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Michele L. Joyner
East Tennessee State University, joynerdh@etsu.edu

Whitney Forbes
East Tennessee State University

Michelle Maiden
Meredith College

Ariel N. Nikas
Meredith College

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A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THE ANTIBIOTIC ERTAPENEM

Michele L. Joyner

Department of Mathematics & Statistics
East Tennessee State University
Johnson City, TN, 37614, USA

Cammey C. Manning

Department of Mathematics & Computer Science
Meredith College
Raleigh, NC, 27607, USA

Whitney Forbes

Department of Mathematics & Statistics
East Tennessee State University
Johnson City, TN, 37614, USA

Michelle Maiden and Ariel N. Nikas

Department of Mathematics & Computer Science
Meredith College
Raleigh, NC, 27607, USA

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Abstract. Ertapenem is an antibiotic commonly used to treat a broad spectrum of infections, which is part of a broader class of antibiotics called carbapenem. Unlike other carbapenems, ertapenem has a longer half-life and thus only has to be administered once a day. A physiologically-based pharmacokinetic (PBPK) model was developed to investigate the uptake, distribution, and elimination of ertapenem following a single one gram dose. PBPK modeling incorporates known physiological parameters such as body weight, organ volumes, and blood flow rates in particular tissues. Furthermore, ertapenem is highly bound in human blood plasma; therefore, nonlinear binding is incorporated in the model since only the free portion of the drug can saturate tissues and, hence, is the only portion of the drug considered to be medicinally effective. Parameters in the model were estimated using a least squares inverse problem formulation with published data for blood concentrations of ertapenem for normal height, normal weight males. Finally, an uncertainty analysis of the parameter estimation and model predictions is presented.

2010 *Mathematics Subject Classification.* Primary: 92C45; Secondary: 34A35, 65L09.

Key words and phrases. Ertapenem, physiologically based pharmacokinetic model (PBPK), free concentration, bound concentration, ordinary differential equations.

1. **Introduction.** Ertapenem is a once-a-day antibiotic commonly used to treat community-acquired and mixed infections [15, 17]. It is part of the class of antimicrobials called carbapenems, which is one of the distinct classes of the β-lactams; β-lactams are used to treat serious infections [23]. Carbapenems are regarded as the most potent class of β-lactams and have the widest spectrum of antimicrobial activity against both gram-positive and gram-negative bacteria [8, 23].

Imipenem and meropenem, which are the other two carbapenems, have an elimination half-life of approximately one hour and are less protein bound; they must be administered several times a day [17, 23]. They also are active against non-fermentative gram-negative bacilli and nosocomial infections [12]. Unlike imipenem and meropenem, ertapenem has a half-life of approximately four to five hours due to high protein binding; approximately 94% of ertapenem is protein bound [8, 23]. This allows ertepenem to be administered just once a day as an intravenous infusion in adult patients [12, 14, 15, 17, 23]. Ertapenem has only limited activity against non-fermentative gram-negative bacilli but is well-suited for use against community-acquired infections [12, 14].

Ertapenem is indicated for use against a wide variety of infections. In the European Union, ertapenem is licensed for the treatment of intra-abdominal and gynecological infections as well as community-acquired pneumonia. In the United States, it is also licensed for the treatment of skin infections and for complicated urinary tract infections [12, 14, 23]. Other uses include treatment of acute pelvic infections and pediatric patients with complicated bacterial infections [12]. Ertapenem may be administered either by intravenous or intramuscular route [12, 23].

Others models for ertapenem have previously appeared in the literature [7, 15]. Our approach will be different as we will use a multi-compartment model based on physiologically-based pharmacokinetic framework. Although it is not the focus of this article, this approach will allow for examination of individuals with different physiological characteristics, such as body mass index, in the future.

In this article, we seek to investigate the uptake, distribution, and elimination of ertapenem following a single one gram intravenous dose. We begin in Section 2 with the development of a physiologically-based pharmacokinetic (PBPK) model from mass balance techniques and incorporate specifics related to the dosing and behavior of ertapenem. In Section 3, inverse problems are performed to estimate the unknown parameters in the model. Numerical simulations are carried out for a normal weight, normal height male and compared to clinical data. Uncertainty analysis is investigated using a bootstrapping technique in Section 4. We will conclude with some final remarks in Section 5.

2. **Model structure and assumptions.** Pharmacokinetic modeling seeks to examine factors that affect absorption, distribution, metabolism, and excretion [3]. PBPK modeling incorporates known physiological parameters such as body weight, organ volumes, and blood flow rates in particular tissues.

In this initial model for ertapenem, we will focus our attention on the tissues and organs most affected by the drug. The PBPK model has separate compartments representing the blood (Bl), kidney (K), adipose or fat (F), the gut (G), and other aggregated tissues (OT) as well as the urine (u) and the feces (f); the kidney and gut have been included so that urine and feces excretion can be considered whereas the adipose compartment is included so the effects of differing body weight can be examined in a subsequent paper. Urine and feces excretion are considered to occur

at linear rates and are represented by $k_u C_K$ and $k_f C_G$, respectively, where k_u and k_f are the first-order linear rate constants for excretion and C_K and C_G represent the concentrations of ertapenem in the kidney and gut. Since hepatic metabolism only plays a minor role in the elimination of ertapenem [17], it is not included in this model. Intravenous (IV) dosing is described by infusion directly into the blood compartment. See Figure 1 for a schematic of the model.

