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ORIGINAL ARTICLE

Integrating *In Vitro*, Modeling, and *In Vivo* Approaches to Investigate Warfarin Bioequivalence

X Zhang[1]*, H Wen[1], J Fan[1], B Vince[2], T Li[3], W Gao[3], M Kinjo[1], J Brown[4], W Sun[4], W Jiang[1] and R Lionberger[1]

We demonstrate the use of modeling and simulation to investigate bioequivalence (BE) concerns raised about generic warfarin products. To test the hypothesis that the loss of isopropyl alcohol and slow dissolution in acidic pH has significant impact on the pharmacokinetics of warfarin sodium tablets, we conducted physiologically based pharmacokinetic absorption modeling and simulation using formulation factors or *in vitro* dissolution profiles as input parameters. Sensitivity analyses indicated that warfarin pharmacokinetics was not sensitive to solubility, particle size, density, or dissolution rate in pH 4.5, but was affected by dissolution rate in pH 6.8 and potency. Virtual BE studies suggested that stressed warfarin sodium tablets with slow dissolution rate in pH 4.5 but having similar dissolution rate in pH 6.8 would be bioequivalent to the unstressed warfarin sodium tablets. A four-way, crossover, single-dose BE study in healthy subjects was conducted to test the same hypothesis and confirmed the simulation conclusion.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Previous work had demonstrated that warfarin sodium tablets could readily undergo a change in crystalline form after brief exposures to higher temperature and humidity but clinical significance (in terms of drug bioavailability) of this product quality variation was unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study applied modeling and simulation to assess the impact of product quality change on its *in vivo* performance.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ In addition to the new insights gained about the impact of product quality on warfarin *in vivo* PK performance, the study also showed the critical role that PBPK absorption modeling and simulation played in the scientific investigation.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ Potential risks to patients from changes in formulation or biopharmaceutics that might alter drug bioavailability can be evaluated through the use of modeling and simulation tools. This impacts decisions in both drug development and drug regulation about when the level of risk calls for the generation of new *in vivo* BE or bioavailability data.

Physiologically based pharmacokinetic (PBPK) modeling and simulation have demonstrated its utility in drug product development[1] and regulatory assessment.[2–5] PBPK absorption models mathematically and mechanistically connect the physicochemical properties of drug substances, *in vitro* performance of drug products, and *in vivo* performance.[5] As the majority of drug products are delivered via oral administration, PBPK absorption modeling and simulation have been routinely used to study the impact of formulation factors or *in vitro* performance on *in vivo* performance, for bioequivalence (BE) assessment, for developing *in vitro* and *in vivo* correlation/relation, and in the implementation of quality by design in drug product development.[4–7] Per 21CFR320.1, "bioequivalence means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." In this article, we will use warfarin sodium tablets as an example to demonstrate the role of modeling and simulation in investigating BE concerns raised about generic drug products.

Warfarin is a weak acid with a pKa value of 5.05 and, therefore, its aqueous solubility is low in low pH conditions and high in high pH conditions. Warfarin sodium is the sodium salt of warfarin, which is available as either the amorphous form or the crystalline clathrate form. The crystalline clathrate form is a warfarin sodium-isopropyl alcohol (IPA) complex; the IPA can be lost in a high relative humidity (RH) environment leading to a transformation into the amorphous form.[8] The conversion from the crystalline to

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[1]Office of Generic Drugs, US Food and Drug Administration, Silver Spring, Maryland, USA; [2]Vince and Associates Clinical Research Inc., Overland Park, Kansas, USA; [3]Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana, USA; [4]Office of Translational Sciences, US Food and Drug Administration, Silver Spring, Maryland, USA. *Correspondence: X Zhang (xinyuan.zhang@fda.hhs.gov)
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the amorphous form is irreversible because IPA is required for the crystal structure stability.[9] We are interested in evaluating whether the loss of IPA could potentially change the *in vivo* behavior of warfarin sodium tablets because the high RH environment is similar to a bathroom condition where medications are commonly stored. Previously, it was reported that the critical quality attributes (CQAs) of warfarin sodium products were significantly affected by manufacturing and formulation variables, whereas CQAs were defined as hardness, disintegration time, assay, content uniformity, IPA, moisture content, crystallinity, and dissolution.[10] It was also observed that the loss of IPA, or the accompanying transition from the crystalline form to the amorphous form, reduced the dissolution rate of warfarin sodium drug products in acidic conditions (e.g., pH 1.2 and pH 4.5) but not in neutral pH (such as pH 6.8).[10] The relationship between the CQAs and *in vivo* performance, which truly defines the clinically relevant CQAs, was not addressed. The slower release in acidic pH conditions due to the loss of IPA in warfarin sodium tablets raised concern of potential bioinequivalence between the stressed tablets and unstressed tablets. The stressed tablets were stored in open bottles in 40°C/75% RH for 24 hours. The unstressed tablets were stored in tightly closed containers in controlled room temperature (about 20–25°C). Because small changes in dose or plasma concentration of warfarin could lead to serious therapeutic failures or serious adverse events, warfarin has been classified as a critical dose drug by Health Canada,[11] or a narrow therapeutic index drug[12] by the US Food and Drug Administration (FDA), in which stricter than regular 80–125% BE standards are applied.

