* PPS input, a PBBM simulation of the human ADME PK was carried out at the clinical dose 50 mg and resulted in a PE of 18.1% for the mean AUC and 7.7% for the mean C_{max} comparing to the observed clinical PK data (20). Comparison of the simulated *in vitro* dissolution and *in vivo* PK profiles to the observed data are shown in Supplemental Figure S4 (A & B), respectively.

Sensitivity Analysis of PPS on Gefapixant PK Exposure

A PSA was conducted using different PPS values as the dissolution rate input for PK simulation to study their impact

Table IV Simulated *versus* Observed PK Parameters for the Free Base Formulation F02 and the Citrate Formulation F04 using the PPS-based PBBM

	AUC _{0–48 h} (h* ng/mL)	C _{max} (ng/mL)	T _{max} (h)	%PE_AUC	%PE_C _{max}
Simulated F02	3856	346	2.1	15	1
*Observed F02	3346	341	3.0	Ref	Ref
Simulated F04	4220	439	2.0	7	12
*Observed F04	3946	391	2.0	Ref	Ref

*Calculated using GastroPlus™

on gefapixant absorption. Using $PPS = 360 \mu\text{m}$ and $T_{lag} = 5 \text{ min}$ as the benchmark reference, the PSA results indicated that the estimated PPS cutoff value for C_{max} fall below 5% at $1900 \mu\text{m}$ or 10% at $2743 \mu\text{m}$, respectively (Supplemental Table S2). The estimated PPS cutoff value for AUC to fall below 5% of that of the reference was greater than $2800 \mu\text{m}$.

In addition, the effect of lag time on absorption of gefapixant were studied at $T_{lag} = 0 \text{ min}$, 15 min and 30 min , respectively, for both 15 and 45 mg doses at the PPS of $2743 \mu\text{m}$, which well covered the PPS of F04A and F04B BE batches and represented the worst case scenario regarding the T_{lag} effect. The simulations at T_{lag} of 30 min should cover the T_{lag} of F04A and F04B formulations ($5 \sim 8 \text{ min}$) as well as the normal gastric emptying time in fasted state. The PBBM result indicated that effect of T_{lag} on exposure was minimal in all simulated conditions (Supplemental Figure S5).

PBBM of pH-Mediated DDI for Gefapixant Formulations

Assessment of the PPI-DDI effect was also conducted as further validation and application.

The PBBM was explored for the simulation of the F01 PPI-DDI study results reported by Gupta (1). The PBBM PK profile fitting was done by incorporating a PPS value of $820 \mu\text{m}$ for pharmacokinetics at normal fasted state and $3900 \mu\text{m}$ for the PPI co-administration, respectively. PE values for both mean AUC and C_{max} are less than 10% of the fitted PK as compared to the observed results (Table V). The F01 dissolution data was not incorporated in the PPS model and PBBM fitting due to high carry-over to the PK profiles and high variability from the previous multi-day dosing administration without wash-out (PK fitting can be seen in Supplemental Figure S6). A stomach pH setting at pH 5.0 in mimicking the conditions with PPIs was also explored without altering other model conditions; the PBBM simulation showed a good fit to the observed clinical PK profiles with a $PE\% < 12\%$ for AUC or C_{max} .

