Chapter 351

Bioequivalence Tests for AUC and Cmax in a 2x2 Cross-Over Design (Log-Normal Data)

Introduction

This procedure calculates power and sample size of statistical tests of bioequivalence using mean ratios from a 2x2 cross-over design. Bioequivalence is measured by to two variables: AUC and Cmax. To conclude bioequivalence, most government regulation bodies require that both variables must pass an equivalence test. Hence, the power calculated here is the joint probability that both AUC and Cmax are shown to be "equivalent". Since AUC and Cmax are calculated on the same data, they are highly correlated. This procedure lets you set this correlation to any value between zero and one.

The details of testing the equivalence of two treatments using log-transformed data from a 2x2 cross-over design are given in another chapter (*Equivalence Tests for the Ratio of Two Means in a 2x2 Cross-Over Design (Log-Normal Data)*) and will only be summarized here.

AUC and Cmax

In a pharmacokinetic two-period two-treatment crossover bioequivalence study, subjects randomly placed into a sequence-group that receives the treatment drug first or the other sequence-group that receives the reference drug first. After a wash-out period, each subject then receives the other drug. Thus, each subject receives both drugs during the study. After a subject receives the drug, a drug plasma concentration profile is obtained. These profiles are then summarized by two variables: AUC and Cmax.

AUC is the area under the concentration-time curve from zero to infinity. AUC measures the total drug exposure (the extent) across time. Cmax is the maximum (peak) serum concentration that the drug achieves.

Together, AUC and Cmax give a reasonable summary of the bioavailability of the drug in the body. In order for two drugs to be declared equivalent, both measures must match.

Equivalence Testing Using Ratios

PASS follows the *two one-sided tests* approach described by Schuirmann (1987) and Phillips (1990). It will be convenient to adopt the following specialized notation for the discussion of these tests.

Parameter	PASS Input/Output	Interpretation
μ_T	$AUC\_\mu_T, C\max\_\mu_T$	*Treatment geometric means*. These are the treatment means of AUC and Cmax.
μ_R	$AUC\_\mu_R, C\max\_\mu_R$	*Reference geometric mean*. These are the reference means of AUC and Cmax.
R_L, R_U	*RL, RU*	*Equivalence Limits.* These limits define an interval of the ratio of the means in which their difference is so small that it may be ignored. Usually, $R_L = 0.8$ and $R_U = 1.25$, but these values may change depending on the circumstances.
R	*R*	*Actual ratio*. This value is calculated using $R = \mu_T/\mu_R$. It is the value at which the power is calculated.

With $R_L < 1$ and $R_U > 1$, the null hypothesis of non-equivalence is

$$H_0: R \leq R_L \text{ or } R \geq R_U.$$

The alternative hypothesis of equivalence is

$$H_1: R_L < R < R_U.$$

Log-Transformation

It has become common practice to take the following steps when performing a bioequivalence test.

1. State the statistical hypotheses in terms of ratios.
2. Transform these into hypotheses about differences by taking logarithms.
3. Analyze the logged data—that is, do the analysis in terms of the difference.
4. State the conclusion in terms of the ratio.

The details of step 2 for the alternative hypothesis are as follows:

$$H_1: R_L < R < R_U \quad \Rightarrow \quad H_1: R_L < \frac{\mu_T}{\mu_R} < R_U \quad \Rightarrow \quad H_1: \ln(R_L) < \ln(\mu_T) - \ln(\mu_R) < \ln(R_U)$$

Thus, a hypothesis about the ratio of the means on the original scale can be translated into a hypothesis about the difference of two means on the logged scale.

When performing an equivalence test on the difference between means, the usual procedure is to set the equivalence limits symmetrically above and below zero. Thus, the equivalence limits will be plus or minus an appropriate amount. The common practice is to do the same when the data are being analyzed on the log scale. However, when symmetric limits are set on the log scale, they do not translate to symmetric limits on the original scale. Instead, they translate to limits that are the inverses of each other.

