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PARTIAL-AREA METHOD IN BIOEQUIVALENCE ASSESSMENT: NAPROXEN

SARFARAZ K. NIAZI*, SYED M. ALAM AND SYED I. AHMAD

University of Karachi, Karachi, Pakistan

ABSTRACT

Regulatory authorities require demonstration of bioequivalence through comparisons of different pharmacokinetic parameters, the area under the plasma concentration–time curve (AUC), the maximum plasma concentration (C_{max}), and the time to reach peak concentration (T_{max}). The applicability and validity of regulatory requirements have been widely criticized on statistical and clinical relevance grounds. For most noncomplicated absorption models, the AUC correlates well with the extent of absorption. However, in nonlinear models of absorption, in mechanisms involving recycling of drugs, and for drugs with long half-life, the use of total AUC (from zero to infinity) can give erroneous and clinically irrelevant results since the area is mostly determined by elimination phase or by recycling. The calculation of total AUC also involves prolonged sampling, adding to the cost and risks associated with bioequivalence studies. The use of C_{max} or T_{max} as a measure of rate of absorption, to correlate with clinical relevance, is widely criticized on logical, technical, and statistical grounds. For drugs used on a multiple-dose basis, C_{max} and T_{max} evaluations become redundant since the average plateau concentration is not affected by these parameters. To resolve the drawbacks in the traditional methodology of bioequivalence evaluation, the use of partial areas in lieu of total AUC, T_{max}, and C_{max} is suggested. This study investigates the logic and robustness of the partial-area method in establishing bioequivalence. We conclude that the 5 h AUC is a more relevant parameter to establish naproxen bioequivalence than AUC_{inf}. We recommend against using symmetrical confidence intervals and report excellent agreement among several methods of calculating confidence intervals, probability values, and nonparametric tests. We suggest that a single-point short-term AUC is a better indicator of the bioequivalence of generic products than the total AUC, C_{max}, and T_{max} as required currently by the regulatory authorities. ©1997 by John Wiley & Sons, Ltd.

KEY WORDS: naproxen; bioequivalence; partial-area methods

INTRODUCTION

Regulatory authorities require evaluation of bioequivalence of products by comparing pharmacokinetic parameters including the area under the curve (AUC), T_{max}, and C_{max}. The AUC is a general indicator of the extent of bioavailability and the C_{max} that of rate of absorption for drugs which are

*To whom inquiries should be directed. Address: Gulf Pharmaceutical Industry, P.O. Box 997, Ras-ul-Khaimah, U.A.E.

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normally released from dosage forms; for controlled release formulations, other paramteres such as plateau time are more appropriate,[1] but these are not included in the official regulatory requirements though these are likely to be addressed in individual drug monographs of bioequivalence testing. The rate of absorption is important since the products showing identical extent of absorption may yield too low or too high concentration levels that can be ineffective or toxic. Frequently, these products also demonstrate problems in dissolution, have narrow therapeutic index, undergo first-pass biotransformation, are absorbed from specific parts of the gastrointestinal tract, are administered in specialized controlled release or delayed release formulations, demonstrate dose-dependent pharmacokinetics, and undergo biliary or other recycling mechanisms. All of these peculiar characteristics can yield differences in the pharmacokinetic properties among and within the study subjects requiring appropriate logical and statistical considerations in their use to establish bioequivalence.

Concerns have been raised regarding the assessment of rate of absorption in bioequivalence evaluations; the utility of C_{max} and T_{max} has been criticized frequently[2–5] since these parameters are determined experimentally and are subject to variation with sampling schedules. The indirect metrics, C_{max} and T_{max}, do not reflect the differences in absorption rate constants and are, as a result, poor choices for comparing rates of absorption. The question arises, 'What is the purpose of bioequivalence demonstration?'[6] Is it to assure pharmaceutical quality or clinical similarity? The limits of acceptability, 80–120%, seem too large for drugs with low therapeutic index and not sufficiently large for drugs with broad therapeutic index. The indirect metrics are also insensitive to changes in the rate of absorption, making them less useful. Additional problems arise where multiple peaks are observed as in the case of biliary recycling or multiple release or where flat peaks are observed as in the case of sustained release products. Furthermore, T_{max} cannot be adequately tested for significant difference using existing statistical models, leaving C_{max} as the only parameter to evaluate absorption rates. The use of mean absorption time (MAT) has also been suggested[3] but this parameter is subject to error, particularly when mean residence times (MRT) are high. Ideally, the absorption kinetics must be evaluated since absorption rate (a function of amount present at the site of absorption) is a continuous variable; however, such evaluations are not necessary for drugs which are quickly absorbed since the process is mostly completed by the time peak concentration is achieved. For drugs administered on a multiple-dose basis, the value of C_{max} is redundant since the plateau levels are not affected by absorption rates. In view of these inconsistencies in the outcome of studies, it is proposed that the use of both T_{max} and C_{max} should be discarded in favour of AUC, which can be made more clinically relevant by evaluating it as partial areas instead of the customary total AUC.

