

Concepts of Bioequivalence and it's Impact on Truncated Area Under Curve (AUC) of Drugs with Long Half Life in point estimate and intra-subject variability

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Abstract

The purpose of this study is to elicit the concept of bioequivalence and investigate the effect of using truncated area under the curve (AUC) method on the bioequivalence of different drugs with long half life in healthy volunteers. Model drugs used were Azithromycin, Mirtazepine, Anastrozole, Letrozole, Clonazepam and Digoxin. More than 12 healthy volunteers, participated in each study using cross over design. Individual disposition kinetic parameters of areas under plasma concentrations (AUC_{0-t} and AUC_{0-inf}), maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) were calculated by non-compartmental analysis using WinNonlin 5.2.1 or higher version. In addition, AUCs truncated at 48, 72 and 96 hour (AUC_{0-48} , AUC_{0-72} and AUC_{0-96}) were calculated and analysed. The 90% confidence intervals for log transformed AUC_{0-t} , AUC_{0-inf} and C_{max} were calculated and 90% confidence intervals for log-transformed AUCs truncated at 48, 72 and 96 hour (AUC_{0-48} , AUC_{0-72} and AUC_{0-96}) were calculated. The intra-subject variability were analysed for all the pharmacokinetic parameters. The study results ascertains that, as different regulatory bodies recommend, AUC truncated at 72 hour can be interchanged with AUC_{0-t} and AUC_{0-inf} . It was further witnessed that intra subject variability was usually less in truncated AUC when compared to that of AUC_{0-t} and AUC_{0-inf} . These results suggests that limiting the pharmacokinetic sample collection period to 72 hour in bioequivalence studies for the drugs having long elimination half-lives is equally accurate, sensitive and an alternative to the conventional approach.

Keywords

Truncated AUC, Partial AUC, Long half life drugs, Intra-subject variability, Bioequivalence

INTRODUCTION

Generics is a term that is often heard of in the recent decades. The innovator pharmaceutical product (innovator drugs) is that which was first authorized for marketing (normally as a patented product). Generic pharmaceutical products (generic drugs) are those which are identical to the innovator pharmaceutical products but manufactured by firms other than the innovator and marketed after the patent expiry of the innovator drug. Prior regulatory approval is mandatory before marketing, which would ensure the interchangeability between innovator and generic drugs, by bioequivalence testing. As incase of innovator drugs, generic drugs can also be recalled from the market if they are proven unsafe or if results of further research arises doubts on its bioequivalence. Hence bioequivalence is a term used when innovator drugs are compared with generic drugs.

To meet the standards of bioequivalence the rate and extent of absorption of the active ingredients from the innovator and generic drugs should be equal or within a defined acceptable range. Two drugs with the same active ingredient can be absorbed differently, depending on the inactive ingredients involved in their production. Using different coatings, fillings, and other ingredients can change the way the medication is absorbed and all of these details must be tweaked before the drug enters testing

Concept of Bioequivalence

As per USFDA, CFR 320.1, bioequivalence is defined as the absence of a significant difference in the rate and extent to

which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study⁽²⁾.

As opined by Dr. Pieter Zanen in 'Bioequivalence and Generic Medicines'⁽⁴⁾, equivalence studies are necessary in two cases:

- **To accept the formulation changes by the originator.** To replace the innovator product with an entirely different product of same therapeutic indication. A good example of the latter is the replacement of CFC's (chlorofluorocarbons) in MDI's (metered dose inhalers) by the ozone-friendly HFA-propellants (hydro-fluoroalkane). Since such a vital change in formulation must incur no change in the therapeutic quality, an equivalence study is obligatory.
- **To accept the formulation of generic medicines.** As in our case, to prove the therapeutic equivalence between innovator and generic drugs and to facilitate their interchangeability.

The most obvious way to prove bioequivalence is by comparing the therapeutic effects of the generic drug in subjects (patients or healthy volunteers) with that of innovator drug. This comes under the pharmacodynamic studies and comparative clinical studies.

