Evaluation of Performance of the Truncated Area Under Curve (AUC) as a Primary Pharmacokinetic Parameter in Bioequivalence Studies

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Abstract

Background and Objective: Prolonged pharmacokinetic sampling is a challenge for successful conduction of the bioequivalence studies for drugs having long elimination half-lives. The regulatory authorities have recommended an alternative to consider the partial AUC (AUC0-t) for studying bioequivalence. However, the results obtained from such truncated approach are not consistent and needs further exploration. We have investigated the suitability of truncated AUC in the field of bioequivalence.

Methods: The bioequivalence studies conducted with conventional approach for Bicalutamide, Topiramate and Amitriptyline having long elimination half-lives were investigated. The pharmacokinetic data obtained from these studies was truncated at 72hrs and 2 half-lives post dose. The 90% confidence intervals constructed for the ratios of means of log-transformed partial AUC (at 72hrs and 2 half-lives post dose) were compared individually with those of the total AUC. The intra-subject variability obtained for partial AUC at 72hrs and 2 half-lives post dose was compared individually for percentage change from that of the total AUC.

Results: No change in the study outcome irrespective of the point of truncation of AUC was observed. The 90% confidence intervals constructed for the ratio of means of log-transformed partial AUC (at 72hrs and 2 half-lives post dose) were well within the acceptable bioequivalence criteria of 0.8-1.25. The intra-subject variability for AUC was not influenced irrespective of the point of truncation of AUC.

Conclusion: Limiting the pharmacokinetic sample collection period to 72 hours in bioequivalence studies for the drugs having long elimination half-lives is equally accurate and sensitive alternative to the conventional approach.

Keywords: Bioequivalence; Truncated AUC; Long half-life; Intra-subject variability

Introduction

In recent years, generic drug products, which are those manufactured by firms other than the innovator, have become very popular. Bioequivalence (BE) studies are the commonly accepted method to demonstrate therapeutic equivalence between two medicinal products. Savings in time and cost are substantial when using bioequivalence as an established surrogate marker of therapeutic equivalence. For this reason, the design, performance and evaluation of bioequivalence studies have received major attention from academia, the pharmaceutical industry and health authorities.

Primarily, the rate and extent of absorption are studied in order to assess the bioequivalence of the generic product to the innovator product. The rate and extent of the absorption of the drug are primarily measured by plotting the plasma concentration-time profile. The area under curve (AUC) and maximum concentration (Cmax) for the test and the reference product are compared. It becomes essential to have an accurate measurement of AUC and Cmax before the results are concluded. In order to have a true and accurate measurement of Cmax, adequate number of sampling points should be placed at and around the anticipated Cmax of the drug. The sampling schedule should be planned to avoid Cmax 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 AUC0-t covers at least 80% of AUC0-∞ (The Investigation Of Bioavailability And Bioequivalence, 2001; Gaudreault et al., 1998). 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 AUC0-∞). It is expected that for the drugs following first order kinetics, three elimination half-lives are required to ensure that more than 80% of the drug is excreted from the body thereby assuring the AUC0-t / AUC0-∞ ratio of 80% or more.

The drug having half-life of more than 24hrs is typically called as a drug with long half-life (Endrenyi and Tothfalusi, 1997). It has been experienced that if the sampling profile is extended beyond 100hrs post dose, the chances of missing samples due to non-reporting of the participant to the study centre are increased. Moreover, the maintenance of the compliance to the protocol specified controlled conditions become difficult with the increased length of the sampling profile. The gastrointestinal transit time for the drug should be completed to ensure the complete absorption of the drug substance from the formulation. Usually, it is 24hrs or at the most 2-3 days (The Investigation Of Bioavailability And Bioequivalence, 2001). For all practical purposes, the drug absorption is over within 72hrs post....

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dose. As the absorption phase of the drug is more sensitive and decisive part of the plasma concentration versus time profile, it is expected that the formulation differences should become generally detectable well before the drug is completely eliminated. The different regulatory bodies involved in the approval of bioequivalence studies have recommended truncating the plasma sampling profile at 72hrs post dose for the drugs having long half-lives for both, cross over as well as parallel study designs. The EMEA has recommended having partial AUC with truncation at 72hrs (AUC_{truncated}) as an alternative to AUC_{full} for comparison of extent of exposure for immediate release formulations (Gaudreault et al., 1998). The USFDA has recommended AUC truncation at 72 hours (AUC_{truncated}) for drugs having long half-life and demonstrating low intra-subject variability (ISV) in distribution. For drugs with long half-lives demonstrating high intra-subject variability in distribution and clearance, AUC truncation is to be handled cautiously (The Investigation Of Bioavailability And Bioequivalence, 2001).