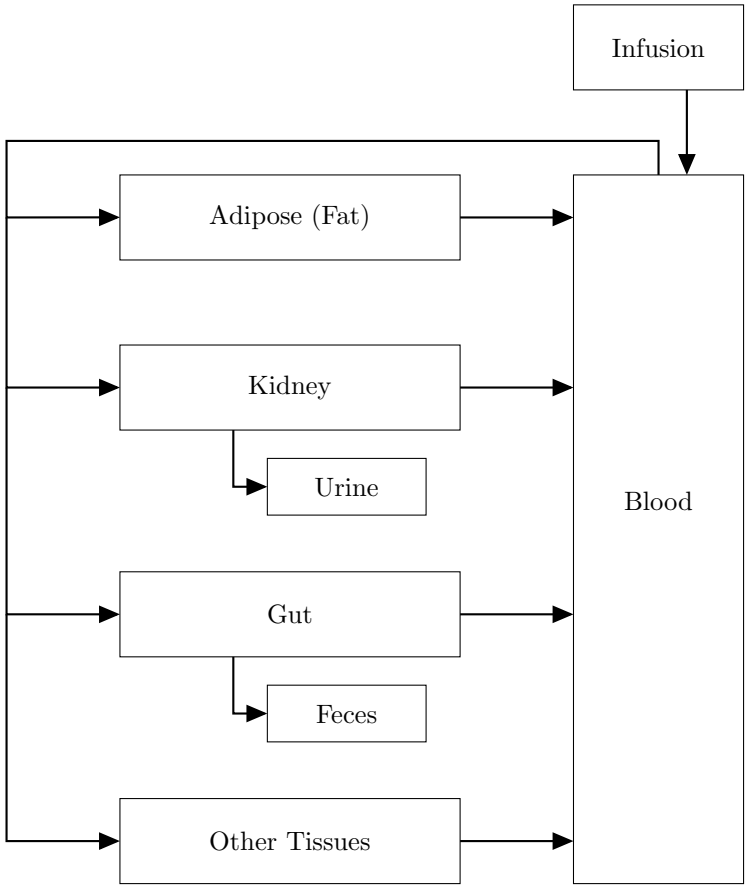


FIGURE 1. Schematic Representation of Compartment Model

Ertapenem is highly bound to human plasma proteins. Only the free, unbound portion of the drug actually saturates the tissues and can be excreted [3]. Moreover, since only the free, or unbound, concentration of the drug is considered to be medicinally effective, we chose to examine both the total concentration and the free concentration in the blood. The total blood concentration, C_{Bl}, is comprised of both the free concentration (C_{Bf}) and the bound concentration (C_{Bound}),

$$C_{Bl} = C_{Bf} + C_{Bound}.$$

Ertapenem exhibits nonlinear binding [10]; therefore, the bound concentration in the blood can be modeled using a nonlinear Michaelis-Menten equation

$$C_{Bound} = \frac{B_m C_{Bf}}{K_d + C_{Bf}},$$

where B_m is the density of binding sites and K_d is the dissociation constant [11, 19]. By substituting the equation for the bound concentration into the equation for the total concentration, we have

$$C_{Bl} = C_{Bf} + \frac{B_m C_{Bf}}{K_d + C_{Bf}}. \tag{1}$$

Thus, the total and free concentrations can be studied without directly calculating the bound concentration. By algebraic manipulation, we obtain the following equation for the free concentration:

$$C_{Bf} = \frac{C_{Bl} - B_m - K_d + \sqrt{(B_m + K_d - C_{Bl})^2 + 4K_d C_{Bl}}}{2}. \tag{2}$$

This equation gives the free concentration in terms of C_{Bl}, B_m, and K_d.

According to work by Merck & Co., "In healthy young adults, the protein binding of ertapenem decreases as plasma concentrations increase, from approximately 95% bound at an approximate plasma concentration of < 100 micrograms (mcg)/mL to approximately 85% bound at an approximate plasma concentration of 300 mcg/mL" [10]. Thus, ertapenem is not flowing quickly out of the blood into other tissues because of the high percentage that is bound; this also impacts how quickly the drug begins to be excreted from the body. This concept impacted our decision to implement an infusion coefficient, α, into the model. Instead of assuming that during the infusion that the outflow from the blood compartment was the entire free portion of the drug, we assumed that only a fraction of the free concentration was leaving the blood; thus, during infusion, the blood flow rate from the blood into each tissue compartment was multiplied by a constant value between 0 and 1, which is referred to as α_I, and after infusion, the infusion coefficient α is set equal to 1. Thus, the infusion constant α is defined by

$$\alpha = \begin{cases} \alpha_I, & 0 < t \leq T_I \\ 1, & T_I < t < 24 \end{cases}.$$

Since ertapenem has a longer half-life than most antibiotics and is being given during a short infusion time, the overall process of uptake and excretion is slow. Thus, since not all physiological characteristics can be taken into account, one way to include these components in our model was to assume that none of the drug was excreted via urine or feces during the infusion. Thus, k_u and k_f were set to zero during the infusion.

