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ORIGINAL RESEARCH ARTICLE

Application of the Finite Absorption Time (F.A.T.) Concept in the Assessment of Bioequivalence

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Abstract

Purpose To formulate a methodology for the assessment of bioequivalence using metrics, which are based on the physiologically sound F.A.T. concept.

Methods The equations of the physiologically based finite time pharmacokinetic models for the one-and two-compartment model with one and two input stages of absorption were solved to derive metrics for the extent and rate of absorption. Simulated data were used to study the proper way for the estimation of metrics. A bioequivalence study was analyzed using these metrics.

Results The rate of drug absorption was found to be equal to the slope of the amount absorbed versus time curve. The amount of drug absorbed at the end of the absorption process, corresponding to the blood concentration at F.A.T. is an indicator of the extent of absorption. The plot of the ratio test/reference of the simulated data for the amount absorbed as a function of time becomes constant beyond the end of drug absorption from the formulation exhibiting the longer absorption. The assessment of the bioequivalence study was based on the slope of the amount absorbed versus time curve for the rate of absorption, while the estimate for the constant ratio test/reference for the amount absorbed was used for the assessment of extent of absorption.

Conclusions The assessment of rate in bioequivalence studies can be based on the estimation of slope of the percent absorbed versus time curve while the constant ratio test/reference for the amount of drug absorbed is an indicator of the extent of absorption.

Keywords bioequivalence · carbamazepine · drug absorbed · extent of absorption · finite absorption time · rate metrics

Abbreviations

AUC	Area Under the blood drug concentration versus time Curve
F.A.T.	Finite Absorption Time
F.D.T.	Finite Dissolution Time
IVIVC	*In vitro in vivo* Correlations
PBFTPK models	Physiologically Based Finite Time Pharmacokinetic models

Introduction

On January 7, 1977, FDA issued final regulations in part 320 (21 CFR 320) establishing definitions and requirements for bioavailability and bioequivalence studies (42 FR 1624). Prior to the definition of bioavailability by FDA, scientists were ambiguous for the proper methodology in terms of the sampling design and the metrics applied for studying bioavailability. One such study was performed by Lovering *et al.* [1] in 1975. The authors of the study focused on the period after administration over which blood level measurements are required to obtain a reliable bioavailability comparison. They analyzed literature data of the following ten drugs: acetaminophen, aminosalicylic acid, chloramphenicol, chlordiazepoxide, digoxin, isoniazid, phenylbutazone, sulfamethizole, tetracycline, and warfarin. They found that reliable bioavailability comparisons among different brands of the drugs could have been made by using truncated concentration time curves since the ratios of areas under the curve changed little between the end of the "absorption

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period" and the time when blood sampling was terminated. A few years later, another study [2], aiming at increasing the power of bioavailability tests, was based on the simultaneous administration of a stable-isotope internal standard of imipramine hydrochloride solution with two brands of imipramine hydrochloride tablets. The plasma concentration of unlabeled imipramine was found to be essentially identical to internal standard for all times points beyond t_{max} for both brand tablets studied. These results unequivocally demonstrate that for all drugs studied in [1, 2] drug absorption terminates at a specific time point and thereafter only elimination of drug operates. In full agreement with this finding are the results of the recently re-examined two digoxin bioavailability/bioequivalence studies [3].

All above results can be interpreted using the finite absorption time (F.A.T.) concept [4–6], which was developed after the questioning of the validity of first-order absorption assumption applied to all oral drug absorption studies since 1953 [7]. The F.A.T. concept was introduced to account for the fact that oral drug absorption cannot last longer than the residence time in the G.I. tract; it is used in conjunction with zero-order absorption kinetics which prevail due to sink conditions caused by the high blood flow in vena cava. The end of the absorption process can either coincide with $t_{\max}$ or may be observed in the declining elimination phase following a one or more zero-order input processes (see Figures in [4–12]).

Besides, the development of the relevant physiologically based finite time pharmacokinetic (PBFTPK) models [8, 9] enabled us to analyze literature pharmacokinetic data for various drugs administered orally, intramuscularly, intranasally, and pulmonary; valid estimates for the duration of drug absorption stage(s) as well as the corresponding zero-order input rates for each one of the absorption stages were derived [8, 10–12]. Furthermore, we recently revamped [13] the roots of biopharmaceutics-pharmacokinetics by modifying in terms of the F.A.T. concept, the Wagner-Nelson [14] and Loo-Riegelman [15] techniques used for the construction of percent absorbed *vs* time plots. We unveiled in [13] that the percent absorbed versus time curves are either bilinear or multi-linear depending on the number of absorption stages with the final change (break) point of the segments equal to F.A.T. This is contrary to the prevailing belief for the last 60 years or so, namely, the exponential character of the percent absorbed versus time plots [14, 15]. These advances were applied recently to revise the classical methodology used for the development of *in vitro in vivo* correlations (IVIVC) [16]. The new approach relies on the concept of Finite Dissolution Time (F.D.T.) and its relationship with the F.A.T. in accord with the biopharmaceutical classification of drugs [16].

Based on the results of the studies [7–13, 16], in this work we build up a reconsideration of the methodologies and metrics used for the assessment of bioequivalence. We formulate a methodology for the assessment of bioequivalence using metrics which are based on the physiologically sound F.A.T. concept.

Theory

According to the FDA definition of 7 January 1977 [17] bioavailability is "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of drug action". Accordingly, the measures of bioequivalence since then are, the area under the curve $[AUC]_0^\infty$ for the assessment of the extent of absorption and $C_{\max}$ as an indicator of the rate of absorption [18, 19]. Although there is a consensus for the validity of $[AUC]_0^\infty$ as a measure of the extent of drug absorption, numerous publications, e.g., [20–31], have shown that $C_{\max}$ is insensitive as an assessor of the rate of drug absorption. Below we analyze the two aspects of the bioavailability-bioequivalence studies, namely, rate and extent, in the light of the new developments [7–13, 16].

Rate

The use of $C_{\max}$ in bioequivalence studies as a rate parameter is inextricably linked with the prevailing hypothesis of first-order drug absorption since 1953 [1, 6, 7]. In fact, assuming one-compartment model disposition, $C_{\max}$ is the blood concentration value when the first-order rate of drug input becomes equal at $t_{\max}$ to the first-order elimination rate. Accordingly, the amount of drug absorbed $A(t)$ normalized in terms of the volume of distribution, V_{d} and expressed in terms of concentration as well as the corresponding rate of absorption as a function of time for drugs with one-compartment kinetics are as follows,

$$\frac{A(t)}{V_d} = \frac{FD}{V_d}\left(1 - e^{-k_a t}\right) \tag{1}$$

$$slope = rate\ of\ absorption = \frac{1}{V_d}\frac{dA(t)}{dt} = \frac{FDk_a}{V_d}e^{-k_a t} \tag{2}$$

where k_{a} is the first-order rate constant, F is the bioavailable fraction of dose D taken. Both equations show the exponential change of the left-hand side parameters of Eqs. 1 and 2 as a function of time. This also applies for drugs obeying two-compartment model disposition; Eqs. 3 and 4 are the counterparts of Eqs. 1 and 2, respectively.