To address the impact of slow *in vitro* dissolution rate on *in vivo* performance in acidic pH conditions, PBPK and absorption modeling and simulations were conducted. Meanwhile, an *in vivo* BE study was initiated with the objectives to test the same hypothesis, and to verify the simulation outcomes. We treated warfarin sodium tablets, 5 mg (Comuadin manufactured by Bristol-Myers Squibb, and warfarin sodium tablets manufactured by Taro Pharmaceutical Industries) in 40°C/75% RH for 24 hours (the stressing condition) to induce the transformation of warfarin sodium tablets and mimic the typical home bathroom condition where medications are commonly stored. This storage condition represents the worst scenario and it is not recommended to store drug products in such conditions. Then *in vitro* tests were conducted to confirm the loss of IPA and change in dissolution rate, whereas other product quality measurements were within the product release specification. A single center, open-label, four-treatment, four-sequence, randomized, single-dose, crossover BE study with pharmacokinetic (PK) endpoints in healthy subjects under the fasting condition was conducted to compare the PK of stressed to unstressed warfarin sodium tablets. Overall, in this article, we studied the impact of product quality change of warfarin sodium tablets on its *in vivo* PK performance using an integrated approach (i.e., *in vitro* testing, bridging modeling, and simulation, and an *in vivo* study). The modeling and simulation work was completed before the *in vivo* study was initiated and the results were confirmed by the *in vivo* study. Meanwhile, we also demonstrated the key role of PBPK and absorption modeling and simulation in investigating product quality and related BE concerns.

MATERIALS AND METHODS

In vitro studies

Assay (potency), impurity, IPA content, and dissolution testing, amorphous content. Warfarin sodium content and impurity in the tablets were measured by a high-performance liquid chromatography method based in part on the analytical method in United States Pharmacopeia-National Formulary (USP-NF) 38-NF 33 (USP-38/NF-33, 2015, Warfarin Sodium Tablets Monograph). IPA content in the warfarin sodium tablets was determined by Pegasus 4D gas chromatography/gas chromatography time-of-flight mass spectrometer (GCxGC; LECO, St Joseph, MI). Warfarin sodium tablets were tested for dissolution in three different media: water, pH 4.5 phosphate buffer, and the two-stage buffer (0.1N HCl for 30 minutes as stage 1, followed by addition of 100 mL of a strong phosphate buffer to raise pH to 7.4 ± 0.1 for the rest of the dissolution test at 50 rpm and 37°C[13]), respectively. The dissolution tests were performed as per USP-NF recommendation in apparatus II. A Rigaku X-ray diffractometer (Cu Kα, $\lambda = 1.5406$ A) was used to carry out several powder X-ray diffraction (PXRD) measurements.

Physiologically based pharmacokinetic and absorption modeling and simulation

Software. Simulations were performed using the commercially available software Simcyp Simulator version 14 (Simcyp Limited, Sheffield, UK) and GastroPlus version 9.0 (Simulations Plus, Lancaster, CA).

Model parameters. Physicochemical parameters, permeability, fraction unbound in plasma, and blood/plasma ratio were obtained from Simcyp established database. The P_{eff} value was adjusted to 12×10^{-4} cm/s given that warfarin was reported to be completely and quickly absorbed from the whole small intestine.[14] The volume of distribution at steady state of warfarin (0.114 L/kg) was predicted using the mathematical model 2[15] implemented in Simcyp (**Supplementary Table S1**).

The dissolution model based on the solubility vs. pH was the Wang-Flanagan equation[16] implemented in Simcyp or the Johnson model implemented in GastroPlus. To integrate the *in vitro* dissolution data obtained from different products at various pH conditions, the Z-factor dissolution model[17] was utilized integrating *in vitro* dissolution testing using a two-stage condition (pH 1.2 for 30 minutes, and then pH 7.5), or a single-stage condition (pH 4.5).

Parameter sensitivity analysis. Sensitivity analyses were performed for solubility, particle size and density, dose, and the Z values for hypothetical *in vitro* dissolution profiles. It was assumed that the warfarin product was 100% dissolved at pH 6.8 for each hypothetical *in vitro* dissolution profile. A 3D parameter analysis was performed to explore the interaction between Z values at pH 4.5 and pH 6.8 and their influence on point estimate (PE) of peak plasma concentration (C_{max}). The Z values were expected to have no impact on area under the curve (AUC) based on the assumption of 100% dissolved at pH 6.8 and an IR formulation for

warfarin product. A 3D parameter sensitivity analysis was also performed to explore the interaction between particle density and particle radius and their influence on C_{max}.

Virtual PK and BE trial simulations. A four-treatment, four-sequence, randomized, single-dose, crossover virtual BE trial simulation with PK endpoints in healthy subjects under fasting condition was conducted to mimic the *in vivo* BE study. The PBPK absorption model based on Z-factors was used to predict warfarin PK and BE to be compared with BE data.

Virtual BE trials were conducted, as previously described,[7] to simulate the passing rate for various pairs of comparison. The virtual BE trials were designed as fully replicated, two-sequence, two-treatment, four-period, crossover BE studies with 30 healthy subjects. A total number of 1,200 healthy subjects were randomly selected and simulated for the unstressed tablets or stressed tablets, based on the Z-factors. For each BE trial, 30 subjects were randomly selected from each treatment, and a total number of 100 trials were conducted for each pair of comparisons. The log-normal statistical distributions of intrasubject variability (S_{WR}) were incorporated in simulated C_{max} ($S_{WR} = 0.10$) or AUC ($S_{WR} = 0.05$).[18] The sensitivity to detect formulation differences was assessed by comparing the passing rate of C_{max} in each study using average bioequivalence (ABE) criteria (90% confidence interval (CI) within 80–125%), and reference scaled average bioequivalence (RSABE).[19]

Bioequivalence study

This was a single center, open-label, four-treatment (A, B, C, and D), four-sequence, randomized, single-dose, crossover BE study with PK endpoints in healthy subjects under the fasting condition. The four treatments were: (A) One warfarin sodium 5 mg tablet (TEST) stored in a closed bottle under controlled room temperature; (B) One warfarin sodium 5 mg tablet (TEST) stored in an open bottle in 40°C/75% RH for 24 hours; (C) One Coumadin 5 mg tablet (REF) stored in a closed bottle under controlled room temperature; and (D) One Coumadin 5 mg tablet (REF) treated under stored conditions in an open bottle in 40°C/75% RH for 24 hours.

