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Pitfalls in Pharmacokinetic Multicompartment Analysis

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When a pharmacokinetic (PK) two-compartment body model with first-order absorption is fitted to blood levels of a drug, the estimates of the PK parameters may have considerable errors and can cause wrong predictions in other features of the system. The objectives of this report were to illustrate this problem, to provide an easy way to prevent wrong estimation, and to investigate the origin of the mistake. A simple way to prevent wrong interpretation of the calculated PK parameters is to inspect the PK profiles visually. Without observing a clear biphasic profile, one should not apply the two-compartment model if the resulting parameters are to be interpreted and used for further simulations. We investigated the origin of this ambiguity in terms of the relative order of magnitude of microconstants (k_a, k_{12}, k_{21}, and k_{10}) and of hybrid constants (A and B). The observed parameter errors will not be of any relevance if the calculated parameters are used only to predict future blood levels over the same time-span. However, if these parameters are used to predict any other characteristic of the system, erroneous predictions may result.

KEY WORDS: compartmental analysis; two-compartment model; first-order absorption; vanishing exponential.

INTRODUCTION

When a pharmacokinetic two-compartment model with first-order absorption (Fig. 1) is fitted to blood levels of a drug, the estimates of the pharmacokinetic parameters may be erroneous due to the ambiguity of parameter assignment (1–4). In the case of a good fit, this error is not relevant as long as the model is used only to predict blood levels. However, when the parameters are used to predict other features of the system such as drug tissue levels, considerable errors in prediction may result. The objective of this report is to illustrate this problem by a simulation of actual blood level data through the aid of computer programs Excel and PKAnalyst and to provide a simple way to predict and prevent this mistake.

METHODS

The blood levels (central and peripheral) for a drug (C) following a two-compartment body model with first-order absorption can be described

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by the following set of equations:

$$C_{\text{Central}} = A * e^{-\alpha t} + B * e^{-\beta t} + D * e^{-k_a t} \quad (1)$$

$$C_{\text{Peripheral}} = \frac{A * k_{21}}{(k_{21} - \alpha)} * e^{-\alpha t} + \frac{B * k_{21}}{(k_{21} - \beta)} * e^{-\beta t} + \frac{D * k_{21}}{(k_{21} - k_a)} * e^{-k_a t} \quad (2)$$

with

$$A = \frac{k_a * Dose}{V_c} * \frac{(k_{21} - \alpha)}{(\beta - \alpha)(k_a - \alpha)} \quad (3)$$

$$B = \frac{k_a * Dose}{V_c} * \frac{(k_{21} - \beta)}{(\alpha - \beta)(k_a - \beta)} \quad (4)$$

$$D = -(A + B) \quad (5)$$

$$\alpha = \tfrac{1}{2}\left[(k_{12} + k_{21} + k_{10}) + \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}}\right] \quad (6)$$

$$\beta = \tfrac{1}{2}\left[(k_{12} + k_{21} + k_{10}) - \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}}\right] \quad (7)$$

where $Dose$ = bioavailable dose; V_c = volume of the central compartment; C_{Central} and $C_{\text{Peripheral}}$ = drug concentration in central and peripheral compartments, respectively; A and B = hybrid constants for intercepts in distribution and elimination phase, respectively; α and β = hybrid constants for slopes in distribution and elimination phase, respectively (with $\alpha > \beta$); k_a = absorption rate constant; k_{12} = distribution rate constant from central compartment to peripheral compartment; k_{21} = distribution rate constant from peripheral compartment to central compartment; and k_{10} = elimination rate constant from central compartment.

Twenty-four combinations of the microconstants (k_a, k_{12}, k_{21}, and k_{10}) with 3, 1, 0.3, and 0.1 hr^{-1} along with $Dose/V_c = 10\ \mu g/ml$ were used as input parameters. The theoretical time–concentration profiles in the central and peripheral compartments were calculated using Excel with these parameters substituted into the Eqs. (1) and (2). A low level of random noise (±5%) was added to the blood levels to generate artificial data at various time points (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, and 24 hr) in the central compartment.

The initial macroconstant parameter estimation was conducted by the stripping algorithm from the PKAnalyst program to prevent any bias. The equations were then fitted by minimizing sum of squares (PKAnalyst) to the two-compartment model to obtain estimated parameters (A, B, α, β, and k_a) with the default rank order $k_a > \alpha > \beta$. A weighting factor of 1 where each concentration is weighted by its reciprocal (WLS[y^{-1}]) was used.