The use of partial areas resolves many of the objections raised against the use of traditional pharmacokinetic parameters. The customary use of total AUC in

bioequivalence measurements by extrapolating to infinity makes the parameters more dependent on elimination, particularly for drugs with long half-lives. It is indicated that the incremental area under the drug level curve representing 10–30% of the total AUC would be more sensitive than either C_{max} or T_{max} or total AUC.[7] The principle of partial areas has been applied to a variety of drugs where T_{max} values were similar but C_{max} values were not.[8] It was suggested that if we restrict observations to T_{max} then the criteria of 80–120% will need to be relaxed since the ratios will not meet the criteria perhaps until an hour or so beyond the C_{max} appearance. It was observed[9] that comparison of area to T_{max} is a poor index since in their studies these parameters showed difference in bioequivalence whereas there was no real difference when the fraction absorbed and the absorption rate constants were compared. However, since the absorption rate constant is not a good index because it relates to transient changes, the use of partial area to T_{max} can be a good index for drugs where absorption rates are important.

The partial areas can also serve as a thermodynamic parameter[10] to represent the rate of absorption. Since the amount of drug remaining in the body is proportional to the AUC from t to Ë, the distribution potential to tissues is reflected in the accumulation function of AUC. How fast a profile accumulates is indicative of its absorption rate and distribution potential. The partial areas before the absorption phase is complete reflect most the absorption characteristics. Thus the use of partial AUC obviates the need to use additional pharmacokinetic parameters to establish absorption rate differences. Short-term AUC calculations also prove useful for drugs that undergo recycling—biliary, salivary, etc, since the calculations are often limited to the appearance of the first peak, and the recycling contribution to the AUC which can confound the real difference in bioequivalence are minimized. This can be an important consideration since the recycling can introduce a large degree of inter- and intra-subject variation; large variations in the AUC values reduce the value of statistical testing, failing to ascertain differences where differences actually exist.

The statistical models suggested by the FDA guidelines[11] acknowledge that standard statistical methodology based on the null hypothesis is not appropriate to assess bioequivalence and that it requires parametric general linear model procedure (GLM-ANOVA) analysis to determine sequence, subject, period, and treatment effects. The sequence effect is tested using the subject sequence mean square whereas all other effects are tested using the residual error. The conclusions are now based on more robust solid statistical tools.[12–14] For AUC, the deviation of 20% of reference is generally accepted[11,34] (for log-transformed data the range is 80–125%). The use however of some very powerful statistical tools such as Bayesian posterior probability and nonparametric evaluations[15] is ignored in the official guidelines. The guidelines do recognize that T_{max} cannot be analysed statistically. One of the most debatable aspects of the official guidelines is the range of

80–120% (80–125% for log-tranformed data) allowed. Studies have demonstrated that for C_{max} a deviation of 30% of reference is more clinically relevant[16] and depending on the nature of drug the range of variation allowed should be tightened or broadened.

In view of the large inter-individual variability in the clearance of many drugs, bioequivalence studies usually follow a two-period change-over design;[17] carry-over effects are accounted for by allowing an appropriate washout period between treatments.[18] A statistical decision procedure which has been adopted by the FDA[11] is based on two one-sided *t*-tests at the nominal 5% level concerning the treatment sequences reference–test and test–reference[19,20] which is equivalent to 90% confidence interval in the bioequivalence range.[21] It has recommended[15] to use a distribution-free (nonparametric) procedure reducing the two-sample situations in which the treatment sequences reference–test and test–reference are compared. It utilizes the equivalence between the rejection of two one-sided hypothesis (bioinequivalence) at the nominal level by means of Mann–Whitney–Wilcoxon tests and the inclusion of the corresponding distribution-free (1–2a) 100 confidence interval in the bioequivalence range.