The criterion to select the stressing condition was to reduce the dissolution of warfarin sodium tablets in acidic pH condition as much as possible while the warfarin sodium tablets still passed the release specifications.

The study was approved by MidLands Independent Institutional Review Board (Overland Park, KS), and the FDA Institutional Review Board, also known as the Research Involving Human Subjects Committee (#14-073D). The study was conducted by Vince and Associates Clinical Research (Overland Park, KS). The study was undertaken in accordance with the Guidance for Industry, E6 Good Clinical Practice of the FDA, and the principles of Declaration of Helsinki.

A total of 32 healthy male and postmenopausal or surgically sterile female subjects between 18 and 55 years of age were enrolled in the study. All subjects were nonsmokers or ex-smokers with a body mass index of 18–30 kg/m^2, and were in good health without significant illness based on medical history, physical examinations, including vital signs, electrocardiograms, and clinical laboratory tests. Subjects were fasting for 10 hours prior to dosing. In each study period, 20 blood samples were collected for PK analysis. PK sampling times were predose, 0.17, 0.25, 0.5, 0.67, 0.83, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours postdose. The washout period was 21 days between each period. Plasma samples were analyzed using high-performance liquid chromatography with tandem mass spectrometry detection at Algorithme Pharma. No assessment of CYP 2C9 or VKORC1 genotypes was conducted because the purpose of the study was to compare the *in vivo* PK of various warfarin sodium products in the same subject (crossover) not between subjects.

The primary comparisons of interest were treatment B vs. treatment C, and treatment C vs. treatment D. The secondary comparisons of interest were treatment A vs. treatment C, treatment B vs. treatment D, treatment A vs. treatment B, and treatment A vs. treatment D.

Pharmacokinetic assessment

For each treatment period, the following PK parameters for each individual were determined by noncompartmental analysis from the warfarin plasma concentration vs. time profiles: C_{max}, the maximum observed plasma concentration, and AUC_{0-72}, the area under the plasma concentration vs. time curve from 0–72 hours. AUC_{0-72} was used in place of AUC_{0-inf} due to the long half-life of warfarin.

Statistical analysis

ABE analyses were performed for the four-treatment, four-sequence, crossover virtual BE simulation and the *in vivo* BE trial. Both ABE and RSABE analyses were conducted for the fully replicated, two-treatment, two-sequence, and crossover virtual BE simulations.

RESULTS

In vitro study

IPA was below the limit of quantification in the stressed warfarin sodium tablets. In general, the IPA content was not detectable after 4 hours of treatment in 40°C/75% RH, open bottle condition for both Coumadin and Taro's warfarin sodium tablets. The PXRD measurement showed that the warfarin crystal-specific peak at 2-theta angle of ~8 disappeared in stressed Coumadin and Taro's warfarin sodium tablets, suggesting a transformation from the crystalline form to the amorphous form (**Supplementary Figure S1**). However, it takes several weeks to completely lose IPA if the bottle is closed in 40°C/75% RH.

Slow dissolution was observed in acidic pH for the stressed warfarin sodium tablets. As shown in **Figure 1**, the dissolution rate of the stressed Coumadin and Taro's warfarin sodium tablets in all three dissolution media: water, pH 4.5 phosphate buffer, and the two-stage buffer medium, was slower than the unstressed tablets. After being stressed in 40°C/75% RH for 24 hours, the average cumulative release of Coumadin warfarin sodium tablets (90.47%) in water was much lower than the average cumulative release for the Taro warfarin tablets (96.98%) at 30 minutes (**Figure 1a**). The same trend was observed in the dissolution with pH 4.5 phosphate buffer and the two-stage buffer medium