Consider an example. Suppose the researchers have determined that the lower equivalence limit should be 80% on the original scale. Since they are planning to use a log scale for their analysis, they transform this limit to the log scale by taking the logarithm of 0.80. The result is -0.223144. Wanting symmetric limits, they set the upper equivalence limit to 0.223144. Exponentiating this value, they find that exp(0.223144) = 1.25.

Geometric Mean

Let Y represent data values in the original scale. In this procedure, the data are transformed to the log scale using X = ln(Y). The hypothesis tests and confidence intervals are performed on X because it is assumed to be normally distributed so standard analysis procedures may be used. Finally, the results are transformed back to the original scale using Y = exp(X).

Let $GM(Y)$ be the *geometric mean* of Y. It may be calculated in several ways including

$$GM(Y) = \left(\prod_{i=1}^{n} y_i\right)^{\frac{1}{n}} = \sqrt[n]{y_1 y_2 \ldots y_n} = \exp\left(\frac{1}{n}\sum_{i=1}^{n} \ln(y_i)\right)$$

For example, the numbers 2 and 8 have an arithmetic mean of 5 and a geometric mean of √(2 × 8) = 4. You can experiment with a few datasets to understand the difference between these two types of means.

Note that geometric mean ≤ arithmetic mean, with equality only when all values are equal.

The geometric mean has the following property:

$$GM\left(\frac{V}{U}\right) = \frac{GM(V)}{GM(U)}$$

Power Calculation

Until recently, the sample size was determined using the maximum of sample sizes calculated using the AUC and Cmax individually. Usually, Cmax would require the largest sample size, so its sample size was selected for the study. This corresponds to setting correlation (see below) between AUC and Cmax to 1.

Lately, several authors have provided formulas that take the correlation between AUC and Cmax into account. Julious and McIntyre (2012) provide a formula for calculating the joint power for testing a superiority hypothesis about AUC and Cmax. Julious (2012) provides the following extension to the case of equivalence tests. We modified this slightly to obtain results for the 2x2 cross-over design.

$$\text{Power(reject non-equivalence for both AUC and Cmax)} = \psi(A_U, C_U, \rho) + \psi(A_L, C_L, \rho) - 1$$

where

$\psi(a_1, a_2, \rho)$ Cumulative bivariate normal probability distribution calculated from $-\infty$ to a_1 and $-\infty$ to a_2

A_U $\dfrac{\sqrt{\left[\ln\left(\frac{\mu_{TA}}{\mu_{RA}}\right)-\ln(R_{UA})\right]^2 n}}{\sigma_{WA}\sqrt{2}} - t_{1-\alpha,n-2}$

A_L $\dfrac{\sqrt{\left[\ln\left(\frac{\mu_{TA}}{\mu_{RA}}\right)-\ln(R_{LA})\right]^2 n}}{\sigma_{WA}\sqrt{2}} - t_{1-\alpha,n-2}$

C_U $\dfrac{\sqrt{\left[\ln\left(\frac{\mu_{TC}}{\mu_{RC}}\right)-\ln(R_{UC})\right]^2 n}}{\sigma_{WC}\sqrt{2}} - t_{1-\alpha,n-2}$

C_L $\dfrac{\sqrt{\left[\ln\left(\frac{\mu_{TC}}{\mu_{RC}}\right)-\ln(R_{LC})\right]^2 n}}{\sigma_{WC}\sqrt{2}} - t_{1-\alpha,n-2}$

ρ Correlation between AUC and Cmax

μ_{TA} Geometric mean of AUC on treatment

μ_{RA} Geometric mean of AUC on reference

σ_{WA} Within Standard Deviation of ln(AUC) = $\sqrt{\text{Within MSE of ln(AUC)}}$

μ_{TC} Geometric mean of Cmax on treatment

μ_{RC} Geometric mean of Cmax on reference

σ_{WC} Within Standard Deviation of ln(Cmax) = $\sqrt{\text{Within MSE of ln(Cmax)}}$

R_{LA} Lower equivalence limit of AUC mean ratio

R_{UA} Upper equivalence limit of AUC mean ratio

R_{LC} Lower equivalence limit of Cmax mean ratio

R_{UC} Upper equivalence limit of Cmax mean ratio

Note that when $\rho = 0$, the joint power is equal to the product of the individual powers of each variable alone. Similarly, when $\rho = 1$, the joint power is near the minimum of the individual powers.

