Pharmacodynamics Analysis using AUC as endpoint in Single Ascending Dose Study

Xusiqiao Cai, Sanofi

ABSTRACT

In pharmacodynamic analysis, the changes from baseline as typical endpoint is used to analyze, while the area under the curve (AUC) value is less commonly used. This paper introduces the analysis method of AUC as an endpoint in a Single Ascending Dose (SAD) study. The AUC values are calculated using the trapezoidal rule, and an alternative method for handling negative PD values using the trapezoidal rule is also provided to ensure the accuracy of the AUC estimation.

The AUC will be log-transformed for the descriptive statistics, ANCOVA analysis and visually explore. The ANCOVA analysis is conducted to explore the relationship between the endpoint and dose levels which allows for an assessment of the impact of dose levels on the PD response while controlling for potential confounding factors. To visually explore PD parameters, plots are also generated to illustrate the relationship between PD parameters and dose levels.

KEYWORDS

PHRMACODYNAMICS, SINGLE ASCENDING DOSE STUDY, TRAPEZOIAL RULE, AUC, ANCOVA

INTRODUCTION

Single Ascending Dose (SAD) study is a type of clinical study design. It aims to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) profile of a new investigation drug. In a SAD study, a single dose of the drug is administered to a small group of healthy volunteers or patients, usually starting with a low dose and gradually increasing it in subsequent cohorts. This study design allows for the assessment of the dose-dependent relationship between drug exposure and various endpoints.

PD analysis plays a significant role in SAD studies by evaluating the drug's effects on the body, such as changes in biomarkers, physiological responses, or clinical outcomes. While the AUC is a commonly employed endpoint in pharmacokinetic (PK) analysis, its application in PD analysis is less common. However, in SAD studies with a relatively short duration, alternative endpoint such as change from baseline may not be adequately capture the drug’s characteristics across different dose levels. In such cases, utilizing AUC as an endpoint offers a better statistical analysis approach for assessing the differences between dose groups, aiding in dose selection and understanding changes in drug effects.

AUC CALCULATION

The AUC value is typically calculated using the trapezoidal rule, which divides the AUC curve into trapezoids and estimates the overall AUC by summing the areas of each trapezoid. This method provides an approximation of the AUC, especially when the time profile of the drug is not a smooth curve. The trapezoidal rule formula is applied to calculate the sub-AUC values in each interval, and the total AUC is obtained by summing these sub-AUC values. For each trapezoidal area that is divided, the formula is as follows:

$$AUC_{(t_i - t_{i-1})} = (t_i - t_{i-1}) \times \frac{f(t_i) + f(t_{i-1})}{2}$$

By adding up each sub-AUC value in the interval we get the AUC value of the entire interval, for example, the AUC value from baseline to T8 (Figure 1):