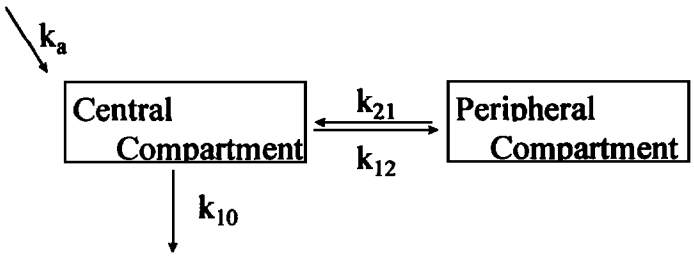


Fig. 1. Schematic diagram of the body as a two-compartment body model. Drug elimination is limited to the central compartment.

The calculated variable k_{21}, in combination with the estimated parameters (A, B, α, β, and k_a), was applied in Eq. (2) to obtain the predicted time–concentration profile in the peripheral compartment. The profiles were then used to compare with the theoretical peripheral compartment profiles.

RESULTS

The two-compartment body model equation for the central compartment resulted in good fits for all of the 24 combinations when fitted to the data (model selection criteria >4.5, correlation >0.997 and coefficient of determination >0.994) (Fig. 2). However, predicted parameters in some examples (Cases 17, 19, 21, and 23) exhibited huge standard deviations which make the predicted parameters unreliable. This issue has been the focus of intensive study in a previous report (1).

When comparing the calculated parameters to the theoretical parameters, the calculated parameters from some examples (Cases 15, 17, 20, 21, 23, and 24) were apparently the results of flip-flop profiles ($k_a < \beta$) where the estimated terminal half-life was prolonged in comparison to the respective half-life after intravenous administration due to the rate-limiting, slower input kinetics (Table I). It is therefore necessary to address the impact of the relative magnitude of the three predicted macroconstants on the peripheral compartment time–concentration profiles before any further data interpretation and application.

It is well known that without an intravenous reference, one cannot assign the estimated parameters from the profiles with first-order absorption. To investigate the impact of the three possible orders of k_a, α and β($k_a > \alpha > \beta$, $\alpha > k_a > \beta$, and $\alpha > \beta > k_a$) on the peripheral compartment profiles, it is necessary to express Eq. (2) in macroconstants only. First of all,

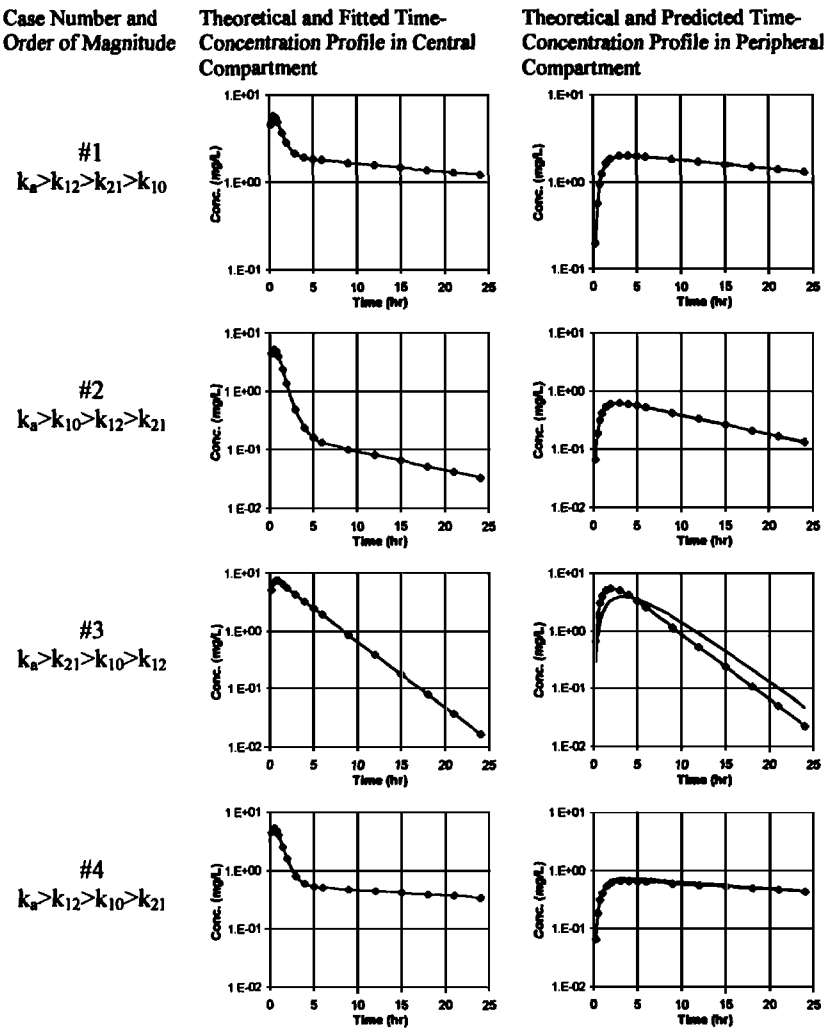


Fig. 2. Comparison of the theoretical (smooth curve) and fitted (point) time–concentration profiles in central compartment and the theoretical (smooth curve with points) predicted (smooth curve without points) time–concentration profiles in peripheral compartment.