The truncated approach is beneficial to the sponsor as it is cost effective. For the Contract Research Organization (CRO), the conduction is relatively easy 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 the clinical phase is more in number thereby meeting the required conduction is relatively easy and the number of subjects completing the study. The EMEA has recommended truncating the plasma sampling profile at 72hrs post dose for suitable drugs, apprehension still exists in CRO given by the regulatory bodies for the truncation of AUC at 72hrs onwards. 

The primary objective of the study is to investigate the robustness of the partial-area method and find appropriate time point for partial AUC calculation by truncating the PK data at various time points. 

The secondary objective is to investigate the change in intra-subject variability for AUC after truncating the PK data at various time points.

Materials and Methods

Drugs

The criteria for drug selection were as below.

1) Elimination half-life of more than 24hrs.

2) Low to moderate intra-subject variability for the primary pharmacokinetic parameters (< 30%).

3) Immediate release formulation.

The representative drugs were Bicalutamide, Topiramate and Amitriptyline having half-lives of approximately 150, 50, and 33 hrs respectively.

Subjects and study designs

The selected bioequivalence studies were conducted at Accutest Research Laboratories Limited, Navi Mumbai, India and had a study population of 24-48 healthy, normal male and female subjects with age between 18-55 years. These studies were conducted with a two period, two treatment, two sequence, single dose, crossover, non-truncated conventional approach. For sample size calculation, SAS, version 9.2 was used for all the studies. The sample size for each study was calculated based on the reported intra-subject variability of the primary pharmacokinetics parameters, considering alpha = 0.05, the bioequivalence acceptable range of 0.8-1.25 and a statistical power of at least 80%. For Amitriptyline, the review report of abbreviated new drug application number 40-218 was referred. As per this report, the highest intra-subject variability of 21% observed for C_{max} demanded the sample size of 20 subjects. Considering the likely dropouts and withdrawals, a total of 28 subjects were enrolled in the study. For Topiramate, the highest intra-subject variability of 23% observed for C_{max} demanded the sample size of 24 subjects. Considering the likely dropouts and withdrawals, a total of 28 subjects were enrolled in the study. This intra-subject variability of 23% for C_{max} was obtained from the in house data of Accutest Research Laboratories Limited, Navi Mumbai, India. As the published data on intra-subject variability for pharmacokinetic parameters for Bicalutamide was scanty at the time of designing the protocol for its study, the sample size was calculated considering the pharmacokinetics of Casodex (the innovator product) and with certain assumptions that there would not be any interaction between formulations and periods, the observations would be log normally distributed and the variances of test and reference parameters would be same. A sample size of 46 subjects was considered as adequate for this study.

All the studies were performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices (GCP). The study protocols were approved by Drushti Independent Ethics Committee, Navi Mumbai, Maharashtra, India.

All the subjects had fulfilled the standard demographic parameters and were devoid of any drug intake 14 days prior to the dosing days. Subjects meeting the inclusion criteria and none of the exclusion criteria had been enrolled in the respective studies. All the studies were conducted in fasting condition. Pre dose 10hrs fasting compliance was maintained prior to dosing in both the periods for all the studies. All the selected studies had adequate coverage of blood sampling profile as evident from the AUC_{0-t}/AUC_{0-inf} ratio of more than 80%. The plasma samples were stored at appropriate storage conditions prior to the bioanalysis. The plasma samples of subjects were analyzed by validated LC-MS/MS method for all the three studies. The linearity ranges for all the analytes were enough to quantify the expected concentration range of drugs from subjects plasma with proposed dose of the study drugs. The details about the bioanalytical methods are provided in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Extraction method</th>
<th>Linearity range ng/mL</th>
<th>Internal standard</th>
<th>Plasma volume for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicalutamide</td>
<td>Solid phase</td>
<td>10.00-1500.00</td>
<td>Nimesulide</td>
<td>0.2 mL</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Solid phase</td>
<td>50.040-3002.394</td>
<td>Phenytoin</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Solid phase</td>
<td>0.814-81.401</td>
<td>Imipramine</td>
<td>0.2 mL</td>
</tr>
</tbody>
</table>

Table 1: Details about bioanalytical methods for study drugs.
The blood samples beyond 48hrs post dose were collected on ambulatory basis. The data of subjects completing both the periods of the study were considered for bioanalysis and statistical analysis. The time deviations from the scheduled time of blood sample collection were considered during the statistical analysis. The SAS version 9.1 was used for the statistical analysis. The 90% parametric confidence intervals were constructed for the ratios of the means of Log-transformed pharmacokinetic parameters \(C_{\text{max}}\), \(\text{AUC}_{0-t}\) and \(\text{AUC}_{0-\infty}\) for the test and reference products. All the studies concluded the presence of the bioequivalence of test product to the reference product as the 90% confidence intervals constructed for the ratios of the means of Log-transformed pharmacokinetic parameters \(C_{\text{max}}\), \(\text{AUC}_{0-t}\) and \(\text{AUC}_{0-\infty}\) fell within the acceptable range of 0.8-1.25.