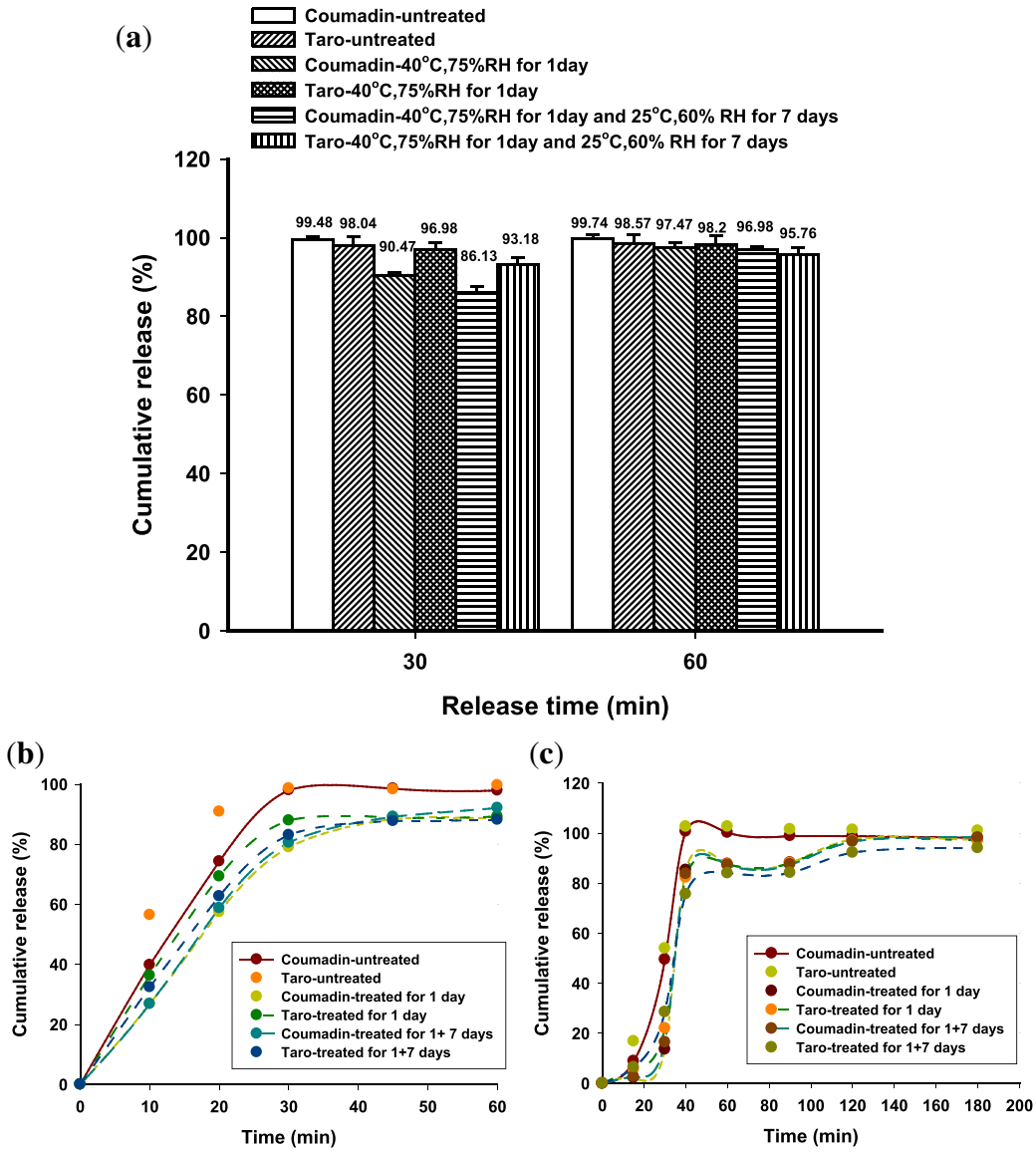


Figure 1 (**a**) Average cumulative release of Coumadin and warfarin sodium tablets (Taro) in water. The warfarin sodium tablets were stored at room temperature ("untreated"), stored in 40°C/75% relative humidity (RH) for 24 hours ("stressed"), or stored in 40°C/75% RH for 24 hours plus in 25°C/60% RH for 7 days. (**b**) Dissolution profiles of Coumadin and warfarin sodium tablets (Taro) in pH 4.5 buffer, and (**c**) in the two-stage buffer. The warfarin sodium tablets were stored at room temperature ("untreated"), stored in 40°C/75% RH for 1 day ("treated"), or stored in 40°C/75% RH for 1 day plus in 25°C/60% RH for 7 days. Treated for 1 day: treated at 40°C/75% RH for 1 day; treated for 1 + 7 days: treated at 40°C/75% RH for 1 day plus at 25°C/60% RH for 7 days.

(**Figure 1b,c**). IPA loss has contributed to decrease in crystallinity of the drug substance in the tablets demonstrated by PXRD (**Supplementary Figure S1**) as well as slower dissolution in water, pH 4.5, and pH 1.2 media. Even when the loss of IPA reduced the dissolution rate at lower pH, the percentage dissolved in higher pH condition (such as pH 7.5) was comparable between the unstressed and stressed tablets at 120 minutes.

There were no significant changes in potency and impurity in the stressed warfarin sodium tablets. The potency for both warfarin sodium products did not change significantly when they were tested immediately after being stressed in 40°C/75% RH, open bottle condition for 24 hours, as well as after 1 week when they were shipped back from the clinical site. Accordingly, no significant change in impurity and appearance was observed for Coumadin or Taro's warfarin sodium tablets (data not shown).

The stressed tablets represent tablets stored in the typical home bathroom condition where medications are commonly stored, which represented a worst case scenario and was not recommended for drug products storage. The *in vitro* behavior generated the hypothesis that the loss of IPA and slow dissolution in acidic pH had significant impact on the PKs of warfarin. PBPK and absorption modeling and an *in vivo* BE study were conducted to test the hypothesis.