$$\begin{aligned}\frac{A(t)}{V_c} = {} & \frac{B_1}{\alpha}\left(k_{10} + \left(\alpha - k_{10}\right)e^{-\alpha t}\right) + \frac{B_2}{\beta}\left(k_{10} + \left(\beta - k_{10}\right)e^{-\beta t}\right) \\ & + \frac{B_3}{k_\alpha}\left(k_{10} + \left(k_\alpha - k_{10}\right)e^{-k_\alpha t}\right)\end{aligned} \tag{3}$$

$$rate\ of\ absorption = B_1(k_{10}-\alpha)e^{-\alpha t} + B_2(k_{10}-\beta)e^{-\beta t} + B_3(k_{10}-k_a)e^{-k_a t} \quad (4)$$

where B_1, B_2, and B_3 are concentration constants, α, β are hybrid constants and k_{10} is the elimination rate constant from the central compartment. In full agreement with Eqs. 1–4, all percent absorbed versus time plots published in the literature since the inception of pharmacokinetics using the Wagner-Nelson [14], Loo-Riegelman [15] techniques and deconvolution approaches [32] are of exponential nature. In parallel, the change over time of the rate of absorption (Eqs. 2 and 4) underscores the difficulty associated with the assessment of "rate" in bioequivalence studies. This was realized from the early days of discussions on bioequivalence in the American Association of Pharmaceutical Scientists in 1972 [33], i.e., "*Assessment of the rate of bioavailability is one of the most difficult problems encountered in bioavailability studies*"; this difficulty is also mirrored on the suggestion by Tucker *et al.* [34] at a large meeting in 1995 "*the ambiguity in the rationale for bioequivalence testing would be removed if the term "rate" was deleted from its definition*". Unfortunately, this difficulty persists through the years until today [6, 20–31].

However, since the recent inception/applications of the F.A.T. concept [4–13, 16], the absorption of a large number of drugs was found to take place passively under sink conditions for a single or multiple successive periods of time, namely, zero-order kinetics controls oral drug absorption for the reasons delineated in [4, 6]. Figure 1 shows a schematic for the percent of drug absorbed, which is generated from the modified in terms of F.A.T. Wagner-Nelson equation (Eq. 2 in [13]) for the test and reference formulations of a hypothetical bioequivalence study assuming one input stage for both formulations and one compartment model disposition.

In the context of Fig. 1, assuming one-compartment model disposition with a single zero-order input for finite time absorption of duration τ, the amount of drug absorbed

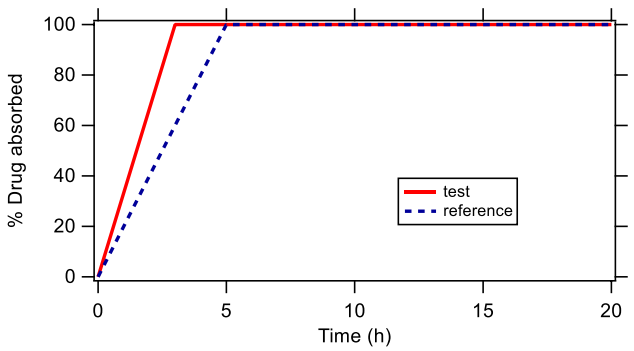


Fig. 1 A schematic of the percent of drug absorbed versus time profiles for the test (T) and reference (R) formulations in a bioequivalence study assuming termination of drug absorption at time $\tau_T = 3$ h and $\tau_R = 5$ h, respectively. The profiles were generated from Eq. 2 reported in [13].

$A(t)$ expressed in terms of concentration $[FD/V_d]$, the corresponding rate of absorption, are given by Eqs. 5 and 6 for the absorbing stage,

$$\frac{A(t)}{V_d} = \frac{A(\tau)}{V_d}\frac{t}{\tau} = \frac{FD}{V_d}\frac{t}{\tau} \quad (5)$$

$$slope = rate\ of\ absorption = \frac{1}{V_d}\frac{dA(t)}{dt} = \frac{FD}{V_d}\frac{1}{\tau} \quad (6)$$

where $A(\tau)$ is the amount absorbed at time τ; it should be noted that for $t > \tau$, $\frac{A(t)}{A(\tau)} = 1$. Equations 5, 6 and Fig. 1 are easily related if the ratio of percent $A(t)/A(\tau)$ is considered. Equation 6 shows that the rate of drug absorption (expressed in concentration/time units) is constant up to time τ and corresponds to the slope of the rising linear segment of each one of the two formulations considered in Fig. 1. In other words, Eq. 6 shows that the rate of absorption can be determined by dividing the total amount of drug absorbed, $A(\tau)$ [which is also equal to $A(\infty)$] divided by the volume of distribution with the duration of the absorption process.

In actual practice, estimates for FD/V_d and τ can be obtained either from the nonlinear fitting of the PBFTPK models to the concentration–time data [9] or the [(absorbed amount)/V_d]-time plots (see relevant plots in the Results section). Subsequently, the drug input rate estimates, $FD/V_d\tau$ can be further used for predictive purposes. However, for the purposes of the current work Eq. 6 has its own merit. It shows explicitly the correct mathematical expression corresponding to the rate concept introduced by FDA in 1977; since then, C_{max} has and still is being used as a rate parameter despite the severe critique for its not pertinent use.

For a one-compartment model with two consecutive zero-order, finite-time absorption stages of duration τ_1 and τ_2, Eqs. 7 and 8 apply [13] for the first stage of absorption, i.e., for $0 < t \le \tau_1$

$$\frac{A(t)}{V_d} = C(t) + k_{el}[AUC]_0^t = \frac{F_1 D}{V_d}\frac{t}{\tau_1} \quad (7)$$

$$slope = rate\ of\ absorption = \frac{1}{V_d}\frac{dA(t)}{dt} = \frac{F_1 D}{V_d}\frac{1}{\tau_1} \quad (8)$$

while Eqs. 9 and 10 are the corresponding formulae for the second stage, i.e., for $\tau_1 < t \le \tau_1 + \tau_2$,

$$\frac{A(t)}{V_d} = \frac{F_1 D}{V_d} + \frac{F_2 D}{V_d}\frac{t-\tau_1}{\tau_2} \quad (9)$$

$$slope = rate\ of\ absorption = \frac{1}{V_d}\frac{dA(t)}{dt} = \frac{F_2 D}{V_d}\frac{1}{\tau_2} \quad (10)$$

where $C(t)$ is the concentration–time function of the one-compartment model with two consecutive zero-order, finite-time absorption stages of duration τ_1 and τ_2, F_1 and F_2 are the bioavailable fractions of the consecutive stages one and two, respectively, while $[AUC]_0^t$ is the area under the blood concentration time curve for the time limits quoted [13]. Both Eqs. 8 and 10 show that the rate of absorption has constant values during each of the two absorption stages.