$$AUC_{T8-Baseline} = \sum_{t=T1}^{T8} AUC(t_i - t_{i-1})$$

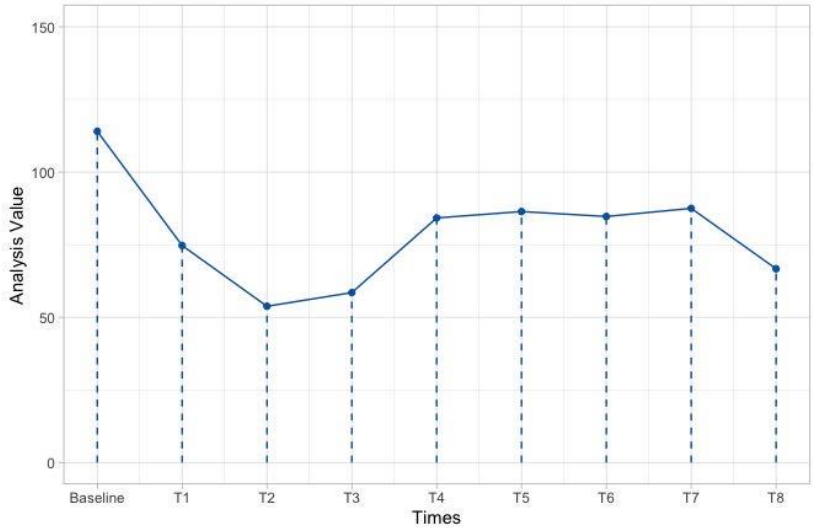

Figure 1. Time profile plot for PD value

SAS CODE 1

```
data auc_calc;
  set input_data;
  by id tpt;
    Xtime = atptn;
    LagTime = LAG(atptn);
    LagValue = LAG(aval);

    if first.id then do;
        LagTime = 0;
        Xtime=0;
        LagValue = 0;
        SumTrapezoid=0;
    end;

    Trapezoid = (Xtime-LagTime)*(aval+LagValue)/2;
    SumTrapezoid + Trapezoid;

    if last.id then output;
run;
```

The code uses the LAG function to obtain the previous value (Detail in Table 1), Xtime=t_i, LagTime=t_{i-1}, aval= $f(t_i)$, LagValue= $f(t_{i-1})$. And Trapezoid is the area of each small trapezoid, SumTrapezoid is the result of accumulating the area of each small trapezoid, and in this example,

the AUC value from baseline to T8 is 3873990. It is important to note that the unit of time (e.g., hours) should be standardized according to the study-specific requirements and adjusted accordingly in the program.

id	atpt	atptn	aval	Xtime	LagTime	LagValue	SumTrapezoid	Trapezoid
001	Baseline	-120	148	0	0	0	0	0
001	T1	60	123	60	-120	148	24390	24390
001	T2	240	117	240	60	123	45990	21600
001	T3	480	121	480	240	117	74550	28560
001	T4	1440	128	1440	480	121	194070	119520
001	T5	2880	137	2880	1440	128	384870	190800
001	T6	10080	135	10080	2880	137	1364070	979200
001	T7	20160	116	20160	10080	135	2629110	1265040
001	T8	30240	131	30240	20160	116	3873990	1244880

Figure 2. AUC calculation detail

HANDLING NEGATIVE PD VALUE

In PD analysis, it is still possible to encounter negative PD values, such as the AUC value of change from baseline value, or some special PD variables, i.e, Complement Alternative Pathway with unit % Activity. In this case, applying the trapezoidal rule directly to calculate the AUC in such cases can lead to errors in the calculated area. Even if the absolute value of the analysis value is used to calculate the sub-intervals AUC, the value corresponding to a triangle (i.e, the red triangle in Fig 3.), but not the right area. Several approaches can be used to address negative PD values:

1. Imputation value: For some specific PD parameters, the medical team will consider relevant medical knowledge to fill in the negative PD value. If the PD parameter has a Lower Limit of Quantification (LLOQ), the imputation value can be set as LLOQ/2, otherwise the imputation can be set as very small value, i.e, 0.001.
2. If the sign of two adjacent observations is opposite, diving the whole area into two right triangles (Fig 4.). For this reason, this sub-AUC calculation is the sum of two triangle areas. For the specific triangle division and the corresponding calculation, there is a detailed calculation method and code in this article of *General AUC calculated based on the trapezoidal rule* (ye, 2016) [1].

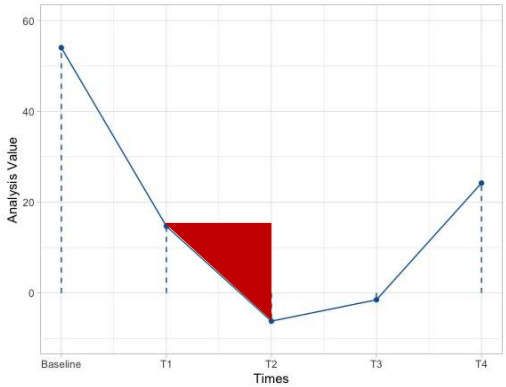

Figure 3. Directly Use trapezoidal rule

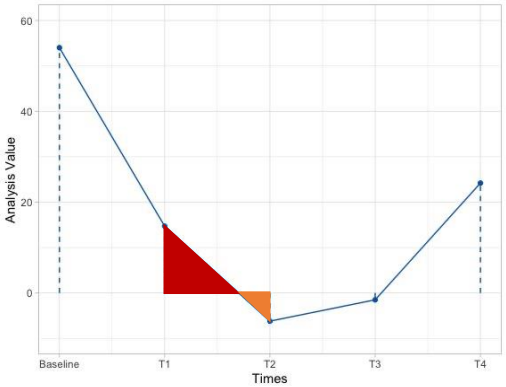

Figure 4. Modified trapezoidal rule

ANALYSIS OF PHARMACODYNAMICS

DESCRIPTIVE STATISTICS

Descriptive statistics will be conducted on the log-transformed AUC values. The following statistics

will be reported for each dose level: number of participants (n), geometric standard deviation (Geo-SD), geometric coefficient of variation (Geo-CV(%)), minimum, maximum. Additionally, individual spaghetti plots will be created for each PD and treatment group to explore the data.