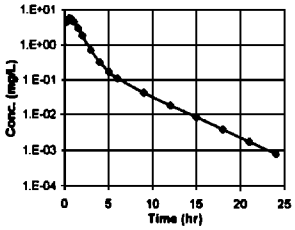
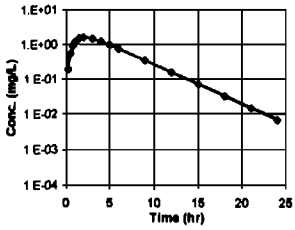
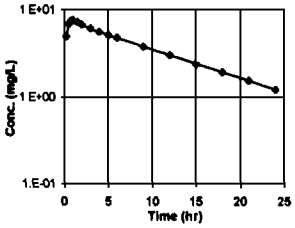
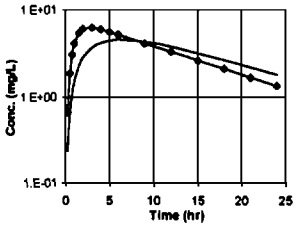
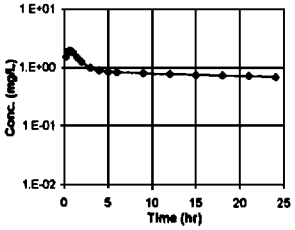
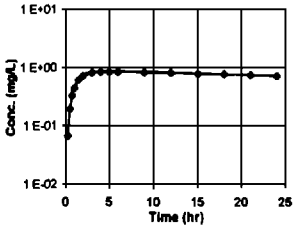
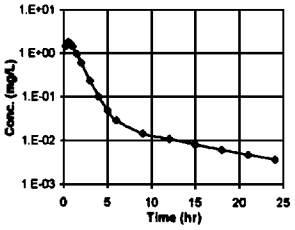
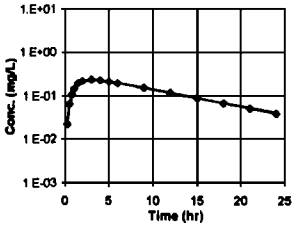
Case Number and Order of Magnitude	Theoretical and Fitted Time-Concentration Profile in Central Compartment	Theoretical and Predicted Time-Concentration Profile in Peripheral Compartment
#5 $k_a > k_{10} > k_{21} > k_{12}$		
#6 $k_a > k_{21} > k_{12} > k_{10}$		
#7 $k_{12} > k_a > k_{21} > k_{10}$		
#8 $k_{10} > k_a > k_{12} > k_{21}$		

Fig. 2. Continued.