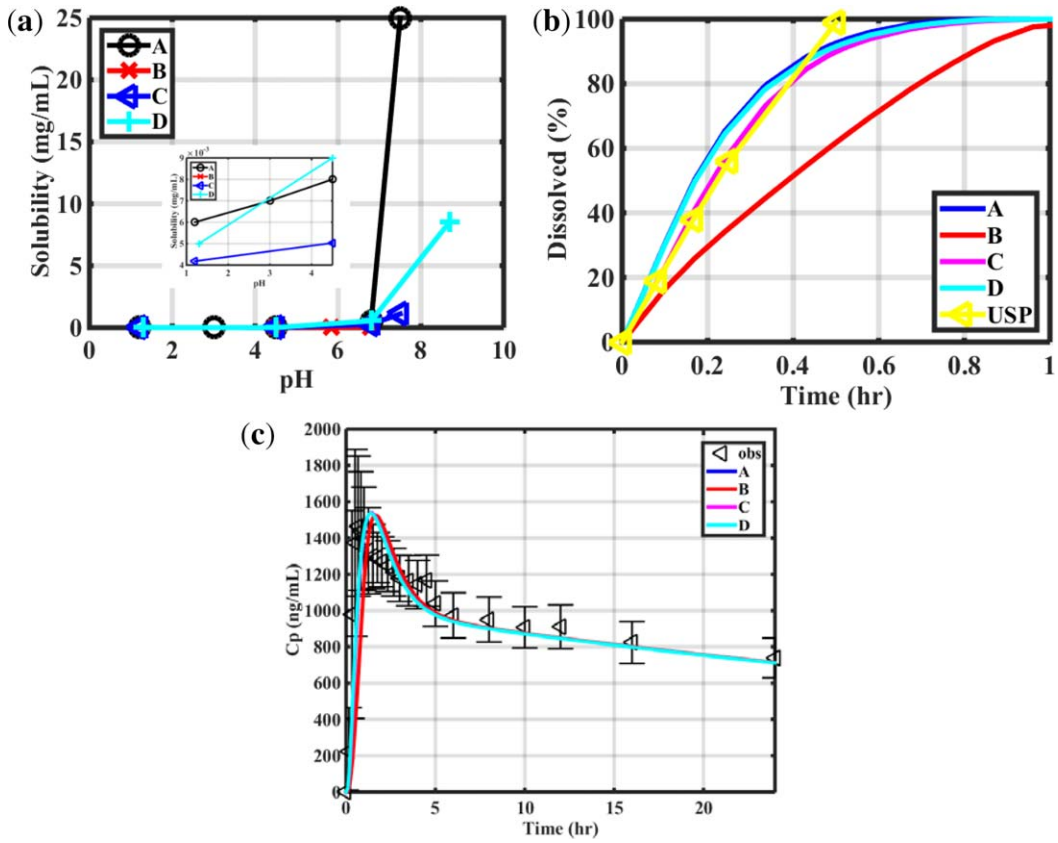


Figure 2 (**a**) Solubility vs. pH profiles from various sources using in the Advanced Compartmental Absorption and Transit (ACAT) model simulation. (**b**) Predicted *in vivo* dissolution vs. time profiles by the ACAT model. (**c**) Predicted plasma concentration vs. time profiles by the ACAT model using solubility data in (**a**).

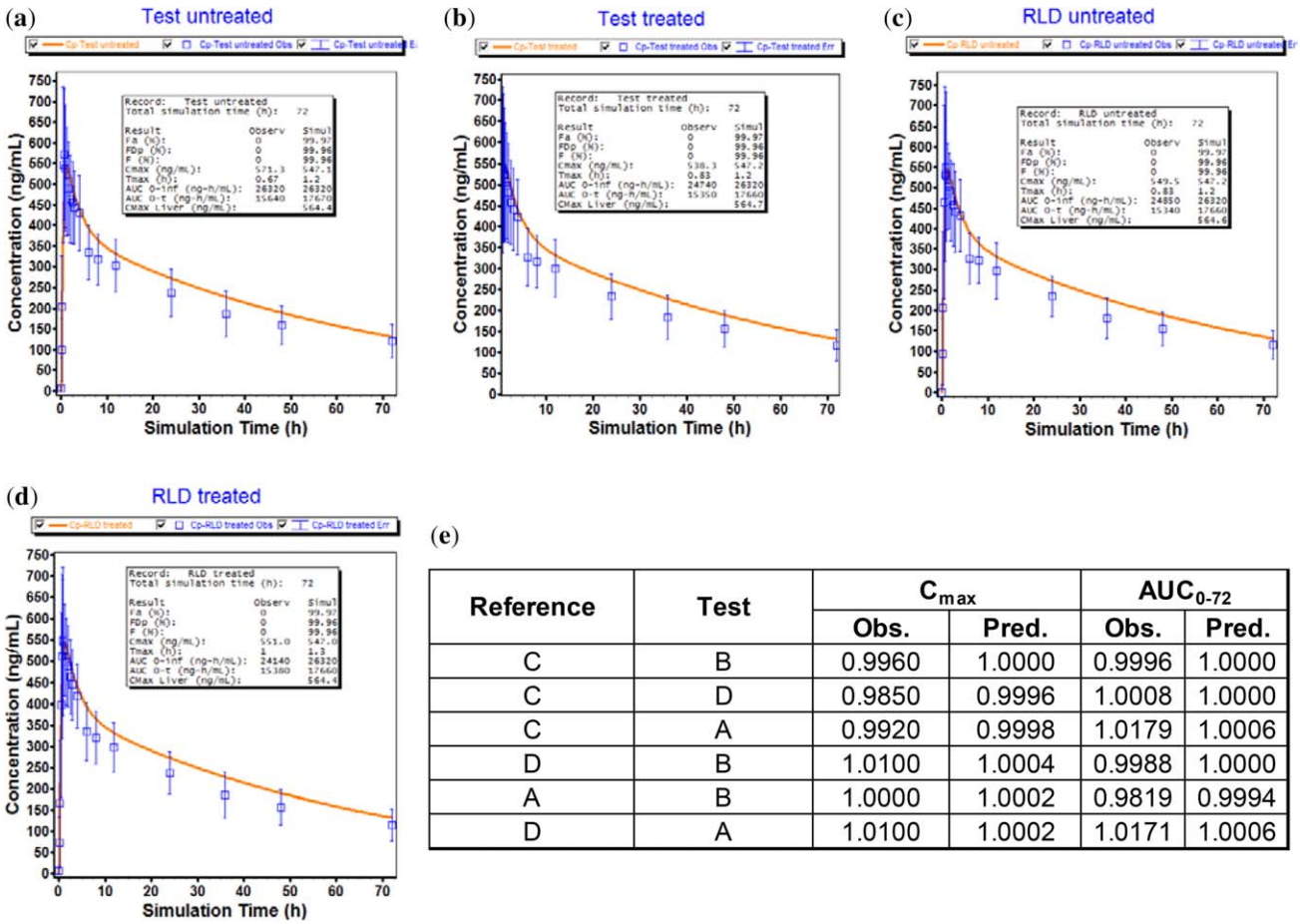
Physiologically based pharmacokinetic and absorption modeling and simulation

PBPK and absorption models predicted warfarin PK profile. PBPK models implemented in both Simcyp and Gastroplus accurately predicted the plasma PK of warfarin (10 mg dose in **Figure 2** and 5 mg dose in **Supplementary Figure S2b**). Both models were thereafter utilized to predict the impact of solubility pH profiles or Z-factor (dissolution rate) pH profiles on warfarin PK performance.