All subjects were considered to be male, and the average height and weight for normal males were used (See Table 1) [25]. The compartment volumes (given in mL) were based on body height and body weight; the calculations for V_{Bl} and V_K were obtained from [22] whereas the one for V_F was from [13] and the V_G was from [18]. The equations for compartment volumes are given by

$$\begin{aligned} V_{Bl} &= \frac{13.1(BH * 100) + 18.05(BW) - 480}{0.5723} \\ V_K &= 15.4 + 2.04(BW) + 51.8(BH)^2 \\ V_F &= \left(1.36 * \frac{BW}{BH} - 42\right) * 1000 \\ V_G &= 0.0171 * (BW) * 1000, \end{aligned}$$

where BW denotes the total body mass and BH is the body height. The International Life Sciences Institute provides a table of tissue densities in humans. For

TABLE 1. Parameter Values Obtained from Literature

Parameter	Value	Units	Reference
BW	72	kg	[25]
BH	1.75	m	[25]
Q_{Total}	$235 * (BW)^{0.71} * 60$	mL/hr	[5]
Q_F	$0.052 * Q_{Total}$	mL/hr	[18]
Q_K	$0.19 * Q_{Total}$	mL/hr	[18]
Q_G	$0.17 * Q_{Total}$	mL/hr	[18]

most soft tissues the value falls between 0.95 and 1.05; only in a few cases are tissue densities outside of the range 0.9 to 1.1 [9]. Thus, we assume total volume of the body was equal to body weight, with a only a change of units needed.

$$BW = Volume * \frac{1kg}{1L} * \frac{1L}{10^3 mL}.$$

Then, we define V_{OT} as the fraction of the total BW not included in the blood, adipose, kidney, or gut compartments

$$V_{OT} = BW * 1000 - (V_{Bl} + V_F + V_K + V_G).$$

The total rate flow in the body was calculated using the subject's body weight [5],

$$Q_{Total} = 235 * (BW)^{0.71} * 60.$$

The flow rates for the adipose, kidney, and gut compartments were a percentage of Q_{Total}, which are given in Table 1 [18]. Q_{OT} was defined as the fraction of Q_{Total} not included in the adipose, kidney, or gut compartments

$$Q_{OT} = Q_{Total} - (Q_F + Q_K + Q_G).$$

The venous-equilibrium model was used for each tissue compartment, which means that in the time that it takes the blood to perfuse the tissue, the drug is able to achieve an equilibrium concentration between the blood and the tissue [3]. Therefore, it was assumed that the concentration in the venous blood leaving the compartment was at equilibrium with the concentration in the compartment, with P_i being the equilibrium partition coefficient for tissue i:

$$C_{venous} = \frac{C_i}{P_i}.$$

Thus, this model requires partition coefficients of various tissues and blood. Partition coefficients for the individual tissue compartments represent the tissue's solubility; they determine the portion of the concentration that can flow from each tissue back into the blood. For example, P_F=1.95 means 1 mL of adipose tissue can hold 1.95 times as much ertapenem as 1 mL of blood. The partition coefficients were obtained by using an algorithm introduced by Poulin and Krishnan [20, 21]. The algorithm is based on n-octanol:water (Ko/w) partition coefficient data. It assumes that the sum of the solubility of a chemical in neutral lipids, phospholipids, and water in a particular tissue or blood is equal to the solubility of the chemical in the tissue or blood, respectively. The equation includes both physiological and drug specific parameters and is given by

$$P_t = \frac{[S_o * N_t] + [(S_w * 0.7P_t) + (S_o * 0.3P_t)] + [S_w * W_t]}{[S_o * N_b] + [(S_w * 0.7P_b) + (S_o * 0.3P_b)] + [S_w * W_b]},$$

where S_w is the solubility of the chemical in water and S_0 is the solubility of the chemical in n-octanol [21]. N_t, P_t, and W_t are the fractions of the tissue volume that are neutral lipids, phospholipids, and water, respectively; whereas, N_b, P_b, and W_b are the fractions of the blood volume that are neutral lipids, phosophilipids, and water. For ertapenem, S_w=0.069230349 mol/m^3 and $S_0 = K_{ow} * S_w$ where K_{ow}=1.66 is the octanol-water partition coefficient of ertapenem [6]. The muscle:blood partition coefficient was used for the other tissue:blood partition coefficient. The values calculated for the partition coefficients in this model are given in Table 2.