Advantages of Bioequivalence studies (using Plasma Concentration-Time Profiles to Claim Therapeutic Equivalence) over pharmacodynamic studies and comparative clinical studies:

- Clinical comparative studies are often tailed by a lack of defined and measurable endpoints. The measurement of the severity of a depression, for example, is a science in itself and a consensus on the best measurement method often does not exist
- For studies on highly variable drugs, to enroll patients in large number is task affixed with practical difficulty
- It is also possible that a trial that is perceived to be therapeutically equivalent, cannot detect small differences that may have clinical relevance.
- The advantages of a pharmacokinetic approach are the superb definition of the endpoint (the plasma concentration of the drug) and lower variability of that endpoint. These characteristics solve many of the problems encountered with clinical testing: they lead to smaller and more powerful trials, which are to the benefit of both the manufacturer and the subject/patient.
- It is time and cost effective when bioequivalence is used as an established surrogate marker of therapeutic equivalence.

The conclusion is that clinical studies are not always the best choice for comparisons of formulations (where small differences are to be expected) and an alternative method had to be developed, which is the pharmacokinetic approach. For these reasons, the design, performance and evaluation of bioequivalence studies have received major attention from academia, the pharmaceutical industry and health authorities.

Role of Bioequivalence in proving therapeutic equivalence

An easy approach to understand the effectiveness of bioequivalence is by considering the fact that, if same number of drug molecules (from innovator and generic products) occupies the receptors, the effect of the drug is going to be similar

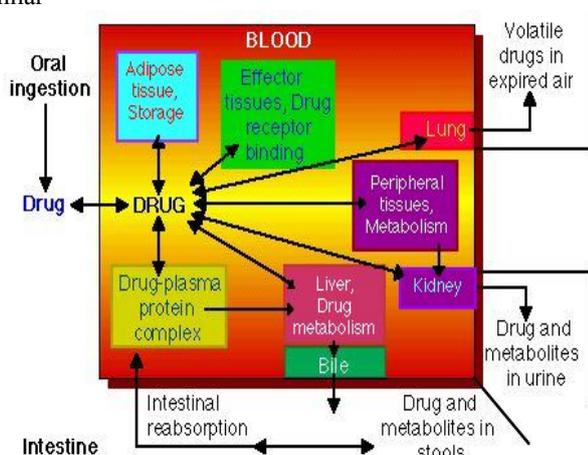


Figure 1: The effect of a drug is related to the number of receptor occupied by drug molecules

Drug molecules are delivered to receptors by systemic circulation, so the number of molecules in the systemic circulation is a measure for the number at the receptor. The parameters governing the plasma concentration of a drug are absorption, distribution, metabolism and elimination of the active drug. Hence measuring and proving these parameters to be equivalents is the best approach towards proving the therapeutic equivalence. This is the objective of bioequivalence studies.

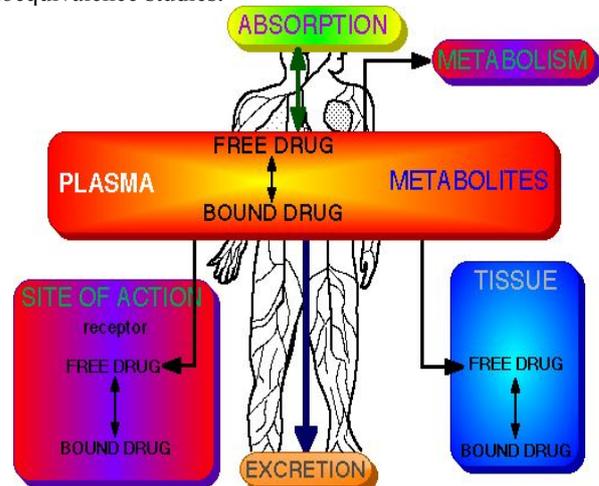


Figure 2: The number of drug molecules present at the receptor is governed by kinetic processes like absorption, distribution, metabolism and elimination

The distribution, metabolism and elimination is supposed to be not influenced by different formulations say A or B. So when the chemical nature of the drug in two different (innovator and generic products) formulations is identical, the distribution and elimination patterns are exactly the same. So any change in the number of drug molecules at the receptor can only be caused by differences in absorption from formulation A or B.