Example 1 – Finding Sample Size

A company has opened a new manufacturing plant and wants to show that the drug produced in the new plant is bioequivalent to that produced in an older plant. A cross-over design will be used to test the equivalence of drugs produced at the two plants.

Researchers have decided to set the equivalence limits for the means ratio at 0.80 and 1.25. Past experience leads the researchers to set σ_W to 0.25 for AUC and 0.3 for Cmax. The significance level is 0.05. The sample size will be determined at a power of 0.8. The correlation between AUC and Cmax is estimated to be 0.75. Correlation values between 0 and 1 will be evaluated. The true ratio is 1.02 for AUC and 1.03 for Cmax.

The font size of the second title row was reduced from 9 to 7 to accommodate this long title all on one line.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 1** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab	
Solve For	**Sample Size**
Power	**0.8**
Alpha	**0.05**
AUC_RU (Upper Equivalence Limit)	**1.25**
AUC_RL (Lower Equivalence Limit)	**1/RU**
AUC_μT (Treatment Geometric Mean)	**1.02**
AUC_μR (Reference Geometric Mean)	**1.0**
AUC_σw (Within Standard Deviation)	**0.25**
Cmax_RU (Upper Equivalence Limit)	**1.25**
Cmax_RL (Lower Equivalence Limit)	**1/RU**
Cmax_μT (Treatment Geometric Mean)	**1.03**
Cmax_μR (Reference Geometric Mean)	**1.0**
Cmax_σw (Within Standard Deviation)	**0.3**
ρ (Correlation Between AUC and Cmax)	**0 0.25 0.5 0.75 1**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Reports

Numeric Results for Testing Equivalence

Solve For: Sample Size
Ratio: R = Treatment Mean / Reference Mean
Hypotheses: H0: R ≤ RL or R ≥ RU vs. H1: RL < R < RU for both AUC and Cmax

Variable	Power	Total Sample Size N	Means: Treatment μT	Means: Reference μR	Means: Ratio R	Equivalence Limits: Lower RL	Equivalence Limits: Upper RU	Within Standard Deviation σw	Correlation Between AUC and Cmax ρ	Alpha
AUC	0.96054	38	1.02	1	1.02	0.8	1.25	0.25	0.00	0.05
Cmax	0.84224	38	1.03	1	1.03	0.8	1.25	0.30	0.00	0.05
Both	0.81310	38								
AUC	0.95562	37	1.02	1	1.02	0.8	1.25	0.25	0.25	0.05
Cmax	0.83053	37	1.03	1	1.03	0.8	1.25	0.30	0.25	0.05
Both	0.80394	37								
AUC	0.95562	37	1.02	1	1.02	0.8	1.25	0.25	0.50	0.05
Cmax	0.83053	37	1.03	1	1.03	0.8	1.25	0.30	0.50	0.05
Both	0.81263	37								
AUC	0.95040	36	1.02	1	1.02	0.8	1.25	0.25	0.75	0.05
Cmax	0.81861	36	1.03	1	1.03	0.8	1.25	0.30	0.75	0.05
Both	0.81129	36								
AUC	0.94423	35	1.02	1	1.02	0.8	1.25	0.25	1.00	0.05
Cmax	0.80511	35	1.03	1	1.03	0.8	1.25	0.30	1.00	0.05
Both	0.80952	35								

Power The probability of rejecting non-equivalence when the means are equivalent. This column gives the individual powers for AUC and Cmax followed by the power of rejecting both variables (this is the power shown on the plots).
N The total number of subjects split between both sequences.
μT, μR The treatment and reference means for both AUC and Cmax at which the powers were calculated. These values are in the original (not logged) scale.
R The ratio of the treatment and reference means for the AUC and Cmax variables. These values are in the ratio (not logged) scale.
RL, RU The lower and upper equivalence limits, respectively. These values are in the ratio (not logged) scale. Mean ratios between these limits are equivalent.
σw The within-subject standard error from the ANOVA model. These values are in the natural log scale.
ρ The correlation between the AUC and Cmax variables.
Alpha The probability of rejecting non-equivalence when the means are actually non-equivalent.