ANCOVA ANALYSIS

ANCOVA (Analysis of Covariance) is a statistical analysis technique that combines the principle of analysis of variance (ANOVA) and regression analysis. It is used to examine the relationship between a dependent variable and one or more independent variables, while controlling for the effects of covariates. The ANCOVA model is particularly useful in situations where there are covariates that may influence the relationship between the independent and dependent variables. For example, in the PD analysis of the SAD study, the baseline PD value of participants may vary cross different treatment groups. By using ANCOVA, researchers can adjust for these baseline differences and better evaluate the true treatment effects.

Refer to the **SAS CODE 2**, the ANCOVA model will be employed to estimate the means and calculate 2-sided 90% confidence intervals (CIs) for the log-transformed AUC values of PD variables. The log-transformed AUC value of PD variables is set to response variable, the log-transformed baseline PD value (pre-administration of IMP) is set to covariate and treatment group is set to factor. Also in order to derive the results of estimated relative difference vs Placebo, the *pdiff* option is set to control("Placebo"), and this option can be set to any study interested group.

SAS CODE 2

```
ods listing;
ods output estimates=estimates
            lsmeans=lsm
            lsmeancl=lsmeancl
            lsmeandiffcl=lsmeandiffcl
            diffs=diff;
proc GLM data=dat;
   class trt;
   model lauc= lbsl trt;
     lsmeans trt/ cl alpha=0.1
                  pdiff=control("Placebo")  stderr  adjust=dunnett;
run;
ods output close;
quit;
```

The main focus will be on the results obtained from the LSMEANCL and LSMEANDIFFCL datasets.

LSMEANCL dataset provides the statistical analysis results related to the Least Square Means Confidence Limits. It includes the Least Squares Means (LSMeans) which represents the results of the estimated mean of log-transformed AUC value for different treatment groups, while controlling for log-transformed baseline value. In addition, confidence limits (CL) is also provided to offer insights into the uncertainty associated with estimates.

	Effect	dependent	trt	LowerCL	LSMean	UpperCL
1	TRT	AVAL	PLACEBO	14.888571	14.968890	15.049209
2	TRT	AVAL	SAR DOSE1	14.614309	14.745034	14.875759
3	TRT	AVAL	SAR DOSE2	14.147636	14.269613	14.391590
4	TRT	AVAL	SAR DOSE3	13.577251	13.701011	13.824771
5	TRT	AVAL	SAR DOSE4	12.668190	12.839868	13.011546