In this study, we conclude that there is no need to extrapolate studies to calculate the total AUC; we used a partial-area method to analyse the bioequivalence data for naproxen, as an example of a complex bioequivalence profile because of its dose dependence of clearance and biliary recycling characteristics.[22–24] We have also compared several methods of statistical analysis and conclude that the symmetrical confidence interval analysis[25] is inappropriate and recommend using other measures of confidence interval and probability calculations including the Bayesian approach. We recommend developing guidelines for specific drugs and classes of drugs regarding the length of time for which the AUC must be calculated; for naproxen bioequivalence we suggest the studies be conducted only up to 5 h of dosing, a much shorter time span than the conventional method of testing its bioequivalence.

MATERIALS AND METHODS

Chemicals

Standard commercial oral dosage forms of naproxen (250 mg tablets) from two manufacturers were used. Naproxen reference standard was purchased from Zan Bon Group, Milan, Italy, and ibuprofen internal reference standard was purchased from Schwelzer Hall (Pvt) Ltd. Singapore. Methanol (HPLC grade, Merck), acetonitrile (HPLC grade, Merck) glacial acetic acid (Merck), and dichloromethane (HPLC grade, BDH) were used for high-pressure liquid chromatography analysis.

Drug administration and blood sampling

Fourteen healthy adult volunteers, aged 21–44 years, and weighing between 51 and 77 kg, were selected. A complete medical history and physical examination, urine analysis, and haematology were obtained for all volunteers within 7 d prior to the initiation of study. The volunteers were instructed to abstain from taking any medication for 1 week prior to and during the study period. The volunteeers were divided randomly into two groups of seven each and given either formulation of naproxen, 250 mg strength. A period of 1 week was allowed for wash-out after which the study was repeated to complete the cross-over design. The drug was administered orally in fasting state with 250 mL water, immediately followed by a continental breakfast. Blood samples (10 mL) were drawn at 0, 1, 2, 3, 4, 5, 6, 8, 10, 24, 36, 48, and 72 h. Blood samples were collected by venepuncture or via an indwelling canula in heparinized blood collected evacuated tubes. The blood samples were centrifuged for 10 min at 3000 rpm and plasma was separated and kept frozen at $-20\,^{\circ}C$ until assayed.

High-pressure liquid chromatographic analysis of plasma samples

Plasma levels of naproxen were analysed by a high-pressure liquid chromatography (HPLC) method developed in this study. Analysis was performed using a system consisting of an auto-injector (SIL–6B Shimadzu, Japan) fitted with a 20 μL loop, a high-pressure pump (LC-6A, Shimadzu, Japan), a spectrophotometric detector (SPD-6A, Shimadzu, Japan) and a data integrator (C-R4A, Shimadzu, Japan). The stainless steel column (300 mm length × 3·9 mm i.d.) used was packed with reversed-phase C-18 Microbondapak (Waters Associates–Millipore, U.S.A.) base and acetonitrile:water (40:60 v/v in 0·1% glacial acetic acid) was used as an eluant with a flow rate of 2·5 mL min^{-1}. The eluant was monitored at 232 nm wavelength. Ibuprofen was used as the internal standard in this study.

Extraction of plasma samples was performed after protein precipitation with phosphoric acid, followed by addition of extraction solution (internal standard in dichloromethane). After centrifugation at 3000 rpm for 10 min, 4 mL of the upper layer were separated and evaporated to dryness under nitrogen stream. The residue was reconstituted in 0·5 mL methanol and 20 μL were injected onto the column. Naproxen and ibuprofen showed excellent separation and resolution at 6·5 and 15·5 min respectively.