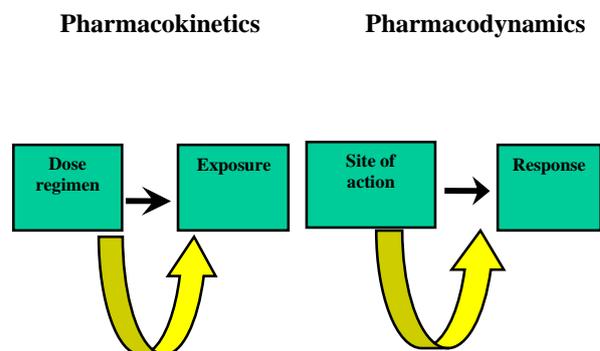


Figure 3: In all subjects/patients distribution, metabolism and elimination are drug specific processes and not formulation dependent As such they are constant and can be ignored in a comparative trial

Since the nature of the absorption process is chemically determined, accounting the fact that if the chemical nature of the drugs are similar their absorption should be similar, it is rationale to judge that, the gastro-intestinal tract will absorb an innovator and generic drug molecule in exactly the same way. So if a difference in absorption dose not exist, this can only be caused by changes in delivery of the drug from the formulations: disintegration of the dosage form and dissolution of the drug. The latter two are known under the term 'pharmaceutical availability'. The possibility of difference due to pharmaceutical availability can be easily curtailed as pharmaceutical availability is under the direct control of the manufacturer.

Thus, the kinetic approach to bioequivalence studies can be rephrased as follows: absorption, distribution, metabolism and elimination are constant within the same volunteer, so differences in the plasma concentrations are due to differences in the pharmaceutical phase. A bioequivalence study is simply a check on identical pharmaceutical phases.

It can be summarized that two drugs with same active ingredient produces same therapeutic effect when the same number of drug molecules from both the drugs bind to the receptor. Since systemic circulation delivers the drug molecules to the receptor, to compare the systemic bioavailability between the two drugs suffices the need. Thus the plasma concentration of drugs at specified point estimates are measured and analyzed. Since the plasma concentrations are governed by absorption, distribution, metabolism and elimination and as the latter are constant within the same subjects, differences in the plasma concentration (= therapeutic effect) are due to differences in the amount of drug absorbed which, in turn, depends on the delivery of drug from the formulation.

The pharmacokinetic parameters measured in a bioequivalence study

Earlier it was argued that a bioequivalence study is a check on the similarity of the release characteristics of formulation A and B. the amount of drug molecules released and the speed of the release are therefore the most important parameters. Rephrased: the rate and extent of the release.

In the in-vivo bioequivalence study these characteristics are determined by measuring the following parameters:

- Area under the plasma concentration-time curve (AUC): It describes the total number of molecules present in plasma and provide information about the extent of the release;
- C_{max} : The maximum plasma concentration and provide the details about the rate of the release;
- T_{max} : The time at which the maximum plasma concentration is reached and it details about the speed of the release;
- $T_{1/2}$: The elimination half-life. It is linked to the elimination of the drug. $T_{1/2}$ is obtained by calculation of K_{el} , the elimination rate constant.

The last three factors fully determine the shape of the plasma concentration-time curve and strategies to compare the shape

of the curve itself instead of these 'derived' parameters are of little use.

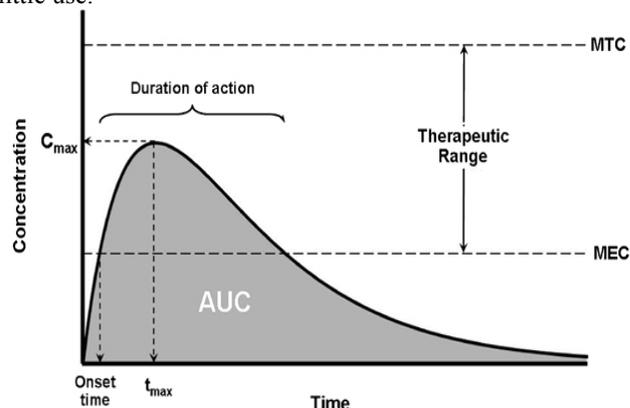


Figure 4: Typical plasma concentration-time curve showing time of the X-axis and the plasma concentration on the Y-axis.

Figure 4 is a typical example of a plasma concentration-time profile of a drug in a volunteer. It clearly distinguishes absorption and elimination phase. When the drug absorbed equals the drug eliminated, C_{max} is present. Before C_{max} is reached (before T_{max}) the absorption is higher than the elimination, after T_{max} the situation is reversed.

Frequently elimination is a so-called first order process, which means that per unit of time a percentage of the drug present in the blood disappears from it. So for example every hour 5% of the drug present in the blood disappears, which means that as the plasma concentration declines, the eliminated drug per time unit also declines.