TABLE 2. Calculated Partition Coefficients

Parameter	Value
P_F	1.95
P_K	1.05
P_G	0.90
P_{OT}	1.01

During the infusion, the rate of infusion is given by

$$R_I = \begin{cases} \frac{D}{T_I}, & 0 \le t \le T_I \\ 0, & t > T_I \end{cases}, \tag{3}$$

where D is the dosage and T_I is the length of infusion. The model is described by the following system of differential equations:

$$\begin{aligned}
V_F \frac{dC_F}{dt} &= Q_F \left(\alpha C_{Bf} - \frac{C_F}{P_F} \right) \\
V_K \frac{dC_K}{dt} &= Q_K \left(\alpha C_{Bf} - \frac{C_K}{P_K} \right) - k_u C_K \\
V_G \frac{dC_G}{dt} &= Q_G \left(\alpha C_{Bf} - \frac{C_G}{P_G} \right) - k_f C_G \\
V_{OT} \frac{dC_{OT}}{dt} &= Q_{OT} \left(\alpha C_{Bf} - \frac{C_{OT}}{P_{OT}} \right) \\
V_{Bl} \frac{dC_{Bl}}{dt} &= Q_F \frac{C_F}{P_F} + Q_K \frac{C_K}{P_K} + Q_G \frac{C_G}{P_G} + Q_{OT} \frac{C_{OT}}{P_{OT}} - \alpha Q_{Total} C_{Bf} + R_I \\
\frac{dA_u}{dt} &= k_u C_K \\
\frac{dA_f}{dt} &= k_f C_G,
\end{aligned} \tag{4}$$

where C_{Bf} and R_I are given by equations (2) and (3), respectively. We assumed there were no background levels of ertapenem in the body, so all initial conditions were zero. The variables and parameters for the model are summarized in Table 3.

3. **Parameter estimation.** The model given by Equations (2) - (4) contains five unknown parameters (K_d, B_m, α_I, k_u, and k_f) which are estimated in this section. Mean plasma concentrations of total and free (unbound) ertapenem at corresponding time points were approximated from graphical data given for healthy adult subjects in Nix et. al. [17] using an extraction program [24] and are given in Table 4. The Nix data [17] corresponded well to the data in [10], but we chose to use the

TABLE 3. Definitions of Model Variables and Parameters

Symbol	Description	Units
C_i	Concentration of ertapenem in tissue i	mcg/mL
C_{Bf}	Concentration of free ertapenem in the blood	mcg/mL
A_u	Amount of ertapenem in urine	mcg
A_f	Amount of ertapenem in feces	mcg
V_i	Volume of tissue i	mL
Q_i	Flow Rate in tissue i	mL/hr
t	Time	hr
P_i	Blood partition coefficient of tissue i	dimensionless
BW	Body Weight	kg
BH	Body Height	m
α	Infusion Coefficient	dimensionless
R_I	Rate of Infusion	mcg/hr
D	Dose	mcg
T_I	Length of Infusion	hr
k_u	First-order rate constant of urine excretion	mL/hr
k_f	First-order rate constant of feces excretion	mL/hr
B_m	Blood receptor constant	mcg/mL
K_d	Dissociation constant	mcg/mL

data from Nix as both total and free concentrations were available in this reference. We split the parameter estimation problem into two parts: 1) estimation of K_d and B_m, and 2) estimation of α_I, k_u, and k_f. Parameters K_d and B_m only appear in the relationship between the free and total concentration; therefore, it is not necessary to use the entire model to determine these parameters. Moreover, if one performs this estimation separate from the entire model, one can use this relationship in future studies (depending on the focus of the study). We note that similar, yet slightly different, parameter estimates are found if one performs a single optimization problem; however, the two-part optimization problem produces a better estimate for the relationship between the free and total concentration. Since free concentration is important in determining the medical effectiveness of the antibiotic, we choose to implement the two-part optimization problem.

TABLE 4. Clinical Data for the Total and Free Concentrations of Ertapenem [17]

Time (t_j) (hr)	Total Concentration ($C_{Bl}(t_j) \equiv y_{1j}$) ($mcg/mL$)	Free Concentration ($C_{Bf}(t_j) \equiv y_{2j}$) ($mcg/mL$)
0.5	160.30	15.48
4	50.57	2.70
6	30.47	1.58
8	20.56	1.10
12	10.47	0.42
18	3.70	0.15

3.1. **Estimation of K_d and B_m.** In Section 2, we introduced two equivalent relationships for the total concentration of ertapenem in the blood, C_{Bl}, and the free or unbound concentration, C_{Bf}, with unknown parameters K_m and B_d. Equation (1) defines the total concentration as a function of the free concentration dependent on the parameter $\mathbf{q} = [K_d, B_m]$, i.e. $C_{Bl}(t) = f_1(t, \mathbf{q})$, where

$$f_1(t, \mathbf{q}) \equiv f_1(C_{Bf}(t), \mathbf{q}) = C_{Bf}(t) + \frac{B_m C_{Bf}(t)}{K_d + C_{Bf}(t)}.$$

Similarly, Equation (2) defines the free concentration as a function of the total concentration, i.e. $C_{Bf}(t) = f_2(t, \mathbf{q})$ where

$$\begin{aligned} f_2(t, \mathbf{q}) &\equiv f_2(C_{Bl}(t), \mathbf{q}) \\ &= \frac{C_{Bl}(t) - B_m - K_d + \sqrt{(B_m + K_d - C_{Bl}(t))^2 + 4K_d C_{Bl}(t)}}{2}. \end{aligned}$$