Pharmacokinetic analysis

The plasma concentration profiles of naproxen (Figure 1) to 72 h were used to calculate areas between sampling intervals by the trapezoidal rule:

$$\mathrm{AUC} = [(C_1 + C_2)/2](t_2 - t_1)$$

Other pharmacokinetic parameters were calculated as follows:

Elimination half-life ($t_{1/2}$) = 0.693/β (β is the terminal rate constant)
Volume of distribution (V_d/F) = (Cl_p/F)/β (F is the fraction absorbed)
Plasma clearance (Cl_p/F) = AUC/Dose
Area under the moment curve (AUMC) =

$$[((C_1t_1 + C_2t_2)/2)\ (t_2 - t_1)] + \ldots + [((C_{n-1}t_{n-1} + C_nt_n/2)\ (t_n - t_{n-1})]$$

The AUCs to 72 h were calculated by adding component areas. The AUC to infinity was calculated by adding to the 72 h AUCs extrapolated AUCs from concentration at 72 h to infinity, calculated by the ratio of concentration at 72 h and the individual terminal slope constant. T_{max} and C_{max} were recorded without extrapolations. Simulated studies were performed by modifying the test formulation plasma values to deliberately introduce bioinequivalence of 60–140%. This was introduced by multiplying the AUC at each sampling time by a factor ranging from 0·6 to 1·4. This modified only the extent of absorption and not the rate of absorption.

Table 1 lists the pharmacokinetic parameters calculated for statistical evaluation purposes. The values of the pharmacokinetic parameters of naproxen calculated in this study were in good general agreement with those from earlier studies.[22,23,26,27]

Statistical analysis

In this study, we analysed the pharmacokinetic parameters using both parametric and nonparametric approaches. The ratios of AUC and difference of T_{max} and C_{max} were analysed using the ANOVA GLM model (SAS Institute, NC, U.S.A.). The confidence intervals were calculated according to various reported methods and included the '*t*' based confidence interval of difference[28] for a significance level of $a = 0{\cdot}05$; the Westlake symmetrical interval;[25] the Mandallaz and Mau confidence interval;[19] the Locke confidence interval;[29] the Anderson–Hauck test[30] which computes the probability in the two one-sided

Table 1. Pharmacokinetic parameters of naproxen in man

Parameters	Naproxen test	Naproxen reference
AUC (mcg mL^{-1} h)	624·0 ± 26·2	654·2 ± 36·3
AUMC (mcg mL^{-1} h)	11 487·9 ± 657·4	12 338·8 ± 786·6
$t_{1/2}$ (h)	15·0 ± 0·92	14·2 ± 0·76
MRT (h)	18·3 ± 0·38	18·8 ± 0·46
C_{max} (mcg mL^{-1})	35·03 ± 1·52	35·92 ± 2·73
T_{max} (h)	2·64 ± 0·44	3·28 ± 0·48
CL_p/F (L h^{-1})	0·410 ± 0·017	0·400 ± 0·020
V_d/F (L)	8·75 ± 0·49	8·01 ± 0·47

confidence interval estimates based on the null hypothesis as required in the FDA guidelines;[11,20,30] and the Bayesian posterior probability[31] based on the sample values that the true relative bioequivalence is contained in acceptable interval. The nonparametric statistics was also calculated to demonstrate bioequivalence[32,15] since the acceptance of bioequivalence based on a *t*-test or analysis of variance may prove erroneous for various reasons including lack of normal distribution.

RESULTS AND DISCUSSION

The bioequivalence of the two naproxen formulations was assessed by comparing the ratio of total AUCs by ANOVA. The confidence interval tests and nonparametric tests concluded bioequivalence of the two formulations studied (Table 2) falling between 80 and 120% for untransformed and 80 and 120% for log-transformed data. The symmetrical range of confidence intervals calculated by the Westlake method[25] yielded higher upper limits by about 10%; however, they were still within the 80 and 120% range used to accept the bioequivalence (Figure 1).