Warfarin PK was not sensitive to the change of solubility, particle size, and particle density but was proportional to the dose (potency/assay). In order to test the sensitivity of *in vivo* PK to the change of critical parameters, parameter sensitivity analyses were performed using both developed models in Simcyp and Gastroplus. Various solubility values were reported from different resources (**Figure 2a**). Therefore, parameter sensitivity analysis was performed for solubility. In both models, it was suggested that unrealistically low solubility could delay the time of maximum plasma concentration (T_{max}), whereas C_{max} and AUC were not sensitive to the change of solubility (**Figure 2c and Supplementary Figure S2b**). Comparing the solubility vs. pH profiles with the *in vivo* dissolution profiles (**Figure 2b**), it was suggested that warfarin sodium tablets reached 100% release within 1 hour after administration even for the lowest solubility vs. pH profile (profile B). Parameter sensitivity analyses on particle size and density suggested that neither parameter had significant impact on warfarin PK parameters (**Supplementary Figure S3a**).

In order to evaluate the appropriate range for assay, we simulated the dose effects on PK. **Supplementary Figure S3b** suggested that warfarin PK parameters (C_{max} and AUCt) change proportionally to the dose (i.e., 10% change in dose led to 10% change in C_{max} and AUCt). Currently, the warfarin sodium tablets USP monograph requires that warfarin sodium tablets contain no less than 95.0% and no more than 105.0% of the labeled amount of warfarin sodium. This is a tighter limit than most drugs because of warfarin's narrow therapeutic index.

BE simulations suggested high passing rate among all pairs of comparison. The dissolution profiles in **Figure 2** for the four treatments in the *in vivo* BE study were used to simulate BE trials. The simulated average PK profiles vs. observed average PK profiles in the *in vivo* BE study are shown in **Figure 3a–d**. In general, the PEs between all interested pairs of comparison were close to 1 (**Figure 3e**). In the 100 fully replicated, two-sequence, two-treatment, four-period, crossover BE trial simulations with 30 healthy subjects, the passing rates for C_{max} were all above 80% using the ABE criteria and the lowest passing rate was



(e)

Reference	Test	C_{max}		AUC_{0-72}	
		Obs.	Pred.	Obs.	Pred.
C	B	0.9960	1.0000	0.9996	1.0000
C	D	0.9850	0.9996	1.0008	1.0000
C	A	0.9920	0.9998	1.0179	1.0006
D	B	1.0100	1.0004	0.9988	1.0000
A	B	1.0000	1.0002	0.9819	0.9994
D	A	1.0100	1.0002	1.0171	1.0006

Figure 3 Simulated pharmacokinetic (PK) profiles and point estimates of the four treatments used in the *in vivo* bioequivalence (BE) study based on the Z-factor model. The four treatments were (**a**) treatment A: one warfarin sodium 5-mg tablet manufactured by Taro stored at room temperature; (**b**) treatment B: one warfarin sodium 5-mg tablets manufactured by Taro treated in 40°C/75% RH, open bottle condition for 24 hours; (**c**) treatment C: one Coumadin 5-mg tablet stored at room temperature; and (**d**) treatment D: one Coumadin 5-mg tablet treated in 40°C/75% RH, open bottle condition for 24 hours. (**e**) Observed and predicted peak plasma concentration (C_{max}) and area under the curve (AUC_{0-72}) using the developed physiologically based pharmacokinetic absorption model. RLD, Reference Listed Drug.

observed when comparing treatments B and C (**Table 1**). The passing rate for C_{max} were all above 78% using RSABE criteria with the lowest passing rate observed when comparing treatments A and B (**Table 1**). The passing rates were all 100% for AUC_{0-72} using ABE criteria and were all above 84% using the RSABE approach with the lowest passing rate observed when comparing treatments A and B (**Table 1**).

PBPK modeling aids in defining in vivo relevant dissolution space. Multiple parameter sensitivity analyses were conducted to map the dissolution space that ensured BE. Multiple Z-factors were tested for pH 4.5 and pH 6.8 conditions to create virtual dissolution profiles. **Figure 4b** showed a three-dimensional (3D) graph describing the relationship between the PE of C_{max} and the Z-factors at pH 4.5 and 6.8. The PEs fell within the range of 0.95–1.00 for the majority region of the space. Based on the RSABE criteria, if the PE of C_{max} was 0.955 and the passing rate was 80% (**Figure 4a**), it required minimum 30% release at 30 minutes in pH 4.5 and 80% release at 30 minutes in pH 6.8 (**Figure 4c,d**).

Table 1 Passing rates for various virtual BE study comparison based on the data from virtual BE trial simulation

	C_{max} (%)		AUC_{0-72} (%)	
T vs R	**ABE**	**RSABE**	**ABE**	**RSABE**
B vs. C	81	80	100	86
D vs. C	96	93	100	87
A vs. C	95	89	100	91
B vs. D	84	79	100	88
A vs. B	85	78	100	84
A vs. D	99	93	100	91

T is the Test formulation, and R is the Reference formulation in each BE simulation.
ABE, average bioequivalence; AUC_{0-72}, 0–72 hour area under the concentration-time curve; BE, bioequivalence; C_{max}, peak plasma concentration; RSABE, reference scaled average bioequivalence.