For a two-compartment model with zero-order, finite-time absorption of duration τ we get Eqs. 11 and 12 for the amount absorbed and the rate of absorption.

$$\frac{A(t)}{V_c} = C(t) + C_P(t)\frac{V_P}{V_c} + k_{10}[AUC]_0^t = \frac{FD}{V_c}\frac{t}{\tau} \tag{11}$$

$$slope = rate\ of\ absorption = \frac{1}{V_c}\frac{dA(t)}{dt} = \frac{FD}{V_c}\frac{1}{\tau} \tag{12}$$

where $C(t)$ is the drug concentration on the central compartment of volume V_c and $C_P(t)$ is the drug concentration in the peripheral compartment of volume V_P, i.e.,

$$C(t) = \frac{FD}{\tau V_c(\beta-\alpha)}\left[\frac{k_{21}-\alpha}{a}\left(1-e^{-\alpha t}\right) - \frac{k_{21}-\beta}{\beta}\left(1-e^{-\beta t}\right)\right] \tag{13}$$

$$C_P(t) = \frac{FDk_{12}}{\tau V_P(\beta-\alpha)}\left(\frac{1-e^{-\alpha t}}{\alpha} - \frac{1-e^{-\beta t}}{\beta}\right) \tag{14}$$

$$[AUC]_0^t = \frac{FD}{\tau V_c(\beta-\alpha)}\left[\frac{k_{21}-\alpha}{a}\left(t-\frac{1-e^{-\alpha t}}{\alpha}\right) - \frac{k_{21}-\beta}{\beta}\left(t-\frac{1-e^{-\beta t}}{\beta}\right)\right] \tag{15}$$

Again, Eq. 12 reveals that the rate of absorption is constant throughout the single absorption stage.

For a two-compartment model with two consecutive zero-order, finite time absorption stages of duration τ_1 and τ_2, we get Eqs. 16 and 17 for $0 < t \le \tau_1$,

$$\frac{A(t)}{V_c} = C(t) + C_P(t)\frac{V_P}{V_c} + k_{10}[AUC]_0^t = \frac{F_1 D}{V_c}\frac{t}{\tau_1} \tag{16}$$

$$slope = rate\ of\ absorption = \frac{1}{V_c}\frac{dA(t)}{dt} = \frac{F_1 D}{V_c}\frac{1}{\tau_1} \tag{17}$$

while for $\tau_1 < t \le \tau_1 + \tau_2$ we get Eqs. 18 and 19

$$\frac{A(t)}{V_c} = C(t) + C_P(t)\frac{V_P}{V_c} + k_{10}[AUC]_0^t = \frac{F_1 D}{V_c} + \frac{F_2 D}{V_c}\frac{t-\tau_1}{\tau_2} \tag{18}$$

$$slope = rate\ of\ absorption = \frac{1}{V_c}\frac{dA(t)}{dt} = \frac{F_2 D}{V_c}\frac{1}{\tau_2} \tag{19}$$

Again, constant values are found for the rate of absorption during each of the two stages, Eqs. 17, 19.

Extent. For the assessment of extent of drug absorption in bioequivalence studies for one-compartment model drugs, we consider first Fig. 1 and then re-plot the corresponding concentration (absorbed/V_d) data as a function of time, Fig. 2a. In this example, both formulations exhibit a single linear absorption profile and different absorption duration and extent of drug absorption. The crux of the matter is that beyond time τ_R assuming $\tau_T < \tau_R$, the ratio of the plateau values becomes constant and corresponds to the relative bioavailability of two formulations, $F_T/F_R = 1.30$, Fig. 2b. For two-compartment model drugs, similar plots of the ratio of the amounts of drug absorbed $A(t)$ of two formulations as a function of time generated from Eq. 11 were obtained, (see Fig. 2b).

It is also interesting to examine the change of AUC of the test and the reference formulation considered in Fig. 2

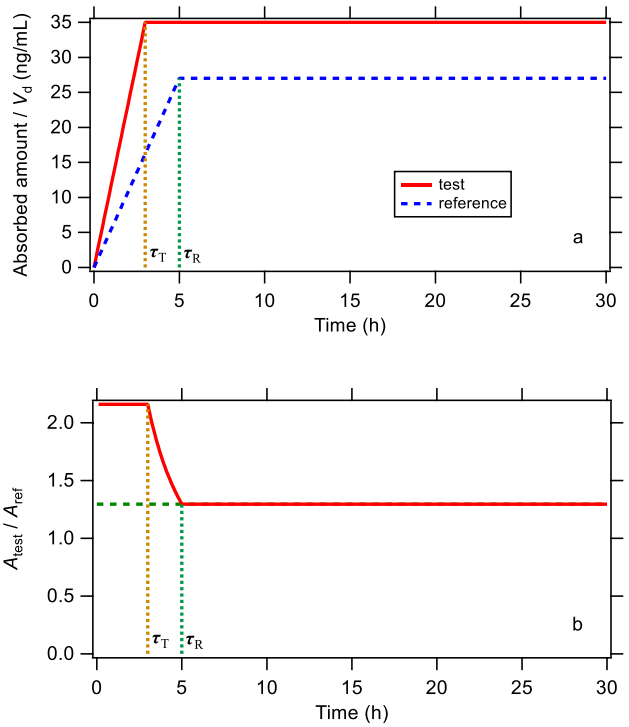


Fig. 2 (**a**) Re-plotted simulated data of Fig. 1 in a concentration (absorbed amount/V_d) versus time format. (**b**) The ratio of the amount of drug absorbed $A(t)$ of two formulations as a function of time; generated from Eq. 5 or Eq. 11 using $F_T D/V_d = 35$ and $F_R D/V_d = 27$. The y-intercept of the extrapolated back horizontal dashed line corresponds to the relative bioavailability of the two formulations, $35/27 = 1.30$.

as well as their ratio as a function of time. The areas under the curve of the two formulations as a function of time are calculated based on Eqs. 20 and 21.

$$[AUC]_0^t = \frac{FD}{V_d k_{el}\tau}\left[t - \frac{1-e^{-k_{el}t}}{k_{el}}\right] \quad t < \tau \tag{20}$$

$$[AUC]_0^t = \frac{FD}{V_d k_{el}}\left[1 - e^{-k_{el}(t-\tau)}\frac{1-e^{-k_{el}\tau}}{k_{el}\tau}\right] \quad t > \tau \tag{21}$$

Figure 3a shows the non-linear increase of AUC as a function of time for both formulations. Figure 3b shows that the ratio initially has a constant value of 2.16 up to time τ_T, which reflects the corresponding ratio of the drug input rates from the two formulations; this is followed by a linear decline up to the end, τ_R of the drug absorption from the reference formulation, while the last nonlinear segment of the plot asymptotically reaches a limit, which corresponds to the relative bioavailability of the two formulations, 1.30.

Methods

We carried out several simulations generating errorless data for the test and reference formulations using various scenarios involving drugs with one or two compartment model disposition, with different duration and extent of drug absorption. For each one of the data sets simulated we constructed the corresponding plots in accord with Figs. 2 and 3. Moreover, we analyzed the data of a bioequivalence study for carbamazepine [35] and an additional set of data of carbamazepine [36] using the approaches based on the F.A.T. concept and delineated above.