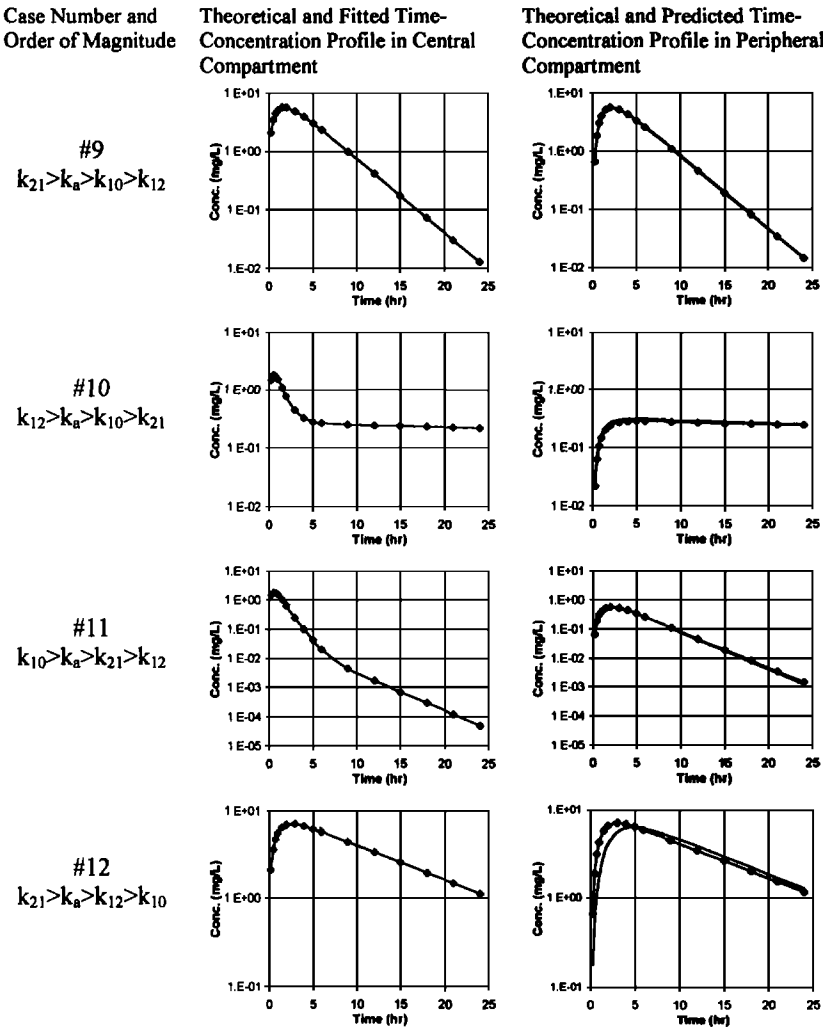


Fig. 2. Continued.

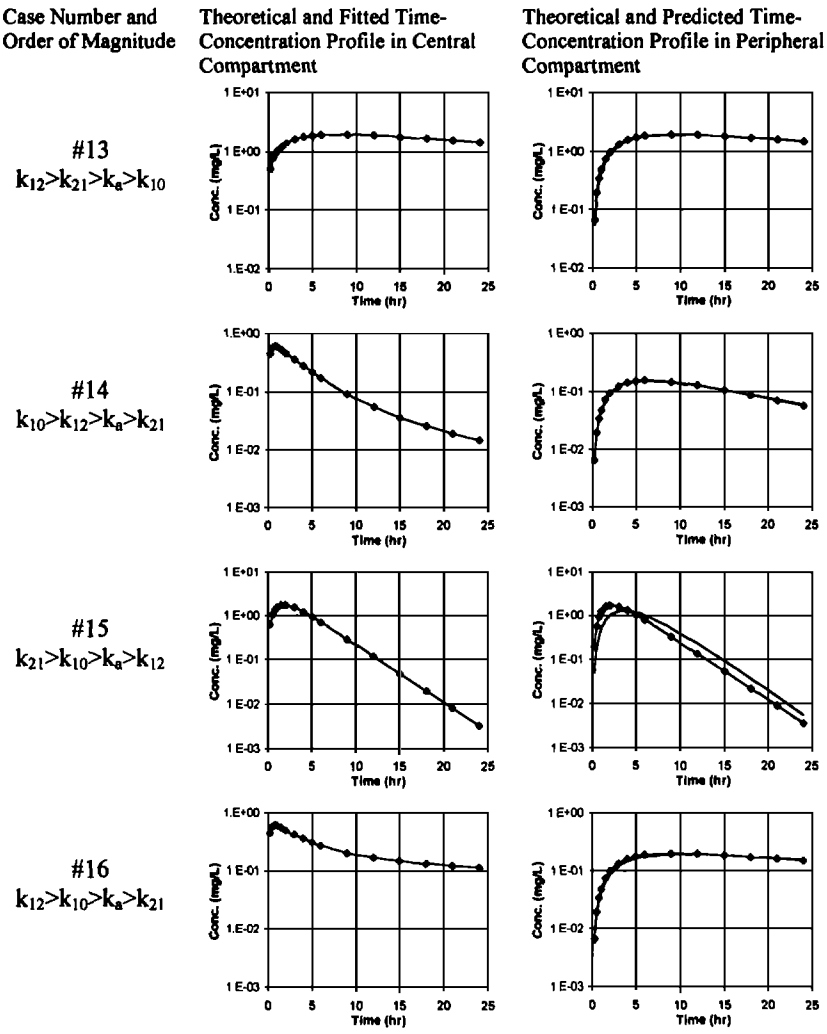


Fig. 2. Continued.