Table 1 lists the values of T_{max} and C_{max} for the two formulations. The C_{max} ratios are required for proof of bioequivalence in FDA protocols. The coefficient of variation was 16% for test, 28% for reference in individual values and about 35% in the ratios of C_{max}: an analysis of variance shows these values

Table 2. Statistical evaluation of the bioequivalence of naproxen

Statistical test	AUC untrans-formed	AUC log trans-formed	C_{max} untrans-formed	C_{max} log trans-formed
ANOVA–GLM[a] *F*-value (CV[b])	2·032 (8·5%)	1·118 (9·54%)	0·0745 (24·16%)	0·0000 (26·89%)
Symmetric CI[5]	89·6–110·4	90·2–109·8	79·5–120·5	78·1–121·9
App. difference CI[28]	88·3–102·4	89·0–104·1	77·6–117·4	80·4–124·3
Mandallaz and Mau CI[19]	88·7–102·5	—	79·5–119·4	—
Locke CI[29]	89·0–102·6	—	80·4–120·4	—
Fleuhler's posterior probability[3] (0·8–1·2)	0·99	—	0·95	0·93
Two one-sided *t*-test[20]	0·99	0·99	0·95	0·93
Nonparametric geometric mean ratio	0·96	—	1	—
Nonparametric 90% CI[15]	88·4–103·2	—	84·4–121·3	—
Nonparametric point estimate[15]	95·5	—	101·6	—

[a]Analysis of variance—general linear model.
[b]Coefficient of variation.
[c]Confidence intervals.

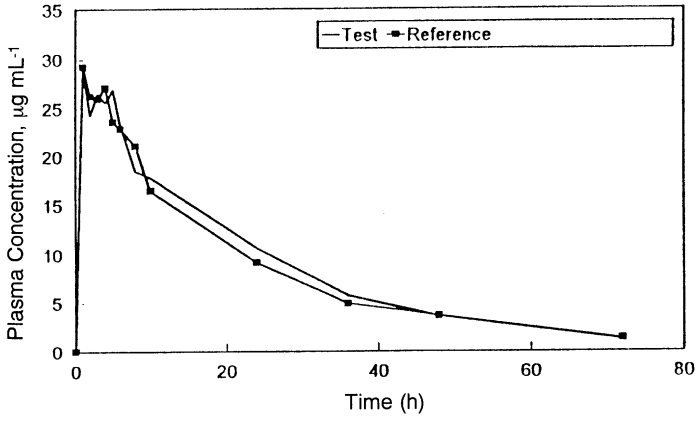


Figure 1. The plasma concentration profile of a naproxen 250 mg tablet

to be statistically not significant (Table 2). The variation in the T_{max} was of much higher magnitude: the coefficient of variation for individual values was 50% for test, 63% for reference, and about 97% for the ratio. However, the FDA protocol does not require statistical testing of this discontinuous parameter. In this study, neither C_{max} nor T_{max} would give any additional statistical inference over what is given by the comparison of total AUC.

Partial areas to all sampling intervals (Figure 2) were tested for confidence limits according to various statistical procedures. A comparison of these interval ratios shows that the conclusion drawn from total AUC analysis would be replicated based on the confidence intervals at sampling times of 5 h and beyond. By this sampling time, all confidence intervals begin to fall within the range of 80–120% of the AUC of reference (Figure 2). This time interval range is beyond the peak concentration ($2{\cdot}64 \pm 0{\cdot}44$ h against $3{\cdot}28 \pm 48$ h) but not too far. This observation is important in comparing with recommendations made in the literature. It has been suggested to calculate the AUC to T_{max}[8] for reference products when using the partial-area method. Chen's study also suggests using a different acceptance criterion than the 80–120% range. We do not agree with either of these suggestions. Basing the cut-off point only on the reference product T_{max} ignores the possibility that the test product may have significantly higher T_{max}. The allowance of 0·5–1 h beyond T_{max} recommended by Chen[8] may not be sufficient and should ideally be based on the nature of the peak. For products giving sharp peaks, extending to T_{max} or an hour past T_{max} may be sufficient, but for products yielding broader peaks, the studies may have to be extended at least 2 h beyond T_{max}. Also changing the acceptance range of 80–120% should not be done arbitrarily. It is understandable why some products will compare better at different ranges but this should be done with clinical justification. In case of naproxen, our recommendation is to follow the area to about 2 h beyond the maximum plasma concentration. This