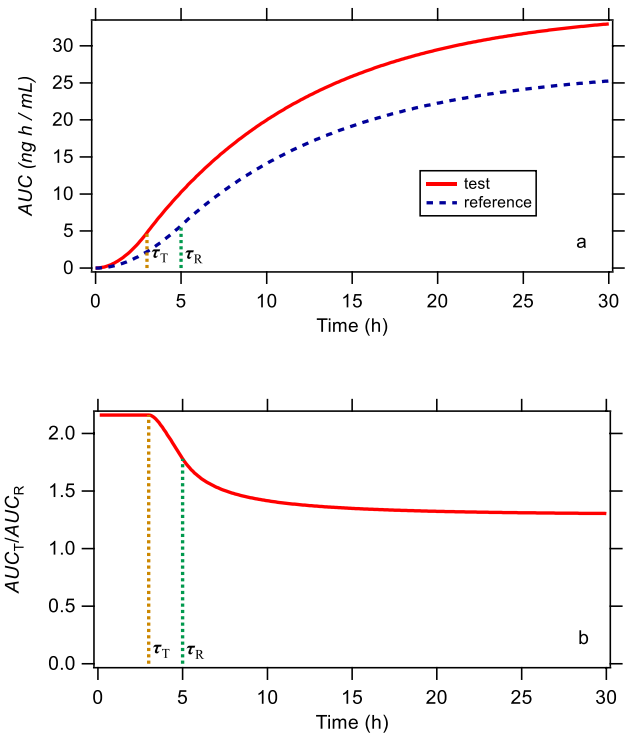


Fig. 3 AUC as a function of time for the same test and reference formulations considered in Figs. 1 and 2 generated using Eqs. 20 and 21 with $k_{el}=0.1\ h^{-1}$ (**a**) and their corresponding ratio (**b**).

Results

Simulated Data

Figures 4 and 5 show the simulated data plots for drugs following kinetics described by a one-and two-compartment model, respectively. In all cases a two-hour difference for the duration of drug absorption between the two formulations was assigned for illustrative purposes. Various test/reference ratios for the extent of drug absorption varying from 85 to 115% were utilized. For two-compartment model drugs we present only the plots of the parameters $(AUC)_T$ and $(AUC)_R$ and their ratio as a function of time since the rest of the plots are identical with their counterparts of the one-compartment model drugs. The common characteristics of all plots for both one- and two-compartment drugs are as follows: i) the percent of drug absorbed increases linearly as a function of time and reaches the plateau (100%) at the end of the duration of the absorption process assigned for the test or the reference formulation; ii) the absorbed amount (expressed in blood concentration units) of drug increases linearly with time and reaches a plateau, which is proportional to the extent of drug absorption at the end of the absorption process from the relevant formulation; iii) the ratio of the amount of drug absorbed from the two formulations (A_{test}/A_{ref}) is constant up to the shorter duration of drug absorption from one of the two formulations, then it either increases or decreases until the longer duration of drug absorption from one of the two formulations and thereafter remains constant and equal to relative bioavailability of the two formulations; iv) the area under the curve AUC increases with time nonlinearly; v) the ratio of the areas, $(AUC)_T/(AUC)_R$ follows the same pattern as the ratio of the amount of drug absorbed from the two formulations (A_{test}/A_{ref}) up to the longer duration of drug absorption and then reaches asymptotically the relative bioavailability of the two formulations. Needless to say, that the slope for all simulated data was found equal to the assigned value $FD/V_d\,\tau$.

Analysis of Carbamazepine Bioequivalence Data

The data of the carbamazepine bioequivalence study [35] were initially digitized. The reference formulation for the two studies in [35] analyzed test1/reference1 and test2/reference2 was Tegretol 400 mg. By analyzing the semi-logarithmic

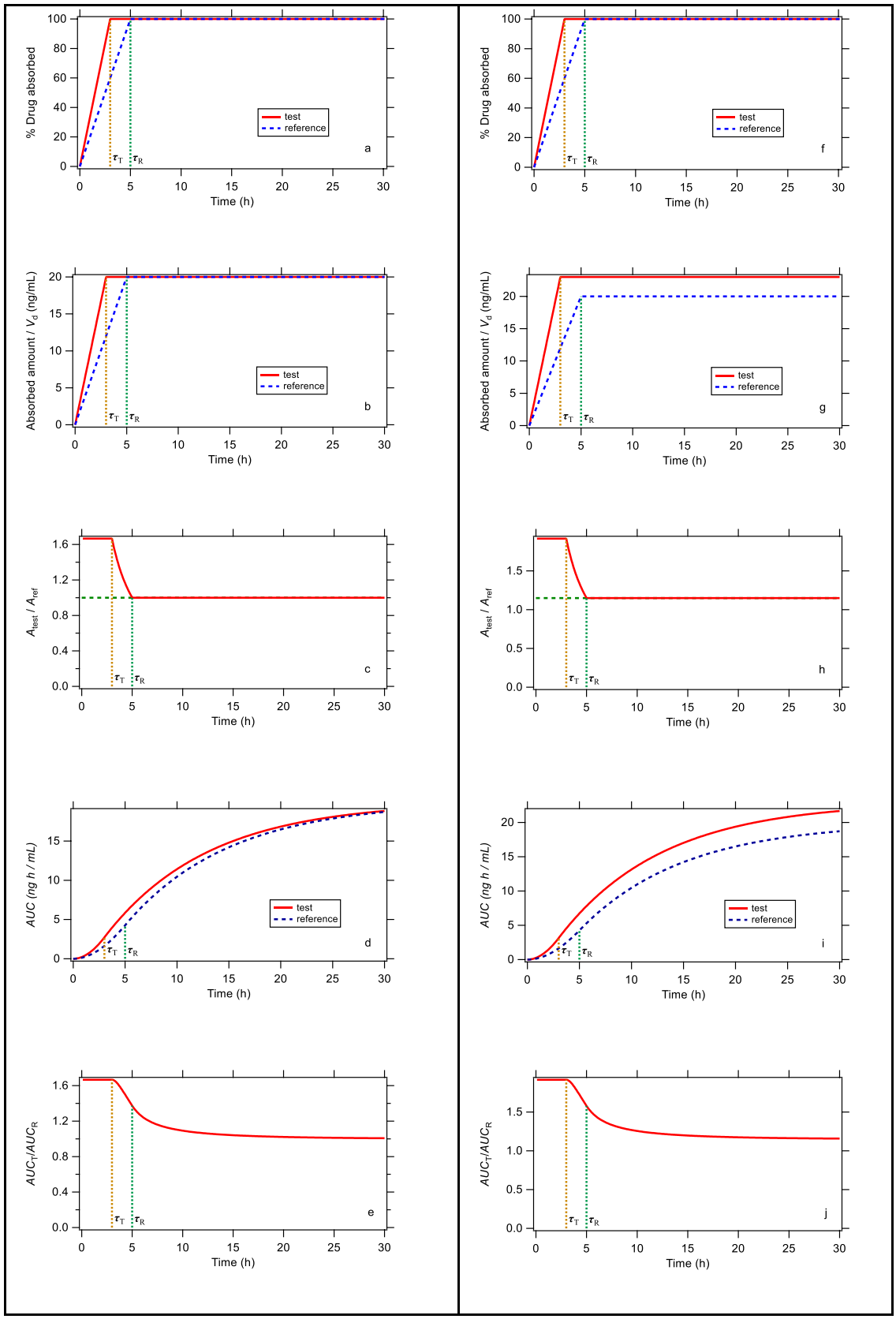


Fig. 4 Simulated data plots for one-compartment model drugs: Key: **a**-**e**. $FD/V_R = 20$ ng/mL, $FD/V_T = 20$ ng/mL, $\tau_R = 5$ h, $\tau_T = 3$ h; **f**-**j**. $FD/V_R = 20$ ng/mL, $FD/V_T = 23$ ng/mL, $\tau_R = 5$ h, $\tau_T = 3$ h; **k**–**o**. $FD/V_R = 20$ ng/mL, $FD/V_T = 17$ ng/mL, $\tau_R = 5$ h, $\tau_T = 3$ h; **p**–**t**. $FD/V_R = 20$ ng/mL, $FD/V_T = 17$ ng/mL, $\tau_R = 5$ h, $\tau_T = 7$ h. In all cases $k_{el} = 0.1$ h^{-1}.