**Data analysis**

For pharmacokinetic and statistical analysis, SAS version 9.2 and Winnonlin version 5.0.1 were used. The \(\text{AUC}_{0-t}\) and intra-subject variability (ISV) for AUC obtained with a non-truncated approach were considered as baseline values for comparison. For the research purpose, we truncated the AUC at 72hrs, and at 2 half-lives post dose and compared it with the baseline value. 90% confidence intervals for the ratio of the population means for log-transformed pharmacokinetic parameters \(\text{AUC}_{0-t}\) were performed at each truncation level and compared with the baseline value. The intra-subject variability for \(\text{AUC}_{0-t}\) was investigated at each level of truncation and compared with the baseline value.

The occurrence of \(C_{\text{max}}\) was within few hours of dosing due to the immediate release nature of all the study formulations. It was not a parameter to get affected with truncations done at different time points as mentioned earlier. In studies with a sampling period of 72hrs and where the concentration at 72hrs is quantifiable, \(\text{AUC}_{0-t}\) and residual area do not need to be reported. Hence the parameters, \(C_{\text{max}}\) and \(\text{AUC}_{0-t}\) were not considered for the data analysis.

**Study results**

The studies for Bicalutamide, Topiramate and Amitriptyline were conducted on 46, 28, and 24 subjects respectively. The studies were successfully completed by 39 (Bicalutamide), 26 (Topiramate) and 24 (Amitriptyline) subjects. The 90% confidence intervals and the mean ratio (Test/Reference) corresponding to the partial AUC at 72hrs and 2 half-lives post dose and total AUC for all the drugs are presented in Table 2. The mean plasma concentration vs. time profile for Bicalutamide, Topiramate and Amitriptyline for untransformed data is presented in Figure 1, Figure 2 and Figure 3 respectively. The intra-subject variability corresponding to the partial AUC at 72hrs and 2 half-lives post dose is Table 3 along with percentage change from baseline.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Partial AUC(_{0-72})</th>
<th>Partial AUC(_{0-2\times t/2})</th>
<th>Baseline value (total AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicalutamide</td>
<td>(C_{\text{max}})</td>
<td>85.99</td>
<td>99.57</td>
<td>86.00</td>
</tr>
<tr>
<td>N = 39</td>
<td>(C_{\text{max}})</td>
<td>96.33</td>
<td>96.13</td>
<td>91.08</td>
</tr>
<tr>
<td></td>
<td>Ratio[%\text{Ref}]</td>
<td>92.48</td>
<td>95.37</td>
<td></td>
</tr>
<tr>
<td>Topiramate</td>
<td>(C_{\text{max}})</td>
<td>99.11</td>
<td>103.86</td>
<td>102.68</td>
</tr>
<tr>
<td>N = 26</td>
<td>(C_{\text{max}})</td>
<td>103.42</td>
<td>100.9</td>
<td>100.43</td>
</tr>
<tr>
<td></td>
<td>Ratio[%\text{Ref}]</td>
<td>98.45</td>
<td>95.79</td>
<td></td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>(C_{\text{max}})</td>
<td>95.14</td>
<td>109.59</td>
<td>110.45</td>
</tr>
<tr>
<td>N = 24</td>
<td>(C_{\text{max}})</td>
<td>102.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not applicable as \(T_{0.2xe} < 2\) is less than 72 hrs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Partial AUC(_{0-72})</th>
<th>Partial AUC(_{0-2\times t/2})</th>
<th>Baseline value (total AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicalutamide</td>
<td>Intra-subject variability</td>
<td>19.50</td>
<td>17.39</td>
<td>17.39</td>
</tr>
<tr>
<td>N = 39</td>
<td>Percent change from baseline</td>
<td>12.13</td>
<td>05.68</td>
<td>NA</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Intra-subject variability</td>
<td>04.94</td>
<td>02.94</td>
<td>04.66</td>
</tr>
<tr>
<td>N = 26</td>
<td>Percent change from baseline</td>
<td>06.00</td>
<td>09.40</td>
<td>NA</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Intra-subject variability</td>
<td>14.33</td>
<td>06.00</td>
<td>14.43</td>
</tr>
<tr>
<td>N = 24</td>
<td>Percent change from baseline</td>
<td>0.69</td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

* Not applicable as \(T_{0.2xe} < 2\) is less than 72 hrs

**Table 2:** Study results after AUC truncation at different time points.

**Table 3:** Intra-subject variability for AUC with percent change from baseline after truncation of AUC at different time points.