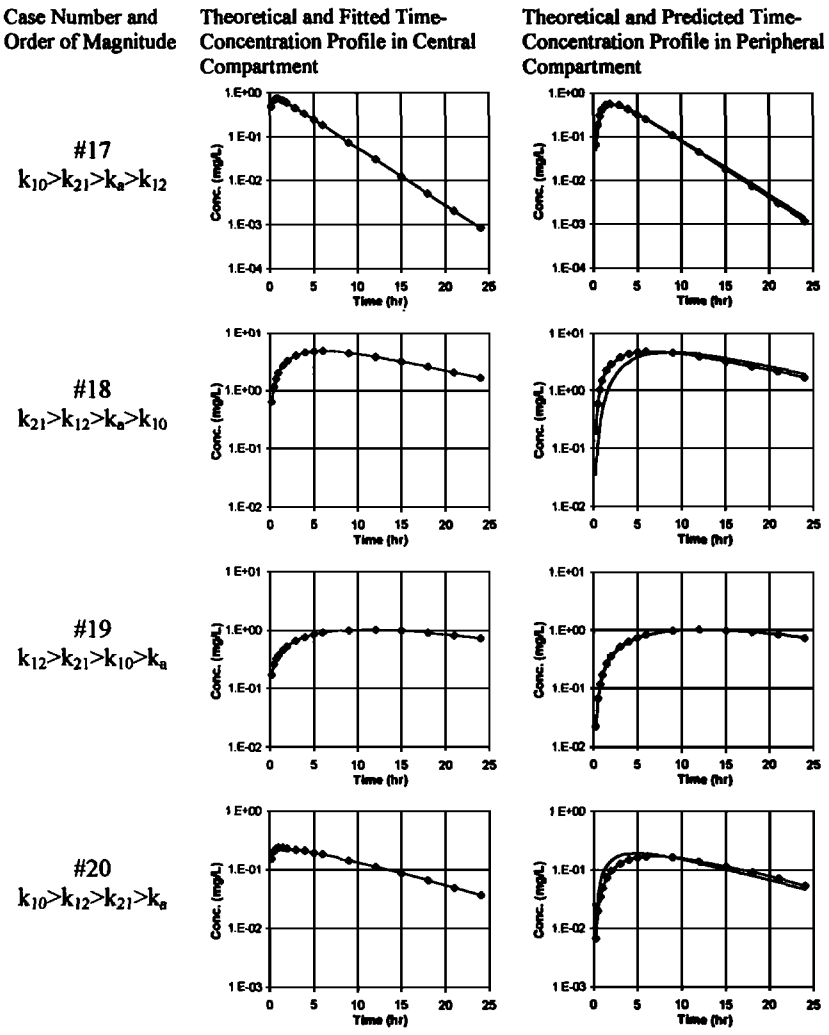


Fig. 2. Continued.

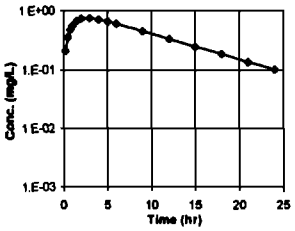
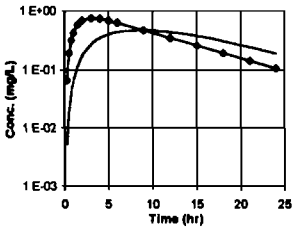
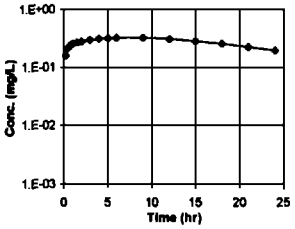
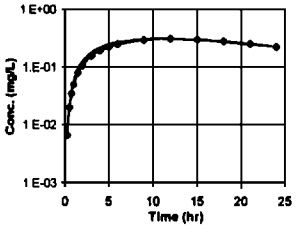
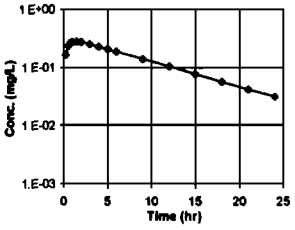
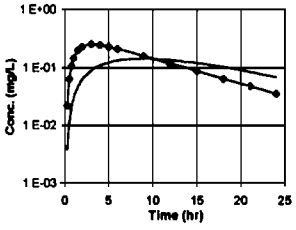
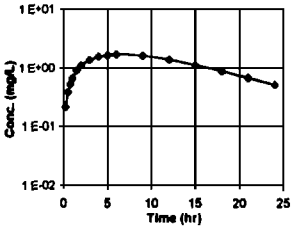
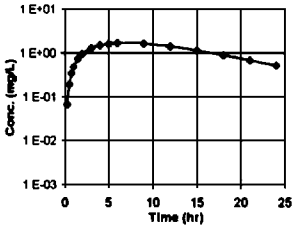
Case Number and Order of Magnitude	Theoretical and Fitted Time-Concentration Profile in Central Compartment	Theoretical and Predicted Time-Concentration Profile in Peripheral Compartment
#21 $k_{21}>k_{10}>k_{12}>k_a$		
#22 $k_{12}>k_{10}>k_{21}>k_a$		
#23 $k_{10}>k_{21}>k_{12}>k_a$		
#24 $k_{21}>k_{12}>k_{10}>k_a$		

Fig. 2. Continued.