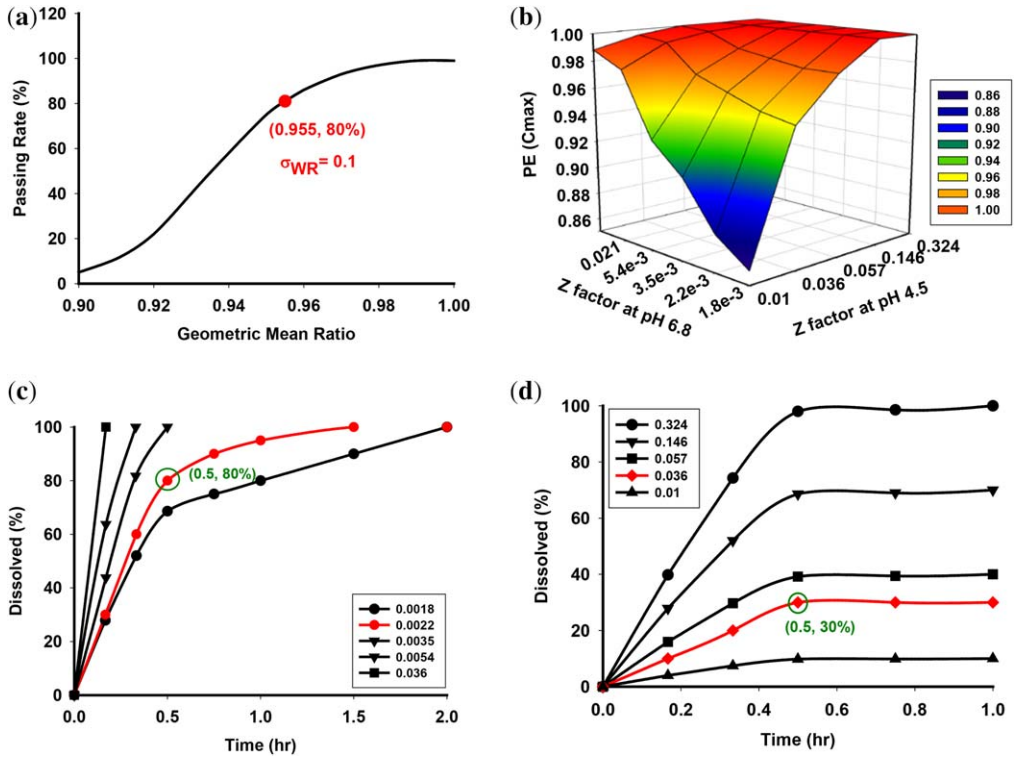


Figure 4 Three dimensional parameter sensitivity analyses for Z-factors at pH 4.5 and 6.8. The color code in the figure indicates different ranges of the point estimates of predicted peak plasma concentration (C_{max}). (**a**) Effect of geometric mean ratio (GMR) on study power for a hypothetical narrow therapeutic index drug when $\sigma_{WR} = 0.10$ and evaluated by reference scaled average bioequivalence approach. The passing rate is higher than 80% when the GMR is greater than 0.955. (**b**) Parameter sensitivity analysis for Z-factor at pH 4.5 and 6.8. (**c**) Hypothetical dissolution profiles with various Z-factors at pH 6.8. (**d**) Hypothetical dissolution profiles with various Z-factors at pH 4.5. PE, point estimate.

In vivo study

PK parameters of the four treatments are very close. The mean PK profiles for four treatments are shown in **Supplementary Figure S4** and the raw plasma concentration data are supplied in **Supplementary Table S2**. Overall, the arithmetic mean values of C_{max} and AUC_{0-72}, and the median values of T_{max} were all very close for the four treatments (**Table 2**). The coefficient of variation (CV%) values for C_{max} and AUC_{0-72} were all $<$30%. Due to the long half-life of warfarin, the elimination phase and AUC_{0-inf} were only estimated for a limited number of subjects.

BE was established among all pairs of treatments based on ABE analysis. The maximum deviation of PEs from 1 for C_{max} was observed for treatment A vs. B (0.979) comparison (**Table 3**). The largest CI for C_{max} was observed for treatment A vs. D (0.909–1.116). Overall, the PEs for all pairs of comparisons were very close to 1.00 and ranged from 0.970–1.020 for both C_{max} and AUC_{0-72}. The 90% CIs were all within (0.900–1.120) for both C_{max} and AUC_{0-72} (**Table 3**).

DISCUSSION

This article demonstrates the key role of modeling and simulation as part of the integrated approach that the Office of Generic Drugs routinely practices to address questions about generic drug substitution. In the warfarin sodium tablets case example, a product quality change was observed and the question of its impact on BE was raised. Warfarin is a narrow therapeutic index drug and patients take it for chronic use, possibly storing them in bathroom conditions characterized by higher humidity. During preliminary investigation, it was observed that tablets color change and slow dissolution in pH 4.5 buffer after warfarin sodium tablets were exposed to high humidity and temperature, which raised concerns regarding potential changes in *in vivo* PK and consequent safety and efficacy performance. Further tests confirmed that IPA was not detectable and warfarin sodium was transformed from crystalline to an amorphous form (**Supplementary Figure S1**) after the tablets were exposed in high temperature and high humidity conditions, which represented a worst scenario and was not recommended for drug products storage but may occur in reality, such as storing the medications in a bathroom.

To address the concern of potential change in *in vivo* PK performance, PBPK absorption modeling and simulation were conducted using *in vitro* dissolution profiles as input to predict *in vivo* performance. Two independent scientists performed modeling and simulation using different software and obtained the same conclusion. Modeling and simulation predicted that the loss of IPA and the slow dissolution in pH 4.5 condition would not affect BE as long as the dissolution in pH 6.8 condition was complete within 120 minutes. The

Table 2 Descriptive statistics of PK parameters by treatment based on all valid measurements