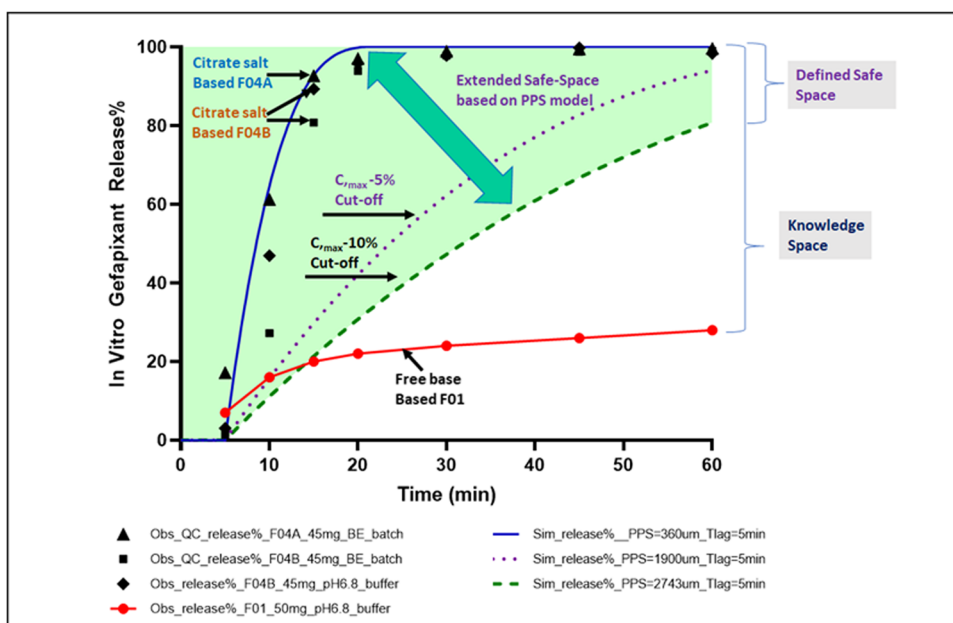
Impact of gefapixant PK for the drug product F04B for potential dissolution slow-down like co-administration with a PPI medicine was assessed at PPS values of $1900 \mu\text{m}$ and $2743 \mu\text{m}$ as shown in Supplemental Table S2 and also in safe space development below.

Table V Comparison of Gefapixant PK Parameters from PPS PBBM versus Observed Data for the Free Base Formulation F01 PPI-omeprazole Study Result using the PPS-based PBBM

	AUC _{0-12 h} (h* ng/mL)	C _{max} (ng/mL)	%PE _{AUC}	%PE _{C_{max}}
PPS PBBM F01 normal fasted (PPS = 820 μm)	3699	437	6.4	2.0
*Observed F01 normal fasted	3475	446	Ref	Ref
PPS PBBM F01 with PPI-fasted (PPS = 3900 μm)	1567	144	9.1	0.7
*Observed F01 with PPI-fasted	1436	145	Ref	Ref

*Calculated using GastroPlus™

Fig. 5 Established clinically-relevant dissolution safe space for gefapixant formulation using the PPS-based PBBM



Development of Dissolution Safe Space and Prediction of Dissolution Failure Edge

An extended *in vitro* dissolution safe-space (Fig. 5) was developed for gefapixant formulations based on the PPS PBBM as well as the clinical BE result of the citrate F04A/F04B formulations and non-bioequivalent PK data of the free base F01 formulation. A knowledge space and dissolution failure edge were obtained upon the outcome of the clinically relevant dissolution safe space. The dissolution safe space with a clinically-relevant dissolution limit (shaded area in green) was established at a PPS cutoff value of 2743 μm for a 10% decrease of C_{max} comparing to the reference formulation as a conservative approach to ensure drug product quality. The percentage of *in vitro* release for a formulation with PPS = 2743 μm is back calculated as approximately 47.3% release in 30 min, or 80% release at 60 min.

A tested formulation is considered to be bioequivalent to the reference drug product when its *in vitro* drug release profile falls into the extended green safe space with $\geq 80\%$ at 60 min.

Discussion

Gefapixant PPS based PBBM successfully simulated the observed PK outcome of the citrate F04A/F04B formulations in the BE study, and the PK results of the citrate F04/free base F02/F01 formulations. All simulation predictive errors of the PK simulation had met the acceptance criteria of $\leq 20\%$. The PBBM was also applied to the assessment of pH-mediated PPI-DDI effects for F01, F04B (equivalent to F04) and achieved a good agreement to their clinical data. A clinically relevant safe space was established using the PBBM to define dissolution failure edge for formulation batches for quality control of scale-up and manufacture process. The safe space was supported by clinical BE (F04A/F04B), rBA study (F04/F02), non-bioequivalent PPI PK data of F01 formulation and widened the knowledge space.