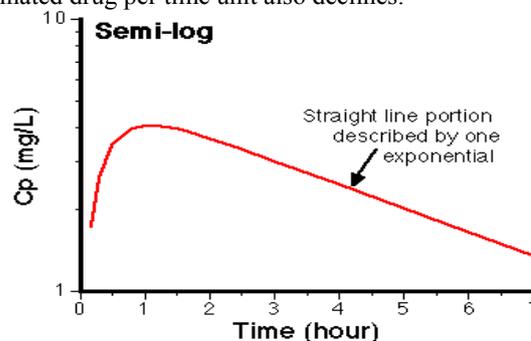


Figure 5: An ln-transformed plasma concentration-time curve, showing a 'linear' elimination phase (actually the elimination is as exponential function)

When elimination is a true first order process, a log transformation of the measured plasma concentrations will render a straight line during the elimination phase, which we shall use later to calculate the elimination half-life.

The calculation of Pharmacokinetic Parameters

Calculation of the AUC_{0-t}

$$AUC_{0-t} = \sum_{i=1}^t (C_i + C_{i-1}/2) (t_i - t_{i-1})$$

Equation 1 calculation of the area under the plasma concentration-time curve

The AUC is calculated by taking the average of two subsequent plasma concentrations (C_i and C_{i-1}) and multiplying that average by the time difference between the consecutive measuring points (t_i and t_{i-1}). All these outcomes are then summed to render the AUC from 0 to the last measuring point. This approach is called the linear trapezoidal approach. The measurement schedule (= sampling schedule) must be designed in such a way that the absorption of the drug is adequately charted, so minimum requirements for the length of the sampling scheme exists. Note that the contribution of widely separated measuring points to the total AUC can be strong, because t_i minus t_{i-1} is then quite large. Measurement errors made during this phase have a significant influence on the results of the study.

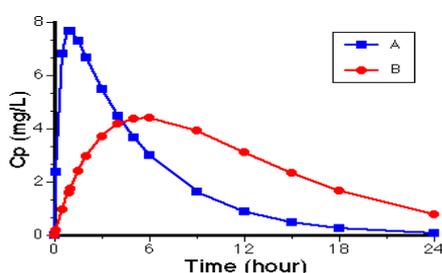


Figure 6: Comparison of two plasma concentration-time curves. The blue upper curve shows higher concentrations.

$$AUC_{0-t} = \sum_{i=1}^t [C_i - C_{i-1}] / \Delta t \ln(C_i / C_{i-1})$$

Equation 2 calculation of the area under the plasma concentration-time curve, using log-trapezoidal rule

Calculation of C_{max}

The calculation of C_{max} is to select the highest value. In order to have a true and accurate measurement of C_{max} , adequate number of sampling points should be placed at and around the anticipated C_{max} of the drug. The sampling schedule should be planned to avoid C_{max} being the first point of a concentration time curve.

The sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if AUC_{0-t} covers at least 80.00 % of AUC_{0-inf} . At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant (which is needed for a reliable estimate of AUC_{0-inf})⁽¹⁾.

Calculation of T_{max}

The calculation of T_{max} is the time point at which C_{max} occurs.

Calculation of K_{el} and $T_{1/2}$

The calculation of K_{el} is an essential part of any bioequivalence study. Above the AUC_{0-t} was calculated but there is a requirement that the absorption phase of the drug has to be adequately described. Generally this is the case when the AUC_{0-t} is > 0.8 , of the extrapolated AUC_{0-inf} . The latter parameter cannot be measured, of course, but is estimated and for that estimate the K_{el} is needed. When AUC_{0-t} is > 0.8 of AUC_{0-inf} it simply means that the sampling scheme was sufficiently long to be sure that the absorption

phase of the drug is indeed adequately described. (For drugs with long half-life this requirement is relaxed). As we discussed earlier, elimination is a first order process and a natural log (ln)-transformation makes it possible to draw a straight line through the elimination phase. The slope of the regression line is now equivalent to K_{el} or the elimination constant.

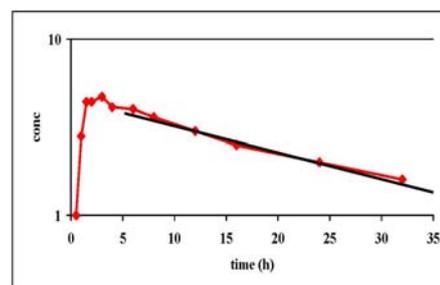


Figure 7: Ln-transformed plasma concentration-time curve showing a regression line through the elimination phase. The slope of the regression line represents the elimination constant.