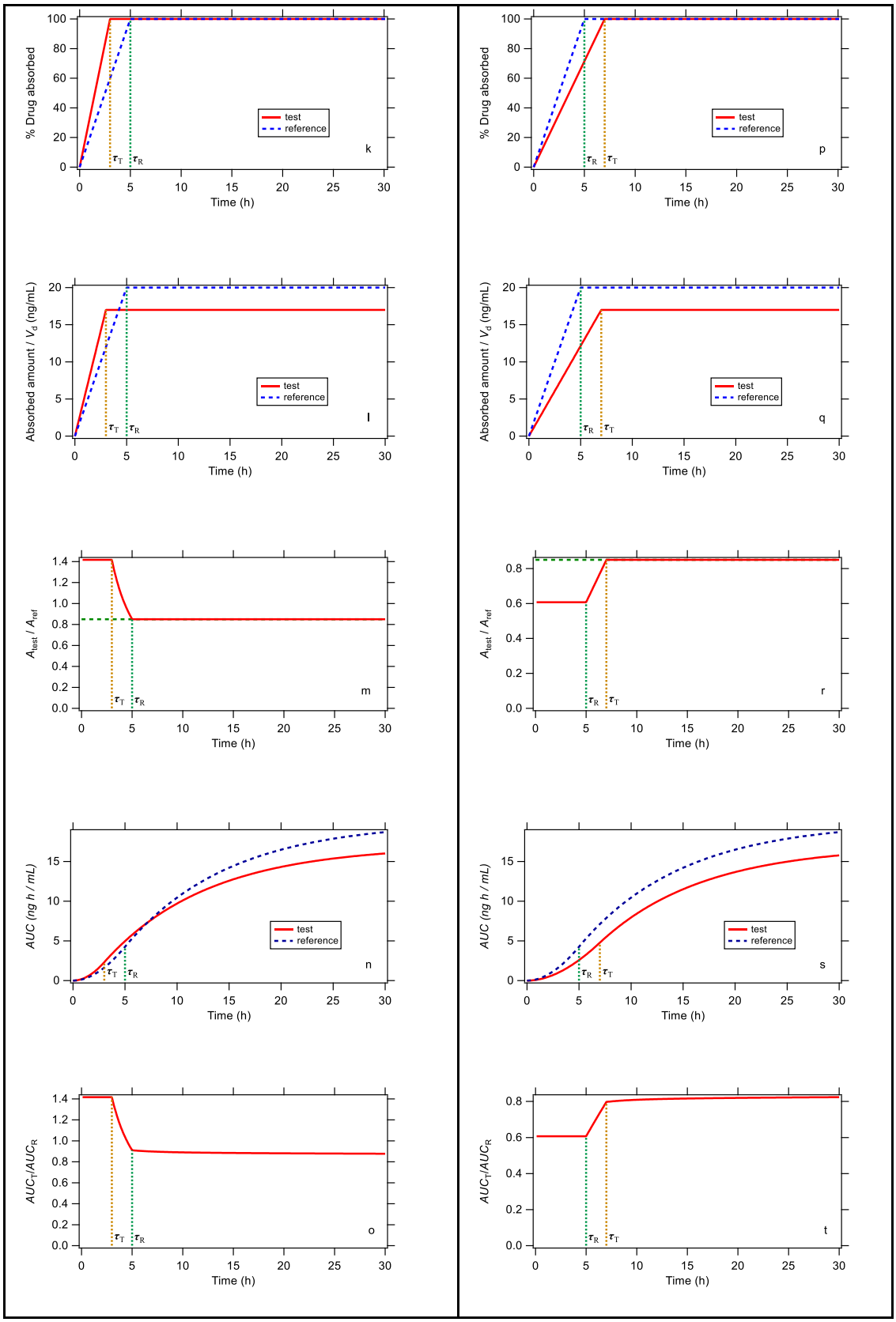
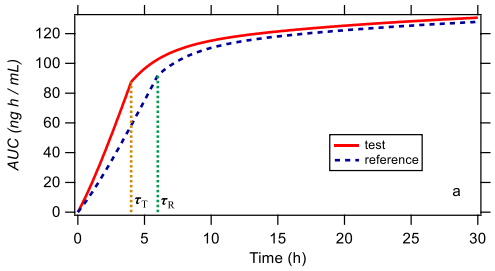
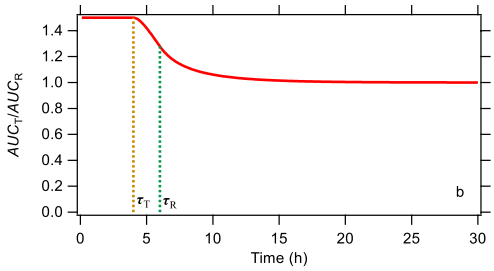


Fig. 4 (continued)


AUC (ng h / mL)
Time (h)
test
reference
a
τ_T
τ_R


AUC_T/AUC_R
Time (h)
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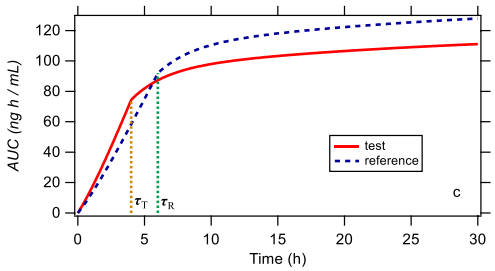
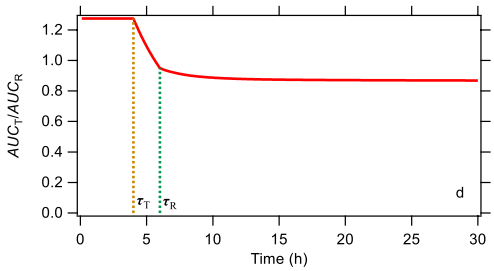
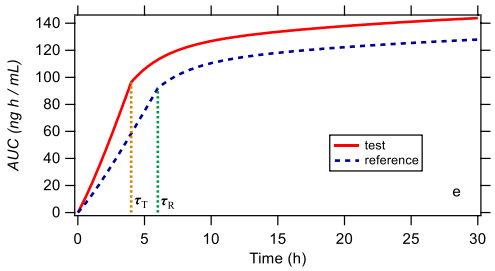