This custom PPS model reported herein has shown a unique advantage by combining drug physicochemical properties along with its physiological conditions in describing drug product's dissolution behavior in an *in vivo* setup where the drug product dissolution takes place. The custom PPS model has been demonstrated to be successful by its well-fitting to all citrate salt formulations – F04, F04A, F04B multi-media dissolution profiles and pH 1.2 F02 dissolution data. As shown in Fig. 4, the fitted product particle size has reflected a good simulation of the multi-media dissolution profiles for the F04A and F04B with a single PPS value. The PPS model also has strengthened the confidence of solubility settings in GastroPlus™

and the biopredictability of this PPS-PBBM on the dissolution of gefapixant formulations. Application of this PPS model on gefapixant formulations indicates it as a useful alternative tool in describing drug product's dissolution. The good agreement as demonstrated by reasonable PBBM simulated PK profiles for independent clinical studies - (a) gefapixant free base F02 tablet, (b) free base suspension in the human ADME study, and (c) citrate salt tablets (F04, F04A, F04B) relative to their observed clinical results - provided additional strong support to the suitability of the developed model conditions for gefapixant formulations. Limitations, however, were observed for the PPS model in simulating the free base F02 formulation's dissolution data in pH 4.5 and 6.8 buffer as previously stated, due to the presence of mixed API phases (free base and *in-situ* salt) in the formulation. This created sigmoidal dissolution profiles that could not be closely fitted using this PPS model and applied for the PK modeling.

Utilizing PBBM in assessment of DDI risk with ARA/PPIs has gained great interests in recent years, especially for weak base drugs where the bioavailability could be reduced in presence of ARA/PPI with elevated stomach pH (6, 9, 10, 12). In normal stomach pH conditions (e.g. pH 1.8), the systemic bioavailability of gefapixant free base formulation was overall similar to the citrate salt formulation as shown in Tables I, IV and V. However, a reduced exposure between the free base formulation and citrate salt formulation was observed when in the presence of a PPI/ARA, omeprazole. With elevated pH stomach conditions, limited solubility of free base form was observed, likely causing lower drug exposure while the citrate salt formulation did not have such PPI effect.

By simulation the PK outcome under elevated stomach pH conditions observed under PPIs, the PBBM can predict the PK outcome and risk of ARA (acid-reducing-agent)-DDI in comparison to normal fasted stomach conditions. The PPS based PBBM herein has successfully simulated the PPI-DDI effect on gefapixant free base formulation F01 with PE values less than 10% for both AUC and C_{max} , demonstrating the successful PBBM conditions and application for gefapixant.

The PBBM result is in a good agreement with the significantly lower drug release of the F01 free base formulation in pH 6.8 dissolution (Fig. 5). Therefore, the F01 formulation is considered non-bioequivalent to the citrate formulations F04, F04A or F04B under PPI coadministration conditions. Low PPI-DDI risk for the commercial drug product F04B was predicted at the PPS settings up to the clinically relevant dissolution limit – 2743 μm (Fig. 5) and with less than 10% reduction in C_{max} . The outcome is in line with the clinical BA PK result of the F04 formulation (Table III), which was bridged indirectly to the commercial formulation F04B with equivalent PK performance. In addition, PBBM projected dose-linear exposure gefapixant PK that corresponds to the

Phase 1 SAD clinical data, supporting suitability of PPS-based PBBM conditions for gefapixant.

PBBM, which links *in vitro* dissolution data across formulations to *in vivo* PK profiles, has found significant application for drug development via quality by design. It can quantitatively define *in vitro* formulation dissolution BE failure limit and make reasonable assessment of its *in vivo* PK performance (2–4, 7, 13, 22–24). A gefapixant PBBM strategy described here was developed for the following purposes: (a) early selection of formulations with desirable PK (free base vs. salt), (b) selection of a formulation without undesirable pH-mediated DDI, (c) establishment of the dissolution limit for BE boundaries and (d) justification and support of dissolution specifications using a PBBM safe space. The gefapixant PBBM workflow (Fig. 2) included *in vitro* and *in vivo* data from gefapixant free base and citrate based formulations from various studies and the human ADME study confirmed its high absorption.