The calculation of half-life is now rather simple. One simply divides 0.693 by the K_{el} to obtain the $T_{1/2}$. The relationship between K_{el} and $T_{1/2}$: $T_{1/2} = 0.693 / K_{el}$. The term 0.693 is derived from $\ln(0.5) = -0.693$ (ignoring signs): K_{el} describes the lowering of the Ln-transformed plasma concentration per unit time and so we have to Ln-transform 0.5 too to obtain the correct estimate of $T_{1/2}$.

$$T_{1/2} = 0.693 / K_{el}$$

Equation 3 Calculation of $T_{1/2}$

Calculation of the AUC_{0-inf}

The next step in process is to extend the plasma concentration-time profile to infinity to obtain the AUC_{0-inf} . The latter parameter is a total mass of drug present in the blood and also serves as a guide for adequate sampling.

To do so, the K_{el} is of course the most logical parameter, next to the last plasma concentration. As mentioned before, K_{el} describes the loss of drug per unit time (h). So division by of C_{last} (mg/l) results in a measure with the unit $mg/l \cdot h$, which is the unit for an AUC. The outcome of this calculation is the AUC from t_{last} to infinity (AUC_{t-inf}), so to obtain the AUC_{0-inf} one has to add AUC_{0-t} and AUC_{t-inf} .

$$AUC_{0-inf} = AUC_{0-t} + C_{last} / K_{el}$$

Equation 4 calculation of the AUC_{0-inf}

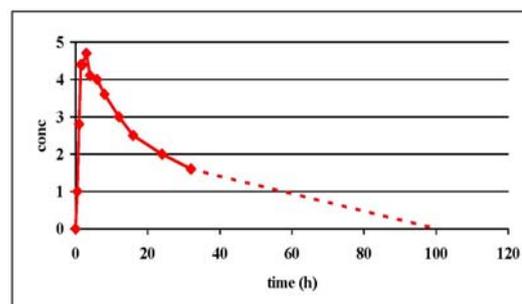


Figure 8: Calculation of the AUC_{t-inf}

Impact of truncated Area Under Curve (AUC) on bioequivalence of drugs with long half life

Preference of truncation

It is now understood that, to establish bioequivalence, the characterization of the rate (C_{max}) and extent (AUC) of test and reference formulation is required. In the case of deviant drugs like, those with long half-lives, the calculation of extent of absorption will be a challenge. For instance, molecules like Azithromycin, Mirtazepine and Anastrozole has long elimination half-lives (more than 24 hr). The establishment of extent of these formulations will be a difficult task, considering the sampling profile determination (where sample collection must exceed up to 10 days from dosing). This operational difficulty in collecting samples may lead to increase in number of dropouts in sample collection and the cost involved in sample collection will be high. In order to overcome this issue, the evaluation of partial AUC is recommended by the different regulatory bodies and very well accepted.

The truncated approach is beneficial to the sponsor as it is cost effective. For the Contract Research Organization (CRO), the conduction is relatively easier and the number of subjects completing the clinical phase is more in number thereby meeting the required statistical power. For the study participant, number of visits for pharmacokinetic (PK) sample donation and the total blood loss is minimized. In this point of view, the partial AUC was calculated to investigate the suitability of truncated AUC in the field of bioavailability and bioequivalence.

Perception of Different Regulatory Bodies about Truncating Area Under Curve (AUC)

The **U.S. Food and Drug Administration (FDA)** Guidance for Industry (U.S. Food and Drug Administration, 2003) entitled, "Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations" discusses about long half-life drugs and appropriate sampling times. The recommendation is stated, "For drugs that demonstrate low intra-subject variability in distribution and clearance, an AUC truncated at 72 hour ($AUC_{0-72\text{ hr}}$) can be used in place of AUC_{0-t} or $AUC_{0-\infty}$ (AUC time zero to infinity). For drugs demonstrating high intra-subject variability in distribution and clearance, AUC truncation warrants caution. In such cases, we also recommend that sponsors and/or applicants consult the appropriate review staff"⁽⁵⁾.

The **EMEA** has recommended having partial AUC with truncation at 72hour (AUC_{0-72}) as an alternative to AUC_{0-t} for comparison of extent of exposure for immediate release formulations (Gaudreault et al., 1998)⁽⁶⁾.

ANVISA (Resolution - RE n° 896, of May 29, 2003- Guide For Relative bioavailability/bioequivalence tests of drug products) has recommended that in the case of drugs presenting long elimination half life (over 24 hours), an alternative collection schedule may be used, of up to 72 hour, allowing the determination of the area under the fragmented curve (ASC_{0-72}), or a parallel study⁽⁷⁾.