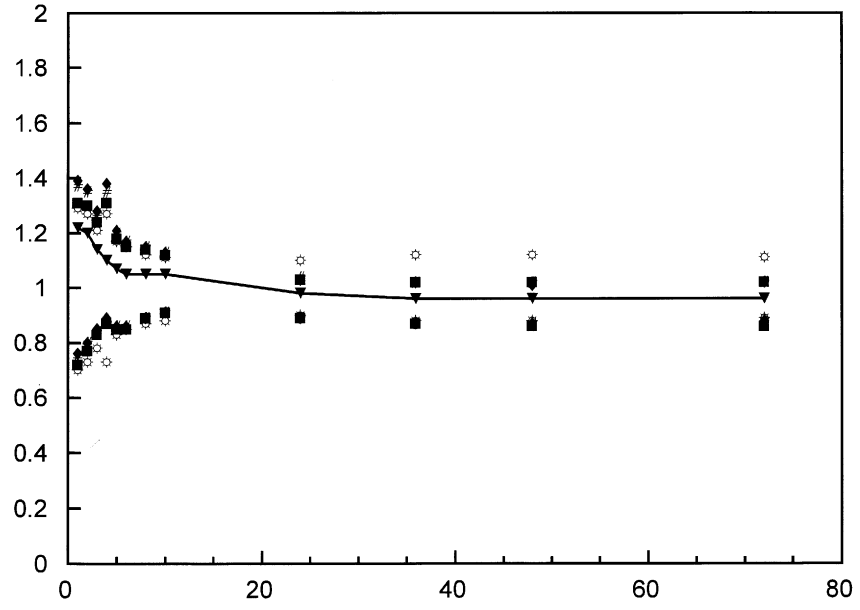
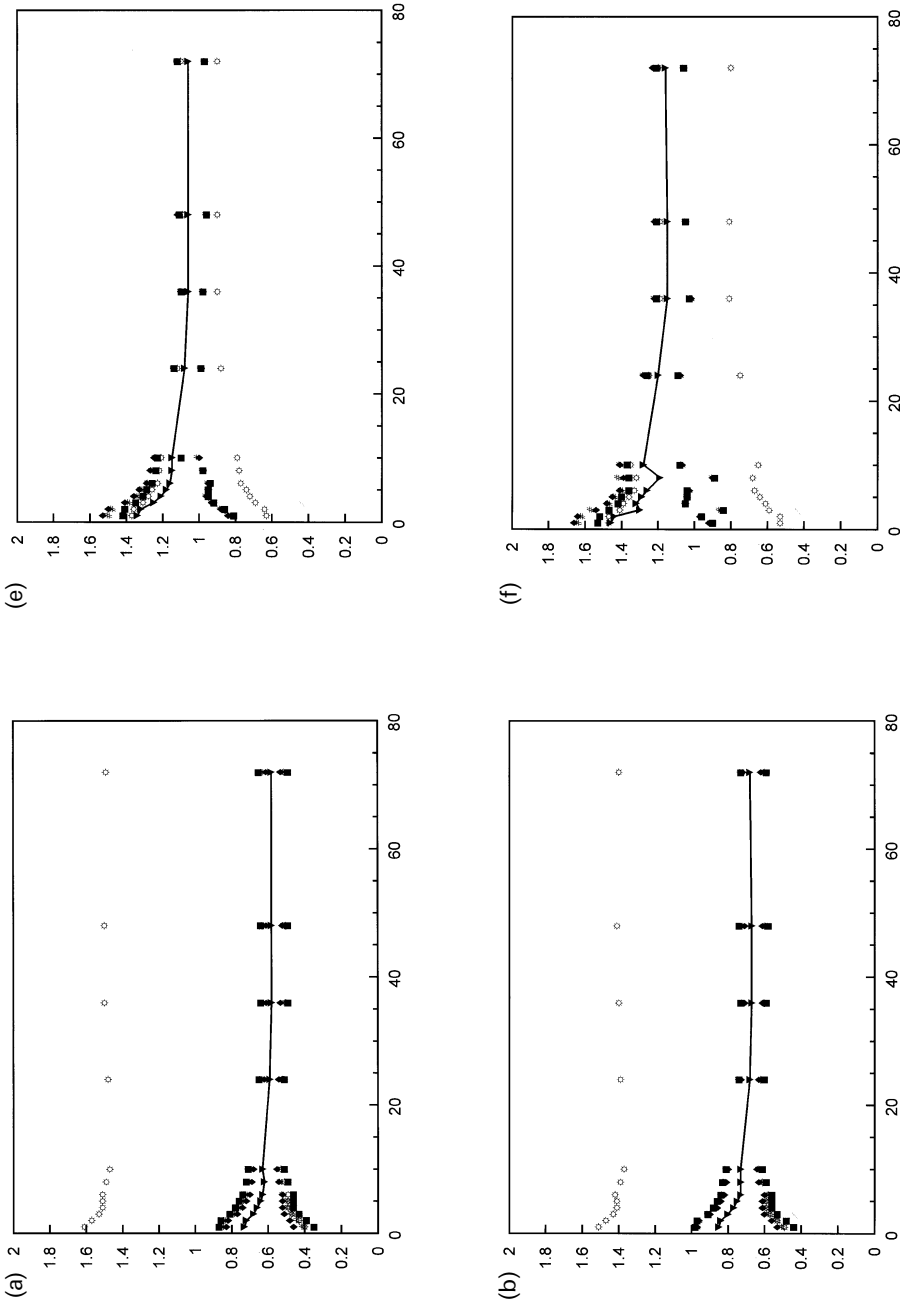