Figure 5 Result of ANCOVA LSMEANCL Datasets

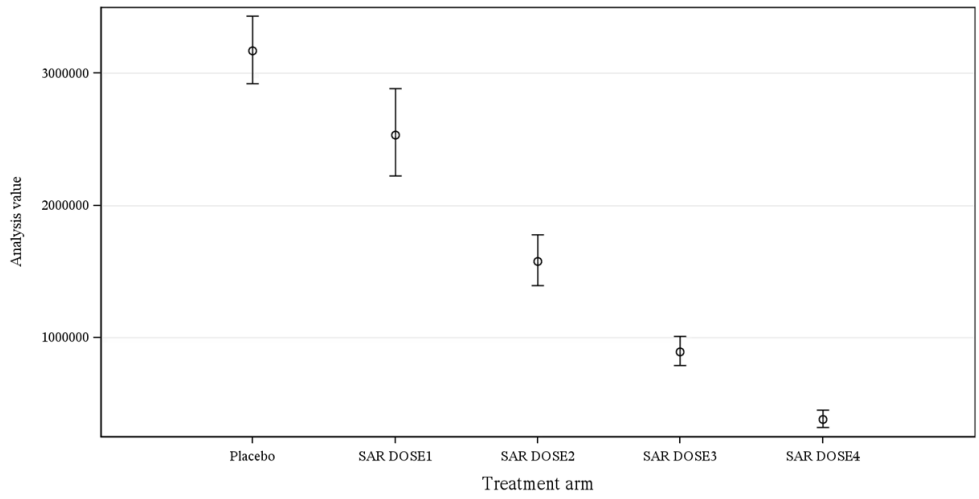

Figure 6. Estimated Geo-Mean and 2-side 90% CI for treatment arms/doses

LSMEANDIFFCL provides the important results related to the comparison of LSMeans difference between different treatment groups. In the example, Placebo group is set to the reference group, so the LSMeans is between different dose group and placebo group. LSMeans differences allow for the comparison of the effects of the independent variable(s) on the dependent variable across different groups. Besides, by analyzing the result of estimated relative difference vs Placebo, one can assess the significance of mean differences. The confidence intervals can be examined to determine if the differences are statistically significant. If the confidence interval does not include zero, it suggests a significant difference between the compared groups. From Figure 6 (Estimated Geo-Mean and 2-side 90% CI for treatment arms/doses), the AUC of PD parameter decreases with the increase of drug concentration. Also the LSMeans difference between different treatment groups (Figure 7) shows the same results, the higher the concentration of the drug, the greater difference with Placebo group.

	Effect	Dependent	i	j	LowerCL	Difference	UpperCL	_trt	trt
1	trt	AVAL	3	1	-0.438813	-0.223856	-0.008899	Placebo	SAR DOSE1
2	trt	AVAL	4	1	-0.900284	-0.699277	-0.498270	Placebo	SAR DOSE2
3	trt	AVAL	5	1	-1.465554	-1.267879	-1.070204	Placebo	SAR DOSE3
4	trt	AVAL	2	1	-2.385767	-2.129022	-1.872277	Placebo	SAR DOSE4

Figure 7 Result of ANCOVA LSMEANDIFFCL Datasets

Note that the LSMEANCL and LSMEANDIFFCL datasets provide results in the log-transformed scale. To present the results, the estimated means and 2-sided 90% confidence intervals will be

transformed back to the original scale by exponentiating the estimations. The estimated relative differences vs Placebo and 2-sided 90% Cis will be transformed back to the original scale and presented as percentage as following:

[exp(Estimated difference vs Placebo in the log-scale)-1]*100

This article provides a way to present the results. Model parameters are to be presented in tabular showing the estimates, and confidence interval (Table 1).

Table1. Model results

	Treatment	Point Estimation	90% CI
Estimated Geo-Mean of $AUC_{D1\text{-}D22}$ (90% CI) (AU)	Placebo	xxx	(xxx , xxx)
	SAR Dose1	xxx	(xxx , xxx)
	SAR Dose2	xxx	(xxx , xxx)
	SAR Dose3	xxx	(xxx , xxx)
	SAR Dose4	xxx	(xxx , xxx)
Relative difference vs Placebo in $AUC_{D1\text{-}D22}$ (90% CI) (AU)	SAR Dose1 vs Placebo	xxx	(xxx , xxx)
	SAR Dose2 vs Placebo	xxx	(xxx , xxx)
	SAR Dose3 vs Placebo	xxx	(xxx , xxx)
	SAR Dose4 vs Placebo	xxx	(xxx , xxx)

CONCLUSION

PD analysis is a critical component of the SAD study. When using the AUC value as the endpoint in PD analysis, it is essential to employ appropriate methods for accurately calculating the AUC value based on the study's requirement. Moreover, performing the ANCOVA model enhances our understanding of the pharmacodynamic response, enabling informed interpretation and decision-making in drug development and clinical research.

REFERENCE

[1] Jianfeng, Y. (2016). *General AUC Calculated Based on the Trapezoidal Rule.*

CONTACT INFORMATION

Your comments and question are valued and encouraged. Contact the author at:

Xusiqiao Cai

39F, Tower 3, in99 Chengdu Yintai Centre, 1199 North Section of Tianfu Road, High-Tech Zone, Chengdu, P.R.C.

Zoe.Cai@Sanofi.com