Health Canada (Notice to industry- Bioequivalence Requirements for Long Half-life Drugs-2005) states that "For drugs which exhibit a terminal elimination half-life greater than 24 hour, bioequivalence standards in comparative bioavailability studies will be applied to AUC_{0-72} and for the purpose of bioequivalence assessment, it will not be necessary to sample for more than 72 hour post-dose, regardless of the half-life. Alternate designs such as parallel studies could be considered"⁽⁸⁾.

Monte Carlo simulations were generally consistent with the experimental data and showed that AUCs truncated at 72 hour performed will be compared to $AUC_{0-\infty}$ as measure of bioequivalence for drugs with long half-life⁽⁹⁾.

However, the recent literature contains several studies for drugs with long half-lives which have investigated the use of AUC_{0-72} as a surrogate for AUC_{0-t} and $AUC_{0-\infty}$. Work presented by Midha and colleagues found that for various drugs limiting the duration of sample collection did not increase the variation of AUC ratios (i.e., $AUC_{truncated}/AUC_{0-\infty}$). Endrenyi and Tothfalusi found that with intra and interindividual variation coefficients of variation (CV) for clearance (CL) up to 25%, the resulting variability of the estimated truncated AUC ratios was generally reduced, as the duration of sample collection in simulated trials was shortened from 4 down to 2 half-lives following drug administration. Their study concluded that the assessment of bioequivalence (BE) for long half-life drugs has undiminished validity when the duration of sample collection is shortened to, at most, 2 half-lives following drug administration. A further investigation into the use of truncated AUC for two-way crossover design experimental data, with median $AUC_{0-\infty}$ intra-subject CVs up to 34% and intersubject CVs up to 45% , concluded that there was discordance in BE conclusions based upon $AUC_{0-\infty}$ versus those with AUCs truncated at less than $1 * T_{max}$. However, for longer sampling times (i.e., those greater than $1 * T_{max}$) there was better agreement. In the same study using simulated data it was found that intra-subject CVs of truncated AUCs changed as a function of time, being higher for AUCs truncated after the first few hours, but then decreasing rapidly to reach a minimum when AUCs were truncated at 96 to 144 hour. An investigation using simulated data with half-lives from 40 to 172 hour and % CV for clearance of 20% concluded that the truncated approach for the estimation of the AUC for long half-life drugs in bioequivalence studies may be useful, but also increases the probability of accepting drugs as being bioequivalent when they are not.

The present study was undertaken to investigate more thoroughly the accuracy of recommendation of the FDA and other regulatory bodies to curb at 72nd hour sampling time for long half-life drugs when a two-way cross over study is used and to determine if there is a true "most informative time" to truncate AUC values in such BE studies. Our primary interest was to investigate the use in two-way cross over studies, since this design is often used to study drugs with very long half-lives.

MATERIALS AND METHODS

Drugs

Drug formulations were Azithromycin, Mirtazepine, Anastrozole, Letrozole, Clonazepam and Digoxin.

Subjects

Healthy, human, adult, male volunteers were enrolled in each crossover studies. All the subjects had fulfilled the inclusion criteria and the exclusion criteria. The sample size for each study was calculated based on reported intra-subject variability of the primary pharmacokinetics parameters available from the literatures or past experience on the molecules of CRO (Clinical Research Organization) considering $\alpha = 0.05$, the bioequivalence acceptable range of 80.00-125.00 % and a statistical power of at least 80.00 %. The volunteers were instructed to abstain from taking any drugs including over-the-counter (OTC) for 2 weeks prior to drug administration and during the study period. Studies were conducted as per the regulatory requirements and Declaration of Helsinki for biomedical research involving human subjects ICH – GCP and GLP guidelines. Study protocols were approved by independent ethics committee.

Study design

The studies were conducted with a two period, two treatment, two sequences, single dose, crossover and non truncated conventional approach.

Drug administration

In each study, following a ten-hour overnight fast, the assigned formulation was administered with about 240 mL of water in sitting posture by trained study personnels. Blood samples were collected up to 24 - 240 hours after dosing in each study and the samples were stored at below -20°C until centrifugation. After centrifugation process the separated samples were stored at a

temperature below -50°C until analyzed by validated and sensitive LC-MS/MS methods. Adequate sampling point and washout period was maintained so that the drug concentration in the biological fluid could be characterized accurately.