We assume the data in Table 4 is a realization y_{*j} of the statistical model

$$Y_{*j} = f_*(t_j, \mathbf{q}_0)(1 + \tilde{\mathcal{E}}_{*j}),$$

where $\mathbf{q}_0$ is assumed to be the vector of "true" parameter values for the relationship between C_{Bl} and C_{Bf} and $\tilde{\mathcal{E}}_{*j}$ is measurement error which is identically distributed with constant variance, i.e. $\mathbb{E}(\tilde{\mathcal{E}}_{*j}) = 0$ and $\mathrm{Var}(\tilde{\mathcal{E}}_{*j}) = \sigma_0^2$. Thus, in the estimation problem for K_d and B_m, we seek an estimator $\mathbf{q}$,

$$\mathbf{q} = \arg \min_{\mathbf{q} \in Q} \sum_{j=1}^{N} \left(f_1^{-2}(t_j, \mathbf{q})[Y_{1j} - f_1(t_j, \mathbf{q})]^2 + f_2^{-2}(t_j, \mathbf{q})[Y_{2j} - f_2(t_j, \mathbf{q})]^2 \right)$$

with corresponding estimate

$$\hat{\mathbf{q}} = \arg \min_{\mathbf{q} \in Q} \sum_{j=1}^{N} \left(\left[\frac{y_{1j} - f_1(t_j, \mathbf{q})}{f_1(t_j, \mathbf{q})} \right]^2 + \left[\frac{y_{2j} - f_2(t_j, \mathbf{q})}{f_2(t_j, \mathbf{q})} \right]^2 \right),$$

where Q is the set of admissible parameter values and $N = 6$ is the number of time steps for which we have data.

In order to insure all parameter values were nonnegative throughout the estimation process, we use a $\ln - \exp$ transformation in the inverse problem. The parameter values are initially transformed by taking the natural logarithm of each value and then the Nelder-Mead simplex method is employed using the fminsearch function in MATLAB [16] to find the optimal values for $\ln q$, where q is the parameter value. Finally, the parameter values are transformed back by taking the exponential. The initial guesses for these parameters were $\ln(K_{d0}) = \ln(15)$ and $\ln(B_{m0}) = \ln(260)$. The rank of the Fisher Information Matrix, $F = \chi^T W \chi$, was used to determine that the parameters were identifiable [4] where the inverse of W is a matrix defined by

$$W^{-1} = \mathrm{diag}(f_1^2(t_1, \mathbf{q}), ..., f_1^2(t_N, \mathbf{q}), f_2^2(t_1, \mathbf{q}), ..., f_2^2(t_N, \mathbf{q})),$$

and

$$\chi(q) = \begin{bmatrix} \frac{\partial f_1(t_1,q)}{\partial K_m} & \frac{\partial f_1(t_1,q)}{\partial B_d} \\ \vdots & \vdots \\ \frac{\partial f_1(t_N,q)}{\partial K_m} & \frac{\partial f_1(t_N,q)}{\partial B_d} \\ \frac{\partial f_2(t_1,q)}{\partial K_m} & \frac{\partial f_2(t_1,q)}{\partial B_d} \\ \vdots & \vdots \\ \frac{\partial f_2(t_N,q)}{\partial K_m} & \frac{\partial f_2(t_N,q)}{\partial B_d} \end{bmatrix}.$$

Figure 2 shows the approximation for $C_{Bf} = f_2(C_{Bl}(t), \mathbf{q})$ using the estimated optimal values $B_m = 243.28\ mcg/mL$ and $K_d = 10.88\ mcg/mL$. In Section 4, we examine the uncertainty in the parameter estimates and the propagation of this uncertainty into the model using a bootstrapping method.