The mean plasma concentration vs. time profile for Bicalutamide.

**Figure 1:** Mean plasma concentration vs. time profile for Bicalutamide.

The mean plasma concentration vs. time profile for Topiramate.

**Figure 2:** Mean plasma concentration vs. time profile for Topiramate.
Discussion and Conclusion

To investigate for the change in study outcome with the truncation of AUC at different time points, the lower and upper limits of the 90% confidence intervals were studied. With regard to AUC, no change in the study outcome is observed with truncation of AUC done at 72hrs, or 2 half-lives post dose. Both, the lower and upper limits of 90% confidence interval fell within the acceptable BE range of 0.8-1.25. Moreover, the degree of change in the 90% confidence intervals for AUC was practically insignificant within each product (Table 2).

Our results are in agreement with the conclusions drawn by various authors and also support the regulatory recommendation to truncate the study at 72hrs post dose for the drugs having long half-life and low intra-subject variability (Guidance for Industry, 2003; The Investigation Of Bioavailability And Bioequivalence 2001; Almeida et al., 2007; Erkent and Koytchev, 2008; Gaudreault et al., 1998; Endrenyi and Töthfalusi, 1997; Najib et al., 2009; Mahmood, 2004).

These results are not in accordance with those concluded by Naji M. Najib et al and Mahmood Iftekhar (Bioequivalence Requirements for Long Half-life Drugs Health, 2005; Marzo et al., 1999). Naji M. Najib et al observed that when the studies were truncated at median $T_{max}$ of the reference product, the 90% confidence intervals for log transformed data fell outside the acceptable limit of 0.80-1.25 for AUC as well as AUC0-72. In our opinion, the point of truncation in all the studies investigated by Naji M. Najib et al was the cause for difference in study results. The median $T_{max}$ for all the drugs investigated by Naji M. Najib et al was within few hours after dosing. Thus, compared to the truncation at 72hr post dose, the truncation at median $T_{max}$ was too early. The sampling points near the $T_{max}$ are very sensitive in determining the $C_{max}$ and AUC. Few subjects might have occurrence of the actual $C_{max}$ after the median $T_{max}$. In such subjects, one can not measure the true $C_{max}$ and subsequently true AUC. This could have been resulted in more failure rate when truncation of the studies was done at median $T_{max}$. Moreover, all the drugs investigated by Naji M. Najib et al were not true representatives of the category 'long half-life drugs'. In the study conducted by Mahmood Iftekhar, a computer simulation analysis was used for demonstrating the suitability of truncated AUC for failed bioequivalence study. He concluded that truncated approach might increase the probability of accepting drugs as being bioequivalent when they are not (Bioequivalence Requirements for Long Half-life Drugs Health 2005). Since only two hypothetical drugs were considered, a firm conclusion could not be put forward. Theoretically, it can happen only for selective drugs having significant recycling or reabsorption of the drug during the elimination phase.

The occurrence of $T_{max}$ was within few hours post dose, and there was no evidence of recycling for all the drugs investigated in our study. Hence, the elimination phase beyond 72hrs of dosing was practically less sensitive to differentiate between the test and reference treatments. Additionally, all these drugs had less pharmacokinetic variability. No change in the study outcome when conducted with conventional or truncated approach can be attributed to these factors.

The ISV is one of the major determinants for the calculation of sample size (number of study participants) for a crossover BE study. From Table 3, it is observed that the ISV for AUC did not change significantly beyond 72hrs PK sampling. Our result is contradictory to the one concluded by Naji M. Najib et al. In the opinion of Naji M. Najib et al, the intra-subject variability for AUC and $C_{max}$ was usually higher when truncated approach was followed thus rendering the inadequate sample size calculation. However, the too early truncation at median $T_{max}$ resulting in failure to capture the true $C_{max}$ and AUC in these studies could be probable factor for such conclusion.

From our results, it is concluded that the ISV for AUC obtained at 72hrs post dose can work as a reliable and sensitive tool for calculating sample size for crossover BE studies. The similar conclusion on inter-subject variability cannot be put forward as all the referred studies were conducted with a crossover design. However, it is expected that the same conclusion can be applied to the inter-subject variability obtained from the studies conducted with a parallel approach; at least for the drugs having low PK variability and showing absence of recycling phenomenon.

In conclusion, our results indicate that it would be reasonable to limit the PK sample collection period to 72 hours in BE studies for the oral formulations of drugs having long half-lives of elimination and low ISV. Furthermore, the intra-subject variability for AUC derived from the truncated approach (partial AUC0-72) can be a reliable and sensitive tool for calculating sample size for BE studies.

References