Figure 2. Partial-area ratios of naproxen 250 mg tablets with confidence intervals as a function of time. (—▼—, AUC Ratio; ■ difference lower/upper limits; #, Mandallaz lower/upper limits; ◆ Locke lower/upper limits; ☼, Westlake lower/upper limits)

time window is needed to bring in the confidence intervals to match the full AUC calculations. This recommendation can be supported by pharmacokinetic criteria also since, for most drugs (not given in controlled release dosage forms), the absorption phase will be mostly completed within 2 h after the peak concentration is observed.

Previous studies suggesting the use of the partial-area method[6] have taken a theoretical approach to assess variations in absorption rate constants. However, no study has measured the impact of forced bioinequivalence. We made this evaluation by modifying the area of the test product within the range of 60–140% and then applied the confidence interval testing (Figure 3). It was interesting to note that regardless of the level of forced bioinequivalence, the 5 h AUC proved to be sufficient to prove bioequivalence. It was further observed that whereas the Westlake symmetrical interval method yielded acceptable ranges of confidence intervals when the products were bioequivalent (Figure 2), extreme deviations were found as the theoretical ratio of areas changed (Figure 3). At ratios greater than one, the lower limits of Westlake deviated by as much as 100%. At theoretical ratios of less than one, the symmetrical upper limits of Westlake showed similar deviation. Therefore, for products which are not bioequivalent, the Westlake symmetric interval would give false results. Though the use of the Westlake symmetrical interval has previously been critized[12,21,33] on theoretical grounds, its failure in assessing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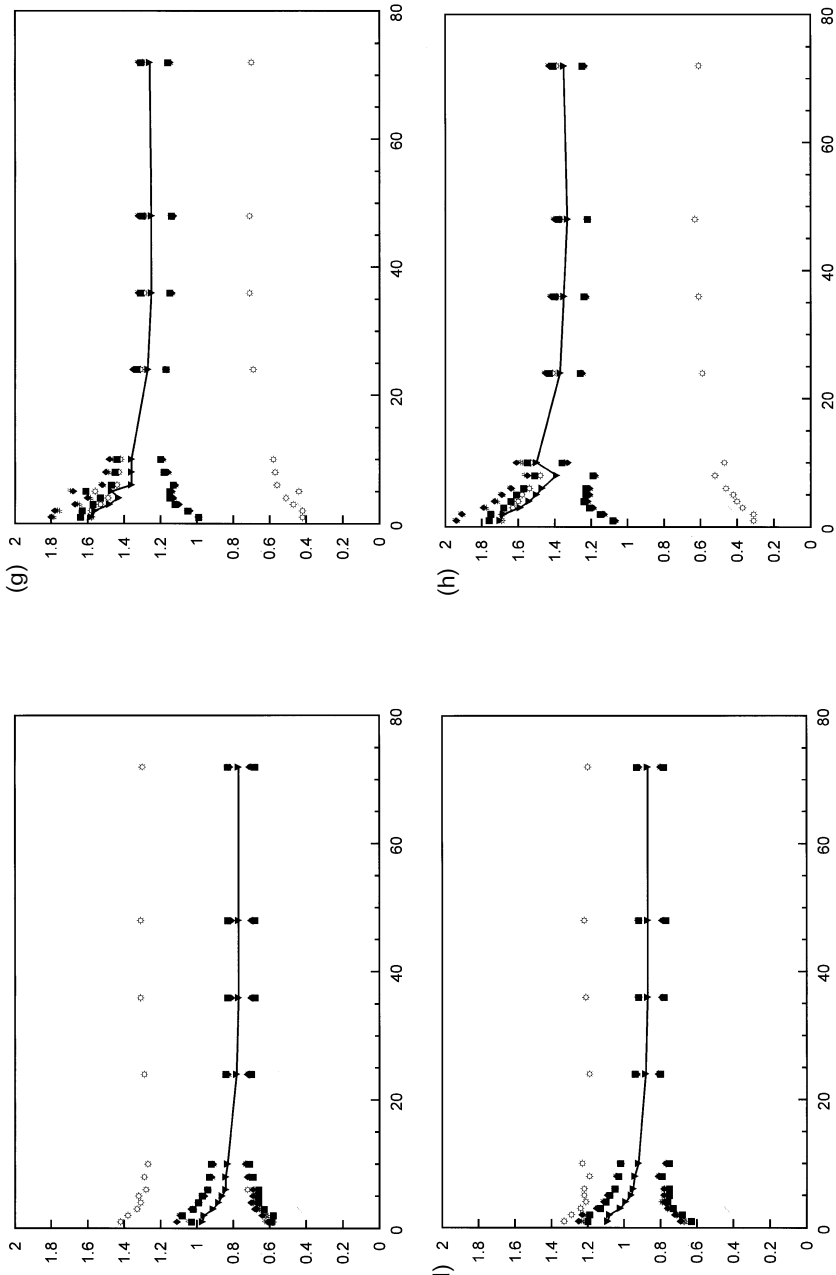