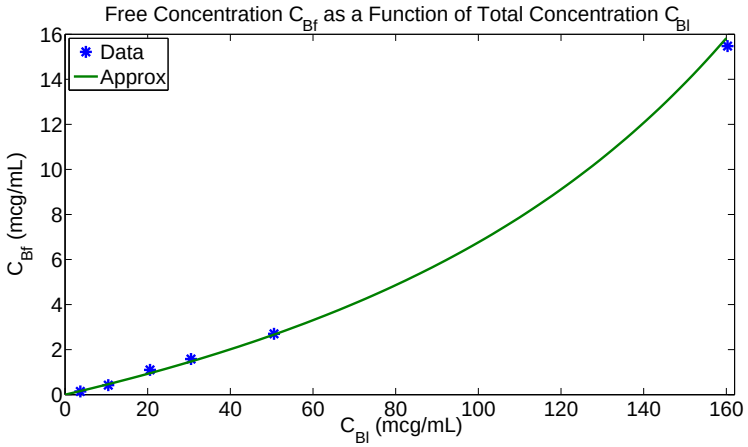


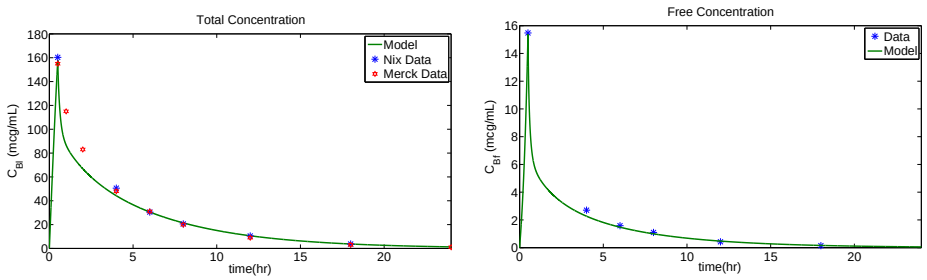
FIGURE 2. Free concentration as a function of total concentration using optimal values for B_m and K_d.

3.2. **Estimation of α_I, k_u, and k_f.** In the previous subsection, we were able to estimate K_d and B_m separately from the model, since these parameters rely strictly on the relationship between the free and total concentrations. To estimate α_I, k_u, and k_f, we must use the entire model given by Equations (2) - (4). As discussed in Section 2, since we assume that during infusion a fraction of the free concentration is leaving the blood, the infusion constant, α, is defined by

$$\alpha = \begin{cases} \alpha_I, & 0 < t \leq T_I \\ 1, & T_I < t < 24 \end{cases},$$

where $0 < \alpha_I < 1$. All other times, we assume the entire free concentration of ertapenem will flow through the body. Therefore, α_I is the parameter we wish to estimate. The parameters α_I, k_u, and k_f were found to be structurally identifiable using the differential algebra approach to identifiability as outlined by Bellu et. al. [2].

The goal of the estimation problem is to find parameter values such that the model is a good approximation of the measured concentrations in the blood (C_{Bl} and C_{Bf}) as well as the measured excretion; therefore, in addition to the data in



(A) Total Concentration (B) Free Concentration

FIGURE 3. Simulation of Equations (2) - (4) using optimal parameter values given in Sections 3.1 and 3.2. Plot (A) illustrates the total concentration C_{Bl}. Plot (B) illustrates the free concentration C_{Bf}.

Table 4, we also use the fact that 80% of the ertapenem dose is typically excreted in the urine within a 24-hour period [10]. As we did in the previous section, we assume the data (including excretion of 80%) is a realization y of a statistical model

$$Y = g(t, \mathbf{q}_0)(1 + \tilde{\mathcal{E}})$$

where $\mathbf{q} = [\alpha_I, k_u, k_f]$, the measurement error $\tilde{\mathcal{E}}_j$ is identically distributed with constant variance, and $g(t, \mathbf{q}_0)$ is the measured, or observed, part of the model at the "true" parameter $\mathbf{q}_0$. We note that $y = [y_{1j}, y_{2j}, 0.8]^T$, $j = 1, ...N$ where y_{*j} is the data given in Table 4, and the observed part of the solution is

$$g(t, \mathbf{q}) = [C_{Bl}(t, \mathbf{q}), C_{Bf}(t, \mathbf{q}), A_u(t)]^T.$$

Since we only have data for C_{Bl} and C_{Bf} at times $t_j = 0.5, 4, 6, 8, 12, 18$ and A_u at time 24, we only consider the observed part of the solution at these time points in the optimization problem. Therefore, we seek an estimator $\hat{\mathbf{q}}$,

$$\hat{\mathbf{q}} = \arg\min_{\mathbf{q}\in Q} \left\{ \sum_{j=1}^{N} \left(\frac{y_{1j} - C_{Bl}(t_j, \mathbf{q}))}{C_{Bl}(t_j, \mathbf{q})} \right)^2 + \sum_{j=1}^{N} \left(\frac{y_{2j} - C_{Bf}(t_j, \mathbf{q}))}{C_{Bf}(t_j, \mathbf{q})} \right)^2 + \left(\frac{0.8 - A_u(24)}{A_u(24)} \right)^2 \right\}.$$

In the parameter estimation problem for α_I, k_u and k_f, we set K_d and B_m equal to the optimal values given in Section 3.1. We then use *fminsearch* and the transformation technique discussed in Section 3.1 to obtain the estimated values $\alpha_I = 0.32$, $k_u = 68588\ mL/hr$ and $k_f = 9639\ mL/hr$. Figure 3 shows the simulation of the model in Equations (2) - (4) using the optimal parameter values with the data; additional total concentration data from Merck & Co. [10] was also plotted in order to compare to data that was not used in the optimization. The comparison between concentration values from the Nix study (Table 4) and the model is given in Table 5; furthermore, the urine excretion is estimated as approximately 80%, the same as the clinical average. The model has an average 6% relative point error when compared to the data.