Assay procedure

The plasma samples were analyzed by validated Liquid Chromatography - Mass Spectrometry/Mass Spectrometry method. Analysis was done only on parent drugs.

Data analysis

Data from subjects who completed the study were included in pharmacokinetic and statistical analysis. The obtained plasma concentrations have been employed for pharmacokinetic analysis by using WinNonlin[®] software (version: 5.2.1 or higher) and statistical analysis by using SAS[®] statistical software (version: 9.1.3 or higher SAS Institute Inc, USA).

Maximum plasma concentration (C_{\max}), Area under plasma concentrations (AUC_{0-t} , AUC_{0-inf}) and confidence interval analysis for log-transformed C_{\max} , AUC_{0-t} , partial AUC at 48, 72 and 96 hr, AUC_{0-inf} were calculated by non-compartmental analysis using WinNonlin[®] software (version: 5.2.1 or higher).

Simulations

Simulations were done to investigate the relationship between known experimental variables and for determining truncated AUC values. Here the experimental data has been simulated for truncated AUCs at 48, 72 and 96 hr and confidence interval analysis for partial AUCs at 48, 72 and 96 hr were calculated by non-compartmental analysis using WinNonlin[®] software (version: 5.2.1 or higher) and the results are given in below table.

Table 1- Point estimates and 90% confidence interval approach and the analysis of intrasubject variability

Molecule	Fast/ Fed	Sample Size (N)	Total No of Sampling Points (h)	Point Estimates and Confidence interval						ISCV (Intrasubject variability)						
				C_{\max} (ng/mL)	AUC_{0-t} (ng ^h /mL)	AUC_{0-inf} (ng ^h /mL)	AUC_{0-48} (ng ^h /mL)	AUC_{0-72} (ng ^h /mL)	AUC_{0-96} (ng ^h /mL)	C_{\max} (ng/mL)	AUC_{0-t} (ng ^h /mL)	AUC_{0-inf} (ng ^h /mL)	AUC_{0-48} (ng ^h /mL)	AUC_{0-72} (ng ^h /mL)	AUC_{0-96} (ng ^h /mL)	T_{\max} (h)
Azithromycin oral suspension (200mg/5ml)	Fasting	76	22 (0-168)	96.40% (88.13- 105.44)	97.97% (92.32- 103.97)	100.11% (96.02- 104.37)	96.72% (91.83- 101.87)	97.38% (92.40- 102.62)	97.54% (92.48- 102.88)	34.05%	22.21%	14.97%	19.35%	19.56%	19.89%	2
Azithromycin oral suspension (200mg/5ml)	Fed	67	22 (0-168)	100.20% (89.28- 112.47)	99.83% (93.08- 107.08)	99.26% (91.45- 107.74)	98.79% (92.74- 105.23)	98.84% (92.74- 105.36)	98.92% (92.74- 105.52)	41.70%	24.67%	26.45%	22.16%	22.39%	22.67%	2
Letrozole tablets 2.5 mg	Fasting	13	23 (0-240)	107.40% (90.90- 126.89)	108.83% (95.58- 123.91)	109.39% (94.59- 126.49)	103.78% (96.15- 112.02)	104.62% (97.18- 112.63)	105.09% (97.76- 112.96)	23.93%	18.53%	20.78%	10.84%	10.46%	10.25%	1
Anastrozole tablets 1 mg	Fasting	12	25 (0-240)	101.39% (91.12- 112.81)	97.44% (90.26- 105.19)	96.35% (88.09- 105.38)	99.02% (94.26- 104.02)	97.81% (92.38- 103.55)	97.42% (91.37- 103.86)	14.50%	10.37%	12.16%	6.67%	7.72%	8.67%	2
Mirtazapine 15 mg film coated tablets	Fasting	28	25 (0-216)	111.01% (100.72- 122.35)	108.07% (99.39- 117.51)	104.99% (97.13- 113.48)	105.79% (98.74- 113.35)	104.67% (96.90- 113.07)	105.41% (97.50- 113.96)	21.58%	18.53%	17.18%	15.21%	17.05%	17.23%	2
Digoxin tablets, USP 0.25mg	Fed	22	19 (0-144)	120.07% (110.79- 130.12)	121.11% (101.85- 144.00)	115.27% (102.42- 129.72)	115.69% (107.42- 124.59)	116.35% (107.65- 125.75)	116.78% (107.35- 127.04)	15.48%	34.08%	22.92%	14.27%	14.96%	16.23%	1-3
Clonazepam orally disintegrating 1 mg tablets	Fasting	30	24 (0-120)	92.94% (85.62- 100.89)	100.54% (94.07- 107.46)	97.51% (94.57- 100.54)	96.93% (93.21- 100.80)	97.06% (93.43- 100.84)	97.13% (93.50% 100.89)	18.81%	15.20%	6.96%	8.90%	8.68%	8.66%	1-4
Clonazepam orally disintegrating 1 mg tablets	fed	30	24 (0-120)	95.08 % (91.00- 99.34)	100.77 % (97.68- 103.95)	102.01% (98.04- 106.14)	98.81% (95.72- 102.00)	99.8 % (96.70- 103.00)	100.12 % (97.09- 103.24)	9.99%	7.07%	9.03%	7.22%	7.18%	6.98%	1-4