Table I. Comparison of Theoretical and Estimated Pharmacokinetic Parameters[a]

	Theoretical value								Fitted value (SD)				
Case	k_a	k_1	k_{21}	k_{10}	A	B	α	β	k_a	A	B	α	β
1	3	1	0.3	0.1	15	2.1	1.4	0.022	3.3 (0.96)	11 (4.2)	2.1 (0.077)	1.2 (0.21)	0.022 (0.0026)
2	3	0.3	0.1	1	18	0.2	1.3	0.076	3.3 (0.53)	16 (3.0)	0.20 (0.031)	1.3 (0.093)	0.077 (0.012)
3[b,d]	3	0.1	1	0.3	2.5	9.3	1.1	0.26	3.5 (0.51)	1.8 (6.4)	8.3 (6.8)	0.42 (0.60)	0.26 (0.039)
4	3	1	0.1	0.3	17	0.58	1.4	0.022	2.1 (0.75)	44 (100)	0.61 (0.027)	1.6 (0.47)	0.026 (0.0031)
5	3	0.1	0.3	1	15	0.45	1.1	0.26	3.2 (0.21)	14 (0.90)	0.45 (0.064)	1.1 (0.037)	0.26 (0.015)
6[b,d]	3	0.3	1	0.1	4.7	7.6	1.3	0.076	5.2 (0.67)	2.3 (0.39)	6.4 (0.49)	0.32 (0.095)	0.066 (0.0039)
7[c]	1	3	0.3	0.1	–3.8	0.87	3.4	0.0088	2.6 (1.0)	4.8 (4.3)	0.88 (0.032)	1.2 (0.37)	0.0096 (0.0024)
8[c]	1	0.3	0.1	3	–4.3	0.032	3.3	0.091	3.0 (0.38)	4.4 (0.46)	0.030 (0.0094)	1.0 (0.047)	0.087 (0.022)
9[d]	1	0.1	3	0.3	–0.19	14	3.1	0.29	4.0 (7.5)	–13 (1.5)	14 (1.2)	0.90 (0.16)	0.29 (0.0096)
10	1	3	0.1	0.3	–4.1	0.27	3.4	0.088	3.9 (0.38)	3.6 (0.24)	0.29 (0.0085)	1.0 (0.041)	0.012 (0.0019)
11	1	0.1	0.3	3	–4.7	0.05	3.1	0.29	3.3 (0.30)	4.7 (0.35)	0.062 (0.045)	1.0 (0.05)	0.3 (0.07)
12[b,d]	1	0.3	3	0.1	–0.42	9.9	3.3	0.091	1.3 (0.45)	–3.2 (5.1)	10 (0.47)	0.56 (0.49)	0.093 (0.0027)
13*	0.3	3	1	0.1	–0.60	2.6	4.1	0.025	3.2 (1.4)	–1.8 (0.11)	2.5 (0.15)	0.30 (0.047)	0.022 (0.0030)
14	0.3	1	0.1	3	–0.80	0.086	4.0	0.075	3.7 (0.43)	0.66 (0.029)	0.14 (0.038)	0.33 (0.023)	0.099 (0.013)
15[b,c,d]	0.3	0.1	3	1	–0.070	–4.3	3.1	0.95	1.3 (0.45)	–4.6 (18)	7.3 (19)	0.45 (0.48)	0.33 (0.10)
16	0.3	3	0.1	1	–0.78	0.20	4.1	0.025	4.1 (0.83)	0.55 (0.027)	0.19 (0.034)	0.26 (0.029)	0.023 (0.0084)
17[c,d]	0.3	0.1	1	3	–1.0	–0.098	3.1	0.95	1.4332 (0.25)	2314 (1027629)	1.0 (0.029)	1.4329 (0.41)	0.29 (0.0038)
18[b,c,d]	0.3	1	3	0.1	–0.21	9.9	4.0	0.075	0.55 (5.8)	–9.7 (19)	10 (3.7)	0.29 (0.34)	0.076 (0.014)
19[d]	0.1	3	1	0.3	–0.19	7.7	4.2	0.071	3.8 (2.2)	13367 (NAN)[e]	–13366 (NAN)[e]	0.08289 (0.0015)	0.08291 (0.0015)
20[b,c,d]	0.1	1	0.3	3	–0.25	–0.17	4.1	0.22	6.1 (2.1)	–0.078 (0.020)	0.31 (0.026)	0.43 (0.23)	0.087 (0.0048)
21[b,c,d]	0.1	0.3	3	1	–0.050	–1.1	3.4	0.88	1.1 (0.064)	–68 (NAN)[e]	69 (NAN)[e]	0.133 (NAN)[e]	0.132 (NAN)[e]
22[d]	0.1	3	0.3	1	–0.23	1.9	4.2	0.071	3.6 (0.71)	–0.30 (0.069)	0.54 (0.076)	0.19 (0.043)	0.040 (0.0056)
23[b,c,d]	0.1	0.3	1	3	–0.29	–0.063	3.4	0.88	3.6 (0.47)	–30 (210916)	30 (210916)	0.1161 (0.52)	0.1159 (1.3)
24[c,d]	0.1	1	3	0.3	–0.070	–6.0	4.1	0.22	4.6 (7.5)	–7.2 (2.7)	7.3 (2.8)	0.20 (0.030)	0.11 (0.03)

[a] Data for theoretical parameters were obtained by substituting 24 different values of the microconstants into Eq. 2 ($Dose = 100$ mg, $V_c = 10$L). Data for estimated parameters were obtained by fitting the equations by minimizing least squares (PKAnalyst) to the oral two-compartmental model.