TABLE 5. Comparison of Model Output and Data

t_j (hours)	C_{Bl} (mcg/mL)		C_{Bf} (mcg/mL)	
	Data	Model	Data	Model
0.5	160.30	157.77	15.48	15.36
4	50.57	44.21	2.70	2.27
6	30.47	30.55	1.58	1.48
8	20.56	21.42	1.10	1.00
12	10.47	10.62	0.42	0.47
18	3.70	3.71	0.15	0.16

4. **Uncertainty analysis.** Due to the small number of data points, asymptotic theory is not appropriate for estimating the uncertainty in the parameter estimates; therefore, in this section, we use a popular bootstrapping technique to investigate the uncertainty in the parameter estimation and how this uncertainty propagates through the model. We construct a family of samples or simulated data using the residuals obtained from the estimates in Table 5. Using the generated sample data, we obtain estimates for the parameters and model simulations using these estimates. The following algorithm is a modified version of the bootstrapping algorithm in Banks et. al. [1] used to determine a distribution of parameter values and corresponding uncertainty in model simulations using 1000 constructed samples.

1. First, estimate optimal parameter values for B_m and K_d using the data in Table 4 and the method defined in Section 3.1.
2. Substitute optimal parameter values B_m and K_d found in Step 1 in the model given by Equations (2) - (4). Then estimate α_I, k_f, and k_u using the methods outlined in Section 3.2.
3. Let $\theta^0 = [B_m^0, K_d^0, \alpha_I^0, k_f^0, k_u^0]$ be the vector of optimal parameter values found in steps 1 and 2. Define the set
$$S = \left\{ \frac{y_{1j} - C_{Bl}(t_j, \theta^{\mathbf{0}})}{C_{Bl}(t_j, \theta^{\mathbf{0}})}, \frac{y_{2j} - C_{Bf}(t_j, \theta^{\mathbf{0}})}{C_{Bf}(t_j, \theta^{\mathbf{0}})}, \frac{0.8 - A_u(24, \theta^0)}{A_u(24, \theta^0)} \right\}, j = 1, ..., N$$
to be the set of 13 standardized residuals.
4. From the set $S = \{s_1, s_2, ..., s_{13}\}$, form a bootstrapping sample $S_b = \{s_1^n, s_2^n, ..., s_{13}^n\}$ by using random sampling with replacement from the set S.
5. Create a sample data set y^n using S_b:
$$y^n = \begin{cases} C_{Bl}(t_j, \theta_{\mathbf{0}}) + C_{Bl}(t_j, \theta_{\mathbf{0}}) s_k^n, & k = 1, ..., 6, \quad j = 1, ...N \\ C_{Bf}(t_j, \theta_{\mathbf{0}}) + C_{Bf}(t_j, \theta_{\mathbf{0}}) s_k^n, & k = 7, ..., 12, \quad j = 1, ..., N \\ A_u(24, \theta_{\mathbf{0}}) + A_u(24, \theta_{\mathbf{0}}) s_k^n, & k = 13. \end{cases}$$
6. Obtain a new estimate θ_0^n:
   - First estimate B_m^n and K_d^n using data y_k^n, $k = 1, ..., 12$ and the method defined in Section 3.1.
   - Then substituting B_m^n and K_d^n in the model given by Equations (2) - (4), estimate α_I^n, k_f^n, and k_u^n using the methods outlined in Section 3.2 and the data y^n.
7. Simulate the model using θ_0^n.
8. Set $n = n + 1$ and repeat steps 4-7 until n=1000.

We calculate 90% confidence intervals for the parameters using the formula

$$[\hat{q} - t_{0.05}(SE), \hat{q} + t_{0.05}(SE)].$$

with critical value $t_{0.05}$ from the *student's t* distribution t^{2N-p} with $2N-p$ degrees of freedom for B_m and K_d and t^{2N+1-p} with $2N+1-p$ degrees of freedom for α_I, k_u and k_f ($N = 6$, $p = 2$) [1]. Table 6 gives the parameter estimates and confidence intervals using the bootstrap method. There is a lot of variability in the confidence intervals, especially for k_u and k_f; however, the variability in the parameter estimates do not generate a significant amount of variability in model predictions for the concentration of ertapenem in the blood as shown in Figure 4 (grey shaded region). Moreover, the mean amount of ertapenem excreted in the urine across the 1000 sample data sets is 81% of the dose with standard deviation of 0.07%. Finally, we can use the statistical model given in Section 3.2,

$$Y = g(t, \mathbf{q}_0)(1 + \tilde{\mathcal{E}}),$$

to estimate a 95% confidence interval for the total concentration of ertapenem using the model in Equations (2) - (4), optimal parameter values found in Sections 3.1 and 3.2, and assuming $\tilde{\mathcal{E}}_j$ is identically distributed with constant variance $\text{Var}(\tilde{\mathcal{E}}) = \sigma_0^2 \approx \hat{\sigma}^2$ where