AUC (ng h / mL)
Time (h)
test
reference
c
τ_T
τ_R


AUC_T/AUC_R
Time (h)
d
τ_T
τ_R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Time (h)
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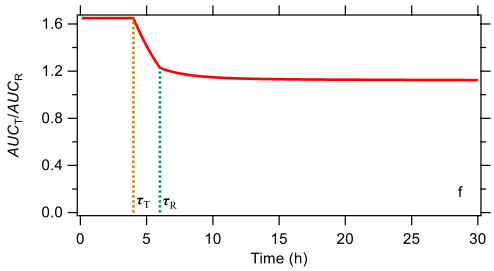
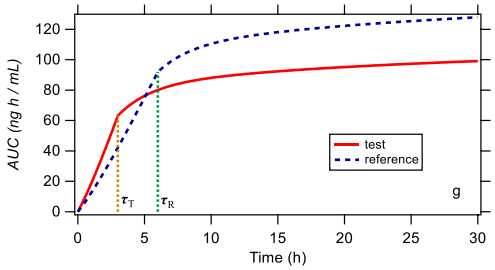

AUC_T/AUC_R
Time (h)
f
τ_T
τ_R


AUC (ng h / mL)
Time (h)
test
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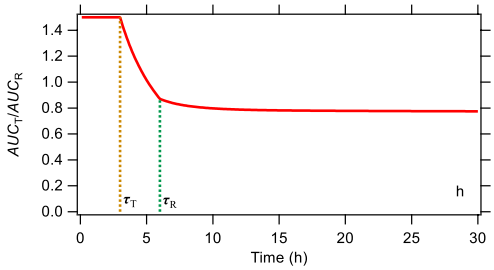
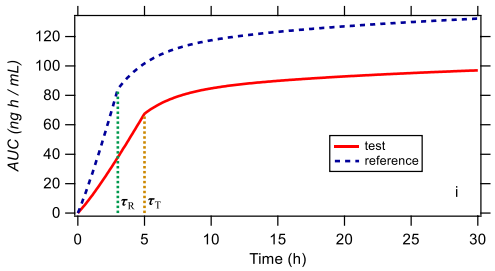
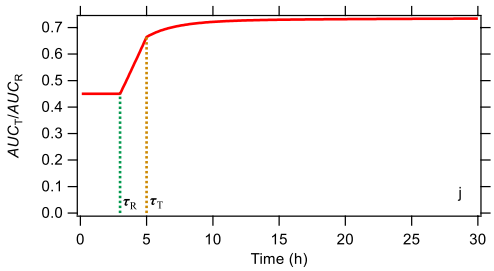

AUC_T/AUC_R
Time (h)
h
τ_T
τ_R


AUC (ng h / mL)
Time (h)
test
reference
i
τ_R
τ_T


AUC_T/AUC_R
Time (h)
j
τ_R
τ_T

◂**Fig. 5** Simulated data plots for two-compartment model drugs: Key: **a**, **b**: $FD/V_R = 20$ ng/mL, $FD/V_T = 20$ ng/mL, $\tau_R = 6$ h, $\tau_T = 4$ h, $\alpha = 0.4$ h^{-1}, $\beta = 0.04$ h^{-1}, $k_{21} = 0.064$ h^{-1}, $k_{12} = 0.126$ h^{-1}, $k_{10} = 0.25$ h^{-1}; **c**, **d**: $FD/V_R = 20$ ng/mL, $FD/V_T = 17$ ng/mL, $\tau_R = 6$ h, $\tau_T = 4$ h, $\alpha = 0.4$ h^{-1}, $\beta = 0.04$ h^{-1}, $k_{21} = 0.064$ h^{-1}, $k_{12} = 0.126$ h^{-1}, $k_{10} = 0.25$ h^{-1}; **e**, **f**: $FD/V_R = 20$ ng/mL, $FD/V_T = 22$ ng/mL, $\tau_R = 6$ h, $\tau_T = 4$ h, $\alpha = 0.4$ h^{-1}, $\beta = 0.04$ h^{-1}, $k_{21} = 0.064$ h^{-1}, $k_{12} = 0.126$ h^{-1}, $k_{10} = 0.25$ h^{-1}; **g**, **h**: $FD/V_R = 20$ ng/mL, $FD/V_T = 15$ ng/mL, $\tau_R = 6$ h, $\tau_T = 3$ h, $\alpha = 0.4$ h^{-1}, $\beta = 0.04$ h^{-1}, $k_{21} = 0.064$ h^{-1}, $k_{12} = 0.126$ h^{-1}, $k_{10} = 0.25$ h^{-1}; **i**, **j**: $FD/V_R = 20$ ng/mL, $FD/V_T = 15$ ng/mL, $\tau_R = 3$ h, $\tau_T = 5$ h, $\alpha = 0.4$ h^{-1}, $\beta = 0.04$ h^{-1}, $k_{21} = 0.064$ h^{-1}, $k_{12} = 0.126$ h^{-1}, $k_{10} = 0.25$ h^{-1}. A_{test}/A_{ref} versus time plots are not presented here since they are identical to the corresponding ones in Fig. 4 (see also the legend of Fig. 2).

plot of carbamazepine data in [35, 36] (Fig. 6) we determined the elimination rate constant, k_{el}, for each formulation. The estimates for k_{el} were used to construct the plots of A_{test}/V and A_{ref}/V as a function of time, Fig. 7. The ratio of the amount of drug absorbed from the two formulations, A_{test}/A_{ref}; and the ratio of the cumulative areas based on the sampling time points, $\Sigma(AUC)_{i,T}/\Sigma(AUC)_{i,R}$ as a function of time are shown in Fig. 8. A summary of the analysis of each one of the data sets is presented in Table I. Based on these results the ratio of the rate of carbamazepine absorption and the relative bioavailability of formulations of the two studies was estimated. We present them in Table II along with the classical metrics of C_{max} and the ratio $(AUC)_T/(AUC)_R$ reported in [35].

Discussion

Simulated Data

Visual inspection of Fig. 4 reveals that the rate of absorption of drug, which corresponds to the slope of either % drug absorbed (Figs. 4 a, f, k, p) or the amount absorbed/V_d (Figs. 4 b, g, l, q) versus time plots is proportional to the value assigned to the input rate parameters $FD/V_R\tau_R$ or $FD/V_T\tau_T$. This is an ideal property for an indicator of drug's absorption rate; accordingly, this observation places an end to the longstanding dilemma for the most proper rate metric since the birth-inception of bioavailability-bioequivalence concepts [17]. All these years, the rate of drug input was misconceived as changing with time due to the prevailing concept of first-order absorption in accord with Eqs. 2 and 4. Therefore, the estimation of the absorption rate can be accomplished with linear regression analysis of the ascending limb of experimental data for the reference and test formulations. Hence, the assessment of rate in a bioequivalence study can be based on the statistical comparison of the initial slopes of the two data sets.

The ratio of the amounts absorbed, A_{test}/A_{ref} as a function of time (Figs. 4 c, h, m, r) show a patent flattening beyond the longest duration of drug absorption from the two formulations. This is also an ideal property for an extent of absorption parameter since the y-intercept of the back-extrapolated plateau value corresponds to the relative bioavailability of the two formulations, which are 1.0, 1.0, 0.85, 0.85 for the examples considered in (Figs. 4 c, h, m, r), respectively.