[b] Unacceptable case with bad prediction in the peripheral compartment.

[c] Flip-flop profile.

[d] Monophasic profile.

[e] Not a number.

k_{21} can be defined as

$$k_{21} = \frac{A\beta*(k_a - \alpha) + B\alpha*(k_a - \beta)}{A*(k_a - \alpha) + B*(k_a - \beta)} \tag{8}$$

Equation (8) can be rearranged to

$$k_{21} = \frac{A\beta k_a - A\alpha\beta + B\alpha k_a - B\alpha\beta}{Ak_a - A\alpha + Bk_a - B\beta} \tag{9}$$

Equation (9) can be further shown as

$$k_{21} = \frac{A\beta k_a + B\alpha k_a - (A + B)\alpha\beta}{-[A\alpha + B\beta - (A + B)k_a]} \tag{10}$$

By substituting Eq. (5) into Eq. (10), k_{21} can be finally expressed as

$$k_{21} = \frac{A\beta k_a + B\alpha k_a + D\alpha\beta}{-(A\alpha + B\beta + Dk_a)} \tag{11}$$

Equation (11) indicates that no matter what the order the macroconstants (ka, α, and β) are, the distribution rate constant (k_{21}) will remain the same. Hence, the drug concentrations in the peripheral compartment will also be equal in all three possible macroconstant combinations ($k_a > \alpha > \beta$, $\alpha > k_a > \beta$, and $\alpha > \beta > k_a$) as shown in Eq. (2). The time–concentration profiles in the peripheral compartment were identical when the estimated parameters from the flip-flop profiles were swapped back to the correct order. From the above derived equations and examples, it can be seen that the order of the macroconstants would not play any role in the time–concentration profiles of the central or peripheral compartment. It should be pointed out that for the three possible macroconstant combinations, three different sets of k_{10}, k_{12}, and V_c [Eqs. (12), (13), and (14), respectively] were derived but identical time–concentration profiles were obtained.

$$k_{10} = \frac{\alpha\beta}{k_{21}} \tag{12}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{10} \tag{13}$$

$$V_c = \frac{Dose*k_a}{A*(k_a - \alpha) + B*(k_a - \beta)} \tag{14}$$

The predicted concentrations in the peripheral compartment were calculated according to the estimated parameters and compared with the theoretical concentrations. Of the 24 cases, 8 (Cases 3, 6, 12, 15, 18, 20, 21,

and 23) had considerable errors in estimating the time–concentration profiles in the peripheral compartment (Fig. 2) and bad parameter fit based upon large standard deviation (Table I). These errors were observed when monophasic time–concentration profiles were seen. On the other hand, for those examples (Cases 1, 2, 4, 5, 7, 8, 10, 11, 14, and 16) that had better estimation of the time–concentration profiles in the peripheral compartment (Fig. 2) and less error in the fitted parameters, biphasic profiles were observed.

DISCUSSION

When one starts to investigate the pharmacokinetic profile of a drug, it is necessary to look at the time–concentration profile to assess what kind of compartment model may be suitable. If a biphasic profile is easily observable, proper parameter estimation is usually possible. However, if a biphasic profile is not detectable, parameter determination may be difficult or impossible without further information. Therefore, a simple way to "prevent" this mistake is to visually inspect the pharmacokinetic profiles. When unable to observe a clear biphasic profile, one should not apply the two-compartment model if the resulting parameters are to be interpreted.

Besides visual inspection it is necessary to investigate the origin of different profiles (monophasic vs. biphasic) that will allow predictions of the curve shape from the magnitude of the microscopic rate constants. The results from the simulations performed in this study indicate that it is the relative magnitude of the redistribution rate constant k_{21} in comparison to the other three rate constants (k_{10}, k_{12}, k_a) that determines the mono- or biphasic behavior of the resulting concentration profile. If absorption is slow in comparison to redistribution ($k_{21} > k_a$), then the resulting profile is monophasic (Cases 9, 12, 13, 15, and 17–24). The system basically provides enough time for the drug to distribute after absorption to mask a distribution phase. If absorption is fast in comparison to redistribution ($k_a > k_{21}$) and there is significant distribution ($k_{12} > k_{21}$), a biphasic pattern can be obtained (Cases 1, 2, 4, 7, 8, 10, 14, and 16). In these cases, the slow redistribution is responsible for the biphasic nature. If absorption is rapid in comparison to redistribution ($k_a > k_{21}$), and there is only little distribution ($k_{21} > k_{12}$), it then depends on the elimination rate constant k_{10} for what curve shape is obtained. If, under these circumstances, $k_{10} > k_{21}$, a biphasic pattern results (Cases 5 and 11). However, if elimination is slow ($k_{21} > k_{10}$), then the curve will look monophasic (Cases 3 and 6) (Table II). Figure 3 shows these relationships in a flow chart.