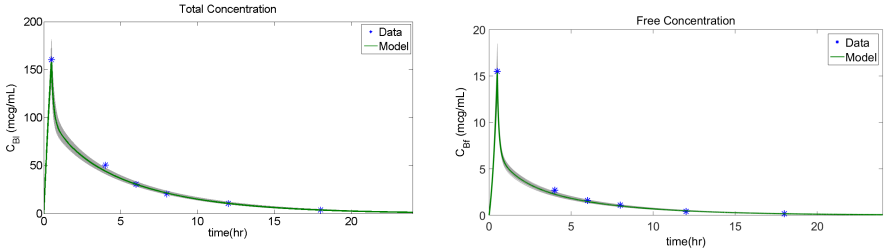
$$\hat{\sigma}^2 = \frac{1}{2N+1-p}\left(\sum_{j=1}^{N}\left(\frac{y_{1j} - C_{Bl}(t,\mathbf{q})}{C_{Bl}(t,\mathbf{q})}\right)^2 + \sum_{j=1}^{N}\left(\frac{y_{2j} - C_{Bf}(t,\mathbf{q}))}{C_{Bf}(t,\mathbf{q})}\right)^2 + \left(\frac{0.8 - A_u(24)}{A_u(24)}\right)^2\right).$$

Figure 5 shows the data (blue stars) for the total concentration of ertapenem in the blood as a function of time, corresponding model simulation with optimal parameters (green line), uncertainty in model simulations due to distribution of parameter estimations (light grey area), and 95% confidence interval in solution assuming a statistical model as above (black dashed line).

TABLE 6. Estimates and Error Bounds for All Parameters using Bootstrapping

$\hat{q}$	Est	SE	90% CI
B_m ($\frac{mcg}{mL}$)	252.53	48.06	[165.42, 339.64]
K_d ($\frac{mcg}{mL}$)	11.35	2.60	[6.65, 16.05]
α_I (unitless)	0.29	0.16	[0.01,0.58]
k_u ($\frac{mL}{hr}$)	71405	15214	[43830 ,98981]
k_f ($\frac{mL}{hr}$)	8875	3837	[1921, 15829]

5. **Discussion and conclusions.** In this paper, we developed a PBPK model for a single gram dose of the antibiotic ertapenem administered intravenously. Ertapenem is a highly bound antibiotic; therefore, we chose to consider the free or unbound concentration separately from the total concentration in the blood. In the future this will allow us to test the effect of varying physiological parameters on the medicinally effective portion of the antibiotic. We fitted the model to published clinical data



(A) Total Concentration (B) Free Concentration

FIGURE 4. Total Concentration C_{Bl} (A) and Free Concentration C_{Bf} (B): Propagation of uncertainty of parameter estimates into the model using the bootstrapping algorithm described in Section 4.

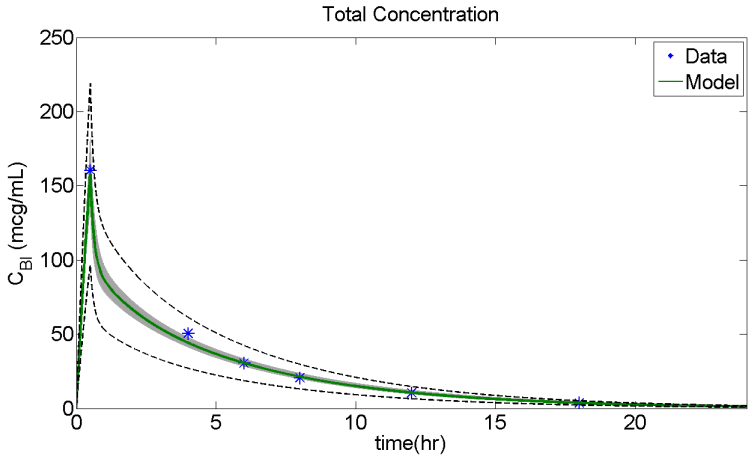


FIGURE 5. This figure shows the data (blue stars) for the total concentration of ertapenem in the blood as a function of time, the corresponding model simulation with optimal parameters (green line), uncertainty in model simulations due to distribution of parameter estimations (light grey area), and 95% confidence interval in solution assuming a statistical model as above (black dashed line).

producing a model fit with an average of only 6% relative point error when compared to the data. Furthermore, we examined the effect of uncertainty in the parameter estimations and the consequence of propagating this uncertainty into the model prediction. We note that all data points, which are averages from the Nix study [17], lie within a 95% confidence interval of the solution curve. If a large number of individual measurements were taken, one can assume that 95% of the data points would lie within the given confidence interval.

The standard PBPK modeling techniques used in the development of the model for ertapenem can be modified and used for other antibiotics. Furthermore, the separation of the free and total concentration in the modeling process allows one to

examine potential cases in which the free concentration may fall below the minimum inhibitory concentration and thus allow for the potential development of resistant bacteria. In a future paper, we use this model to examine the potential effects of varying parameters such as weight, height or gender on this minimum level of free concentration; this would allow us to better predict what the most effective dosing might be, which would hopefully then produce the most timely healing of the infection and minimize antibiotic resistance.

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E-mail address: joynerm@etsu.edu
E-mail address: manningc@meredith.edu
E-mail address: wforbes2@vols.utk.edu
E-mail address: michelle.maiden@colorado.edu
E-mail address: annikas@ncsu.edu