The ratio of the parameters $(AUC)_T/(AUC)_R$ shown in Figs. 4 e, j, o, t (for one-compartment model drugs) and Figs. 5 b, d, f, h, j (for two-compartment model drugs) exhibit a nonlinear change as a function of time asymptotically reaching the relative bioavailability value of the two formulations. It should be noted that the cumulative areas ratio plots are constructed directly, without any treatment, from the experimental data. This type of plot allows an easy/rapid rough estimation for the cessation of the absorption process of the two formulations.

Analysis of Carbamazepine Bioequivalence Data

The plots in Fig. 6 clearly demonstrate that carbamazepine follows one-compartment model disposition in all five formulations studied. The estimates of the elimination rate constant are listed in Table I. The plots of the cumulative amount of carbamazepine absorbed (expressed in terms of concentration) in Fig. 7 of the four formulations of the bioequivalence study [35] depict more than one input stage, long duration of the absorption processes ranging from 16.1 to 32.2 h, and quite similar plateau values for A_∞/V_d. Further visual inspection of the plots of %absorbed for each one of the formulations (Figs. 8a, b, f and g) as well as the ratios A_{test}/A_{ref} and $\Sigma(AUC_i)_T/\Sigma(AUC_i)_R$ (Figs. 8 c, h, e and j) as a function of time, shows quite similar patterns. The comparison of the estimates for the ratio test/reference of the extent metric $[AUC]_0^\infty$ with the novel extent metrics $[AUC]_0^\tau$ and A_∞/V_d in Table II verifies that similar results are obtained using either the classical $[AUC]_0^\infty$ or the novel metrics $[AUC]_0^\tau$ and A_∞/V. The same conclusion applies to the ratio test/reference of rate metrics, C_{max} versus the initial slope, Table II.

Regulatory Implications

The results presented above can have an impact for the assessment of rate and extent of absorption in bioequivalence studies. So far, the assessment of bioequivalence relies on model independent approaches. In this study, minimal modeling work based on the modified [13] Wagner-Nelson [14] and Loo-Riegeleman [15] plots is utilized to provide a novel approach for the assessment of bioequivalence. In the era of the Model-Informed Drug Development (MIDD) [37–39], this study paves the way for the incorporation of bioavailability and bioequivalence as new topics in the MIDD initiative and expand it towards a Model-Informed Drug Development & Assessment (MIDDA) by employing quantitative-modeling

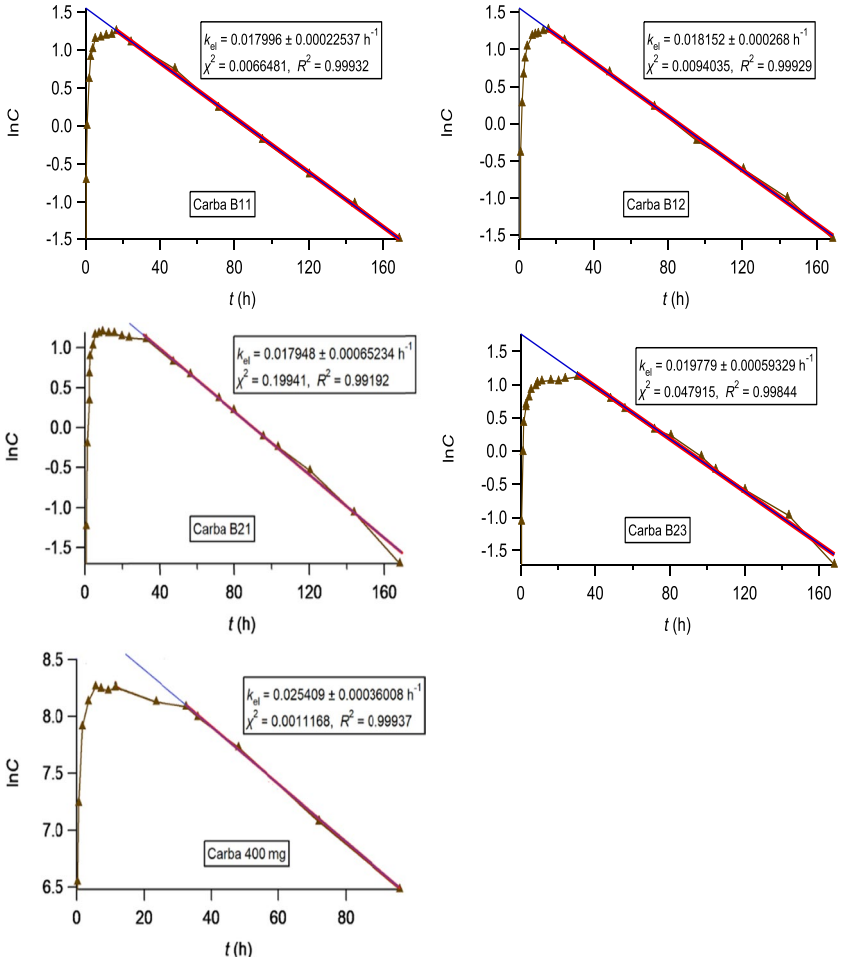


Fig. 6 Semi-logarithmic plots of carbamazepine data reported in [36, 37].

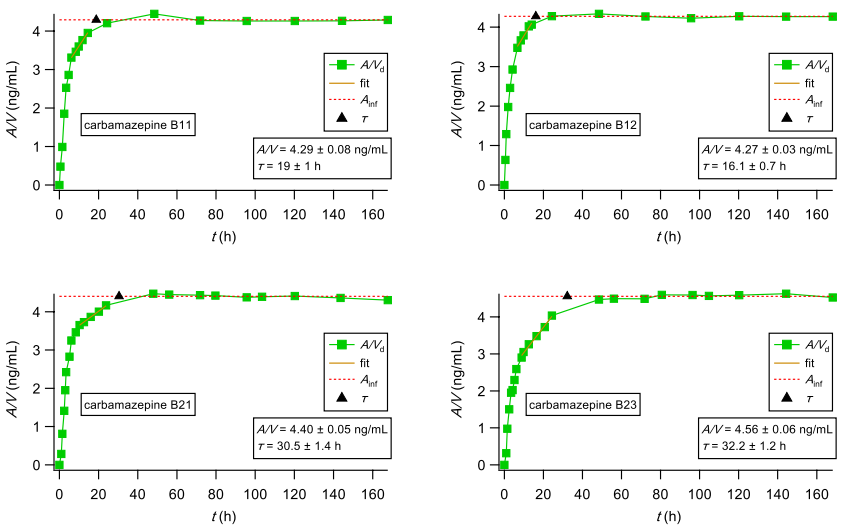


Fig. 7 Plots of A_{test}/V and A_{ref}/V as a function of time for the two carbamazepine studies reported in [35]. Redrawn from [16].

methodologies for the assessment of bioavailability and bioequivalence. The word "Assessment'' in the abbreviation MIDDA encompasses both the minimal modeling and the novel bioequivalence metrics introduced in this study.