Figure 3. Partial-area ratios with confidence intervals as a function of time at different levels of forced bioinequivalence: (a) 60%; (b) 70%; (c) 80%; (d) 90%; (e) 110%; (f) 120%; (g) 130%; (h) 140%. Open stars represent the Westlake symmetrical intervals. For other intervals see the text

bioequivalence when products are not bioequivalent is clearly demonstrated in the simulations made in this study. This forced bioinequivalence was introduced only in the extent of absorption because it has been demonstrated that an alteration in the rate of absorption of naproxen does not affect the bioavailability.[22,35,36]

We have demonstrated using detailed analysis of the AUC that for bioequivalence evaluation of naproxen products the partial-area method can be successfully used and the studies need not be conducted for the customary multiple half-lives. Whereas the half-life serves a useful purpose in the development of therapeutic regimen, its utility in bioequivalence trials is questionable. Since the purpose of bioequivalence determination is to compare the extent of absorption that is clinically relevant, classically, the rate of absorption has been included as one of the parameters for bioequivalence evaluation.[6] The FDA[11] requires the use of C_{max} and T_{max} as indicators of the absorption rates. Not only are these unrealistic markers of absorption rate in complex absorption kinetics but, in instances where drugs are given on a multiple-dose basis, as most drugs are, these parameters are irrelevant in determining the clinical efficacy of products. The use of area beyond the pseudo-distribution equilibrium state is more influenced by the elimination phase of the drug and thus confounds the importance of the AUC as a marker of amount of drug absorbed. The area under the curve during the absorption phase better reflects the variation in the rates of absorption,[10] making the use of partial areas a more clinically relevant parameter.

It is suggested that for naproxen we can use a partial-area method to 5 h sampling time and no statistical advantage would be gained by continuing the study beyond the 5 h sampling. Similar evaluations should be made for other drugs also but a good starting point is to analyse blood levels to at least 2 h beyond the value of T_{max} for both test and reference, whichever is higher, and not merely 0·5–1 h for reference only as suggested in the literature.[8] In using the partial-area method, we also recommend the use of the Bayesian approach in analysing the probability function; this allows variable calculation of various intervals. We also recommend using nonparametric tests to evaluate the data that may not be normally distributed.

The approach of using the partial-area method can result in substantial cost savings to generic drug manufacturers besides being a more meaningful statistical parameter. If the purpose of bioequivalence studies is to demonstrate clinical relevance, partial-area methods with specific ranges of variability specific to the class of drugs would offer an ideal tool to assess the quality of products.

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