	Treatment A (*N* = 29)		Treatment B (*N* = 28)		Treatment C (*N* = 28)		Treatment D (*N* = 30)	
Parameter (Units)	**Mean**	**(CV%)**	**Mean**	**(CV%)**	**Mean**	**(CV%)**	**Mean**	**(CV%)**
C_{max} (ng/mL)	652.16	(18.7)	652.34	(18.6)	655.61	(18.5)	652.06	(22.0)
ln (C_{max})	6.4628	(3.0)	6.4636	(2.9)	6.4685	(3.0)	6.4557	(3.5)
T_{max} (hours)[a]	0.67	(0.50–3.00)	0.67	(0.50–3.00)	0.67	(0.25–8.00)	0.83	(0.50–3.00)
AUC_{0-72} (ng·h/mL)	15,640.46	(22.5)	15,352.81	(22.1)	15,339.90	(21.2)	15,382.12	(21.4)
ln (AUC_{0-72})	9.6324	(2.4)	9.6156	(2.3)	9.6146	(2.4)	9.6177	(2.3)
$AUC_{0-\infty}$ (ng·h/mL)	11,198.81	(NC)[b]	18,127.21	(38.3)[c]	13,927.67	(58.0)[d]	16,038.63	(60.4)[e]
ln ($AUC_{0-\infty}$)	9.3236	(NC)[b]	9.7449	(4.6)[c]	9.4496	(6.5)[d]	9.5819	(6.7)[e]
λ_Z ($hours^{-1}$)	0.0261	(NC)[b]	0.0271	(46.0)[c]	0.0248	(17.0)[d]	0.0226	(20.7)[e]
T_{half} (hours)	26.52	(NC)[b]	28.78	(36.3)[c]	28.38	(17.0)[d]	31.32	(20.7)[e]

Notes: A total number of 32 subjects were enrolled in the study. Subject 003 was withdrawn due to a positive drug screen on check in at period 3. Subject 006 was withdrawn due to an abnormal electrocardiogram found at period 2. Subject 019 was withdrawn due to a positive drug screen on check in at period 4. Subject 008 was excluded from the pharmacokinetic (PK) and statistical analysis due to co-administration of other medications. Subject 031 was excluded from the PK and statistical analysis following bioanalysis due to a quantifiable predose concentration in period 4 that was higher than 5% of the C_{max} value for that same period, as predetermined in the study protocol.

$AUC_{0-\infty}$, area under the curve to infinity; AUC_{0-72}, 0–72 hour area under the concentration-time curve; C_{max}, peak plasma concentration; CV, coefficient of variation; NC, not calculated; T_{max}, time of maximum plasma concentration.

[a]Median; [b]$n = 1$; [c]$n = 3$; [d]$n = 2$; [e]$n = 2$.

pKa value of warfarin is around 5.2. Therefore, the solubility of warfarin is low in low pH conditions, and high in high pH conditions. The pH in the majority of the gastrointestinal tract is above 6.0, but not in the stomach where the pH is low (about 1.2). As warfarin has a long half-life, even if the tablets have slow dissolution in acidic conditions after being exposed in high temperature and high humility conditions, complete absorption from the proximal small intestine (pH above 6.0) is expected provided that dissolution in high pH media was not affected.

Modeling and simulation results were completed before the *in vivo* study was initiated. The *in vivo* study was a four-treatment, four-sequence, crossover, BE study with PK endpoints. The main concern was the BE between the generic product or the Reference Listed Drug (RLD) tablets being stored in bathroom-type conditions and the RLD being stored normally. The primary comparisons were conducted between the warfarin sodium 5 mg tablets (test) treated under the stressing condition and Coumadin 5 mg tablets (RLD) stored under controlled room temperature; and between the Coumadin 5 mg tablets (RLD) treated under the stressing condition and Coumadin 5 mg tablets (RLD) stored under controlled room temperature. The *in vivo* study indicated that all pairs of comparisons were all fairly close to 1.0, suggesting that difference observed in *in vitro* tests had minimum impact on *in vivo* PK. The simulation predictions were confirmed by the *in vivo* study.

After we gained more confidence with the model, we further utilized the PBPK absorption model to map an *in vivo* relevant *in vitro* dissolution space (**Figure 4**), which can be used as a surrogate of *in vivo* performance for warfarin sodium tablets as it is impossible to perform *in vivo* studies on every suspected batch and to test every hypothesis.

In summary, in this study, we gained new knowledge about the impact of the loss of IPA in warfarin sodium tables on their *in vivo* PK performance as well as demonstrated the key role of PBPK absorption modeling and simulation played in bridging *in vitro* observation and *in vivo* performance, and regulatory assessment. The model successfully predicted BE for warfarin sodium tablets with product quality changes. The FDA had enough confidence in using modeling and simulation in the initial risk assessment to defer any potential action until the *in vivo* study. The Office of Generic Drugs has routinely integrated modeling and simulation components in the scientific investigation process and the FDA continues to gain experience and confidence in utilizing the advanced modeling and simulation tools in regulatory review.

Table 3 Average BE analysis

	AUC_{0-72}		C_{max}	
	GMR	**90% CI**	**GMR**	**90% CI**
Primary comparisons				
B vs. C	0.998	(0.968–1.030)	1.007	(0.957–1.059)
C vs. D	0.996	(0.965–1.028)	1.009	(0.941–1.082)
Secondary comparisons				
A vs. C	1.017	(0.979–1.056)	0.990	(0.906–1.082)
B vs. D	1.014	(0.974–1.056)	1.014	(0.974–1.056)
A vs. B	1.015	(0.990–1.041)	0.979	(0.916–1.048)
A vs. D	1.014	(0.974–1.056)	1.007	(0.909–1.116)

Notes: The primary comparisons of interest represent the primary concerns (i.e., whether the test product being stored in the patient in-use condition, such as the tablets were stored in the bathroom) shows BE to the reference product being stored at room temperature, and whether the reference product being stored in the patient in-use condition shows BE to the fresh reference product. The secondary comparisons of interest covered all the other possible scenarios (i.e., whether the test product is BE to the reference product under the normal storage condition, whether the test product is BE to the reference product if both are stored under the patient in-use condition, whether the test product being stored in the patient in-use condition shows BE to its self being stored in the normal storage condition, and whether the test product being stored under the normal storage condition is BE to the reference product being stored under the patient in-use condition).

AUC, area under the curve; BE, bioequivalence; CI, confidence interval; C_{max}, peak plasma concentration; CV, coefficient of variation; GMR, geometric mean ratio.

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