In addition to the prediction of curve shape based on the microscopic rate constants, it was of interest to find out if a prediction was also possible

Table II. Effect of Absorption, Distribution, and Excretion on the Pharmacokinetic Profile

Case	Absorption	Distribution	Excretion	Expected profile
9, 12, 13, 15, and 17–24	Slow	—	—	Monophasic
1, 2, 4, 7, 8, 10, 14, and 16	Fast	Much	—	Biphasic
3 and 6	Fast	Little	Slow	Monophasic
5 and 11	Fast	Little	Fast	Biphasic

based on the magnitude of the hybrid constants. It has been noted that a small amount of noise added could be sufficient to disguise the two-compartment nature of the model, and the best least-squares fit reduced the model to the one-compartment case in which k_a and α were essentially equal (3). Our results show that the constants α and β are not good indicators for the mono- or biphasic character of the curve. In several cases (Cases 9 and 11, as well as 2 and 6, or 3 and 5) the same values for α and β resulted in mono- or biphasic shapes without consistency. The intercepts A and B, however, seem to be much better predictors. The results show that if both A and B are negative, a monophasic curve is observed (Cases 15, 17, 20, 21, 23, and 24). In all other cases, it depends on the respective magnitude of the absolute values of A and B. If the absolute value of A is larger than that of B ($|A| > |B|$), a biphasic pattern results (Cases 1, 2, 4, 5, 7, 8, 10, 11, 14, and 16); otherwise if $|B| > |A|$, the curve is monophasic (Cases 3, 6, 9, 12, 13, 18, 19, and 22) (Table III).

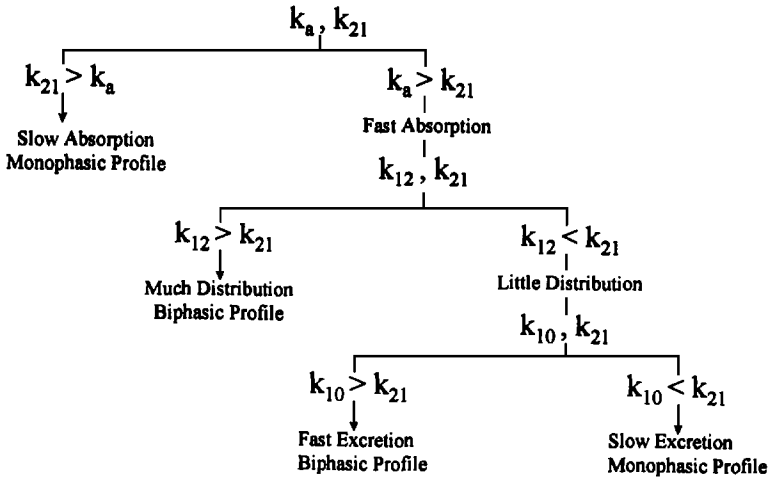


Fig. 3. Decision chart to predict biphasic nature of a two-compartment body model with first-order absorption.

Table III. Effect of Hybrid Constants (A, B) on Pharmacokinetic Profile

Case	A	B	Expected profile
15, 17, 20, 21, 23, and 24	(−) Negative	(−) Negative	Monophasic
1, 2, 4, 5, 7, 8, 10, 11, 14, and 16	$\|A\| > \|B\|$		Biphasic
3, 6, 9, 12, 13, 18, 19, and 22	$\|B\| > \|A\|$		Monophasic

All of the 24 cases adhere to the described rules in terms of hybrid constants and microscopic constants. To further challenge the systems, an additional 120 different combinations of the microconstants (10, 5, 1, 0.5, and 0.1 hr^{-1}) were tested. The described rule held in 118 examples. The two exceptions (k_a, k_{12}, k_{21}, k_{10}, A, and B were 10, 0.5, 1, 0.1, 4.3, 6.4 and 5, 0.5, 1, 0.1, 5.3, 6.5, respectively) observed were at the situation when absorption is fast and elimination is slow with little distribution (similar to Cases 3 and 6) which should have produced monophasic curves but which can with certain parameter values also produce biphasic curves. However, in the vast majority of the cases the proposed rule worked.

The above parameter errors generally will not be of any consequence as long as the parameters are used only to predict future blood levels over the same time span. If, however, the parameters are used to predict any characteristic of the system other than blood levels, errors in the estimated parameters may result in appreciable errors in these predictions. The above examples were simulated to prove this hypothesis.

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