Although the proposed slope of %absorbed versus time plot fits very nicely to the notion of rate introduced in FDA's definition of bioavailability in 1977, concerns are raised for the necessity of rate considerations in view of

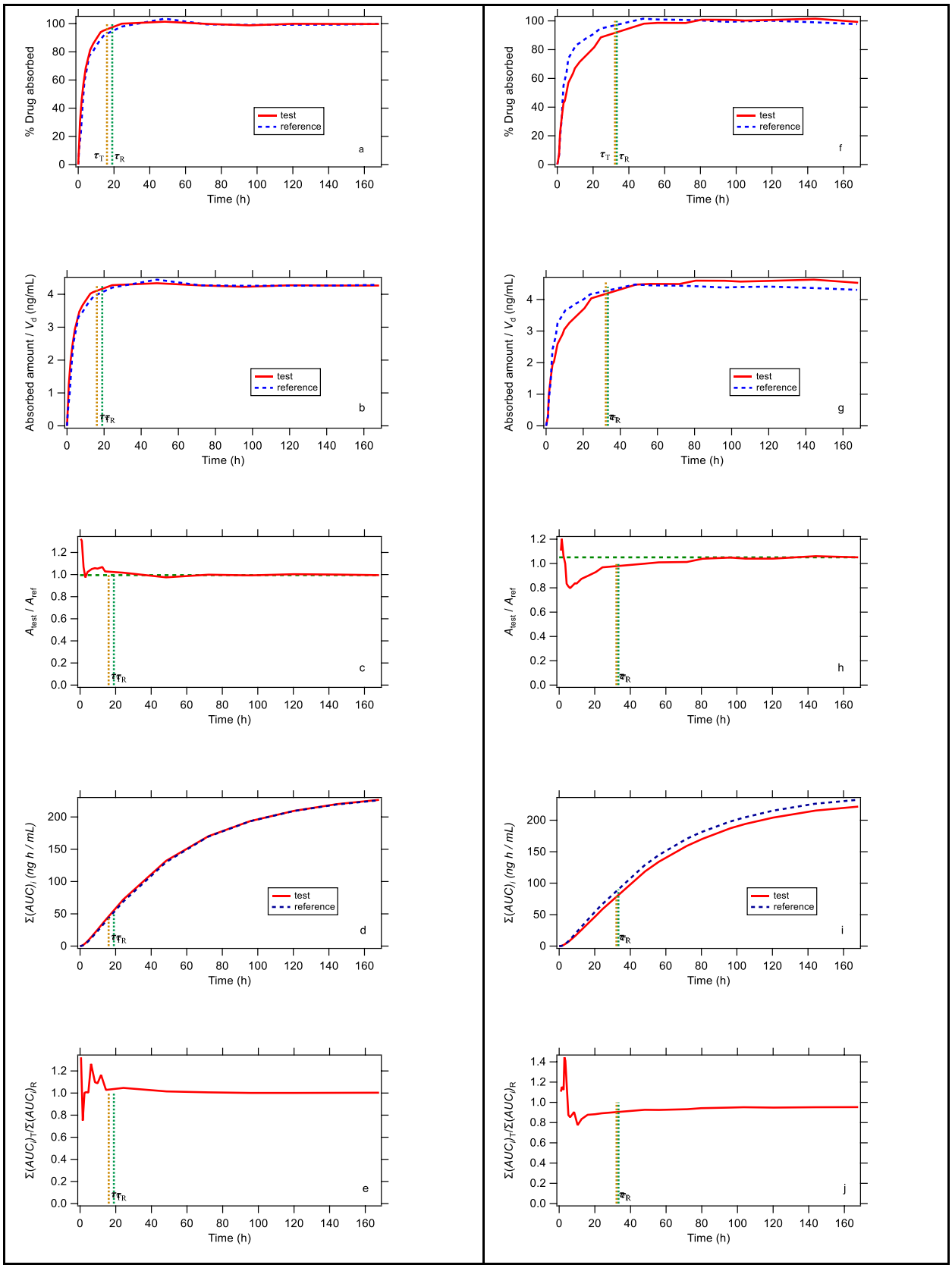


Fig. 8 Plots of A_{test}/A_{ref} and $\Sigma(AUC_i)_T$ /$\Sigma(AUC_i)_R$ as a function of time for the two carbamazepine studies reported in [35]. **a** – **e**: samples B11 and B12, **f** – **j**: samples B21 and B23.

Table I Parameter Estimates ± SD Derived from the Analysis of Carbamazepine Data [35, 36]

Formulation	Ref	Initial slope (ng/mL h)	A_∞ / V_d (ng/mL)[a]	τ (h)	k_{el} (h^{-1})
B11	[35]	0.72 ± 0.03	4.29 ± 0.08	19 ± 1	0.0180 ± 0.0004
B12	[35]	0.82 ± 0.10	4.27 ± 0.03	16.1 ± 0.7	0.0179 ± 0.0002
B21	[35]	0.72 ± 0.06	4.90 ± 0.05	33 ± 2	0.0204 ± 0.0006
B23	[35]	0.60 ± 0.06	4.74 ± 0.09	32.6 ± 1.6	0.0208 ± 0.0011
400 mg	[36]	1358 ± 43[b]	5520 ± 115	31 ± 3	0.023 ± 0.002

[a] Apparently, the blood concentration units used in [35] should be µg/mL, rather than the quoted ng/mL

[b] Based on the first 4 data points

Table II Test/Reference Ratios of the Parameters Indicated for Carbamazepine [35]

Data set	C_{max}	$[AUC]_0^\infty$	$[AUC]_0^\tau$	A_∞/V	Initial slope
B12/B11	1.04	1.003	1.03	0.982	1.14
B23/B21	0.89	0.954	0.909	0.962	0.83

the physiologically sound concept of F.A.T. For example, the currently used metric C_{max} does not mirror the rate of absorption (20–31) and is mainly being used as a safeguard for pharmacological reasons. However, carbamazepine's C_{max} corresponds to a pseudo steady state at t_{max} since in three sets of data $t_{max} = \tau$ and one set of data $t_{max} < \tau$, see Fig. 6 in [16]. Besides, the prolonged duration of carbamazepine absorption, unmasked by the analysis using PBFTPK models [16], underscores not only the importance of F.A.T. in rate of absorption considerations but also rules out the need for a controlled release formulation for carbamazepine [40]. These observations taken together lead either to the abolishment of the term rate in the definition of bioavailability and its use in bioequivalence studies or its replacement with the duration of drug's absorption derived from the percent absorbed versus time plots. In either case, it is advised to use C_{max} only for its pharmacologically related meaning applying boundary limits for the ratio test/reference mean C_{max} values without criteria of statistical difference, following a case-by-case scenario. Plausibly, these boundary limits can be shorter for critical drugs.

As far as the extent of absorption is concerned, the results of the study clearly show that the ratio of the amounts absorbed, A_{test}/A_{ref} is a proper metric for its assessment. Our results show that the assessment of the extent of absorption can be reliably based on truncated concentration–time data, namely, not earlier than the time point of the formulation exhibiting the longest duration of absorption. Our work theoretically substantiates the pioneering study of Lovering *et al.* [1] on the use of truncated data for the assessment of the extent of absorption published in the early 1970s prior to the definition of bioavailability by FDA.

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Declarations

Conflict of Interest The authors declare no conflict of interest.

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