The developed gefapixant PBBM successfully fitted to *in vitro* dissolution profiles for the early and late formulations, simulated their *in vivo* clinical PK profiles, and matched with the BE/ τ BA PK study outcome with low predictive errors (< 15%, Tables III and IV). The PBBM established bioequivalence safe space was used in support of gefapixant formulation drug product quality control. As illustrated in the dissolution safe space plot (Fig. 5), it is predicted that gefapixant formulation batches are bioequivalent to the F04B commercial drug product, without requiring additional clinical BE studies, when their *in vitro* drug release > 80% at 60 min under the QC dissolution model conditions. By incorporating of the free base F01 PPI PK data, the safe space was widened to a broader knowledge space for a better risk assessment of formulation PK performance. Despite not being a part of the controlled BE crossover study with the citrate F04A and F04B formulations, the gefapixant F01 data provided a valid case, in which its pH 6.8 dissolution profile is outside of the calculated safe-space limit of < 80% at 60 min. Both the clinical PK data and PBBM showed PK exposure of the F01 had exposure decreased by 50% under PPI coadministration while the citrate formulations had demonstrated no exposure drop with or without PPI presence. This supports the clinical relevancy of the PPS PBBM based safe space.

Biowaiver of BE study can be supported by well-defined PBBM and *in vitro* dissolution data. Cases of virtual BE utilizing PBBM for bioequivalence evaluation have been reported in literature (4, 6, 13, 15). Virtual simulation of BE studies can be performed using PBBM in subjects who are randomly selected with difference physiological conditions (11). Such PBBM allows quick assessments of BE possibility of success by varying stomach pH, stomach emptying rate, GI transit time, as well as physiological factors to inform bridging risk and facilitate further decision making on clinical studies.

Gefapixant citrate, despite a BCS IV classification, has high apparent solubility and rapid dissolution profiles from the citrate salt based formulation, and combination of moderate to high fraction absorbed can make gefapixant drug product perform more like a BCS I or III compound. The latest ICH M9 guidance (18) supports BCS based biowaivers to drug products where the drug substance(s) exhibit high solubility and, either high permeability (BCS Class I) or low permeability (BCS Class III). Gefapixant PBBM as shown have successfully fitted *in vitro* dissolution at multiple pH conditions and correlated to the PK outcome of the relative BA study and the BE study for the early free base F01, F02 formulations, Phase 3 F04A formulation and the commercial drug product F04B. This indicates a quite reasonable PK outcome from the PBBM. Therefore, *in vivo* risk can be predicted using the PBBM safe space, which can be used as support for biowaiver of clinical studies. Potential applications like minor changes of formulation composition, excipient grade and manufacture process can be justified for clinical study waiver by demonstration of no PK impact using PBBM. In summary, gefapixant PBBM and BE safe space reported herein provide an opportunistic application in potential model based biowaivers (21).

Conclusions

The physiologically based biopharmaceutical model reported herein has shown it as a useful tool in establishment of a safe space for gefapixant formulation bioequivalence. PBBM can use independent study data, including those of a radiolabeled human mass-balance study. PK impact and quality of commercial formulation batches can be reasonably assessed by *in vitro* assessment of their dissolution behavior and application of the clinically-relevant dissolution safe space. Potential biowaiver of clinical PK study can be well supported utilizing this established PBBM for bridging future formulation changes without conducting clinical PK studies. Furthermore, the proposed PBBM is suitable for application in post-approval space to assess the impact of scale-up and post-approval changes on gefapixant formulation bioperformance.

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MW, TH, WZ, DW, KR, FK: generation of experimental data and its curation, writing/revising content, and final editing of the version to be published.

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Declarations

Conflict of Interest The authors are stock-holders of Merck & Co., Inc., and declare no conflict of interest.

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