RESULTS

To investigate for the change in study outcome with the truncation of AUC at different time points, point estimate, 90% confidence interval and intra subject variability were studied. The out come of the analysis are summarized in table 1. As shown in table 1, point estimate, the 90 % confidence intervals and intra-subject variability for log transformed AUC_{0-t} , AUC_{0-inf} and C_{max} were calculated and presented.

In this research, we investigated the effect of using truncated area under the curve method on the bioequivalence of different drugs in healthy volunteers. The experimental data of AUC_{0-t} was simulated for the calculation of AUCs truncated at 48, 72 and 96 hour (AUC_{0-48} , AUC_{0-72} and AUC_{0-96}).

The experimental data showed that conclusion concerning bioequivalence were identical between AUCs truncated at 72 hour and AUC_{0-t} , AUC_{0-inf} in all the studies. The results of this simulation with the experimental data showed that AUC truncated at 72 hour performed well when compared to AUC_{0-t} and AUC_{0-inf} as a measure of extent of bioequivalence for drugs with long half-lives.

It has been observed in all the studies that AUC truncated at 72 hour (AUC_{0-72}) has shown the better point estimates and 90% confidence interval. There was a marginal increase in the point estimate only in Anastrozole study and it was found to be similar as the degree of change in confidence interval was insignificant. Irrespective of all the studies, it was observed that AUC_{0-72} has provided the better intra-subject variability than AUC_{0-t} and AUC_{0-inf} .

With regard to AUC_{0-48} and AUC_{0-96} , point estimate, the 90 % confidence intervals and intra-subject variability have been analysed and the resulted are presented in table 1 and found to be almost similar to AUC_{0-72} .

DISCUSSION AND CONCLUSION

The common measures used in a bioequivalence study are area under the curve (AUC) and the maximum plasma concentration (C_{max}). Estimation of AUC requires frequent blood samples. For long half-life drugs, sampling for long periods of time may become cumbersome. To resolve this issues truncated AUC method was recommended by regulatory authorities in bioequivalence studies for long half-life drugs. The suggested length of time for the truncated AUC is 72 hour post dose and the study design can be either two-way cross over design or parallel design. Many studies have been conducted to show that truncated AUC till 72 hour is a suitable approach. Our results are in agreement with the conclusions drawn by various authors and also support the regulatory recommendation to truncate the study at 72hr post dose for the drugs having long half-life.

Based on the results obtained, it was concluded that the assessment of bioequivalence for long half-life drugs would not be adversely affected by limiting the duration of an investigation and consequently, by using truncated AUCs.

For an oral immediate release product with a long elimination half-life drug (approximately >24 hrs), applicants can conduct a single-dose, crossover study, provided an adequate

washout period is used. If the crossover study is problematic, BE applicants can use a BE study with a parallel design. For either a crossover or parallel study, sample collection time should be adequate to ensure completion of gastrointestinal transit of the drug product and absorption of the drug substance (which usually occurs within approximately 2 to 3 days). C_{max} and a suitable truncated AUC can be used to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hour (AUC_{0-72hr}) can be used in place of AUC_{0-t} or AUC_{0-inf} . For drugs demonstrating high intrasubject variability in distribution and/or clearance, AUC truncation should not be used.

In conclusion, our results indicate that it would be reasonable to limit the PK sample collection period to 72 hour in BE studies for the oral formulations of drugs having long half-lives of elimination. Furthermore, the intra-subject variability for AUC derived from the truncated approach (AUC_{0-72}) is reliable and sensitive.

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