Summary Statements

A 2×2 cross-over design will be used to simultaneously test whether the treatment AUC is bioequivalent to the reference AUC, and whether the treatment Cmax is bioequivalent to the reference Cmax, based on the ratios of the geometric means (R_{AUC} = AUC_μT / AUC_μR, where AUC_μT is the treatment AUC geometric mean and AUC_μR is the reference AUC geometric mean, and R_{CMAX} = Cmax_μT / Cmax_μR, where Cmax_μT is the treatment Cmax geometric mean and Cmax_μR is the reference CMax geometric mean). For the AUC equivalence test, the ratio equivalence limits are 0.8 and 1.25 (H0: $R_{AUC} \le 0.8$ or $R_{AUC} \ge 1.25$ versus H1: $0.8 < R_{AUC} < 1.25$). For the Cmax equivalence test, the ratio equivalence limits are 0.8 and 1.25 (H0: $R_{CMAX} \le 0.8$ or $R_{CMAX} \ge 1.25$ versus H1: $0.8 < R_{CMAX} < 1.25$). Each of the equivalence comparisons will be made using two one-sided tests on the data after a natural-log transformation (the original-scale data is assumed to follow a log-normal distribution). The Type I error rate (α) is to be 0.05. The AUC within-subject standard deviation of the natural-logged data values is assumed to be 0.25, and the Cmax within-subject standard deviation of the natural-logged data values is assumed to be 0.3. The correlation between the AUC and Cmax variables is assumed to be 0. To detect an AUC mean ratio (R_{AUC}) of 1.02 (AUC_μT = 1.02, AUC_μR = 1), and a Cmax mean ratio (R_{CMAX}) of 1.03 (Cmax_μT = 1.03, Cmax_μR = 1), with 80% power to simultaneously reject both null hypotheses, the total number of subjects needed will be 38, to be split evenly between the two sequences.

Dropout-Inflated Sample Size

Dropout Rate	Sample Size N	Dropout-Inflated Enrollment Sample Size N'	Expected Number of Dropouts D
20%	38	48	10
20%	37	47	10
20%	36	45	9
20%	35	44	9

Dropout Rate	The percentage of subjects (or items) that are expected to be lost at random during the course of the study and for whom no response data will be collected (i.e., will be treated as "missing"). Abbreviated as DR.
N	The evaluable sample size at which power is computed (as entered by the user). If N subjects are evaluated out of the N' subjects that are enrolled in the study, the design will achieve the stated power.
N'	The total number of subjects that should be enrolled in the study in order to obtain N evaluable subjects, based on the assumed dropout rate. N' is calculated by inflating N using the formula N' = N / (1 - DR), with N' always rounded up. (See Julious, S.A. (2010) pages 52-53, or Chow, S.C., Shao, J., Wang, H., and Lokhnygina, Y. (2018) pages 32-33.)
D	The expected number of dropouts. D = N' - N.

Dropout Summary Statements

Anticipating a 20% dropout rate, 48 subjects should be enrolled to obtain a final sample size of 38 subjects.

References

Blackwelder, W.C. 1998. 'Equivalence Trials.' In Encyclopedia of Biostatistics, John Wiley and Sons. New York. Volume 2, 1367-1372.
Chow, S.C. and Liu, J.P. 1999. Design and Analysis of Bioavailability and Bioequivalence Studies. Marcel Dekker. New York.
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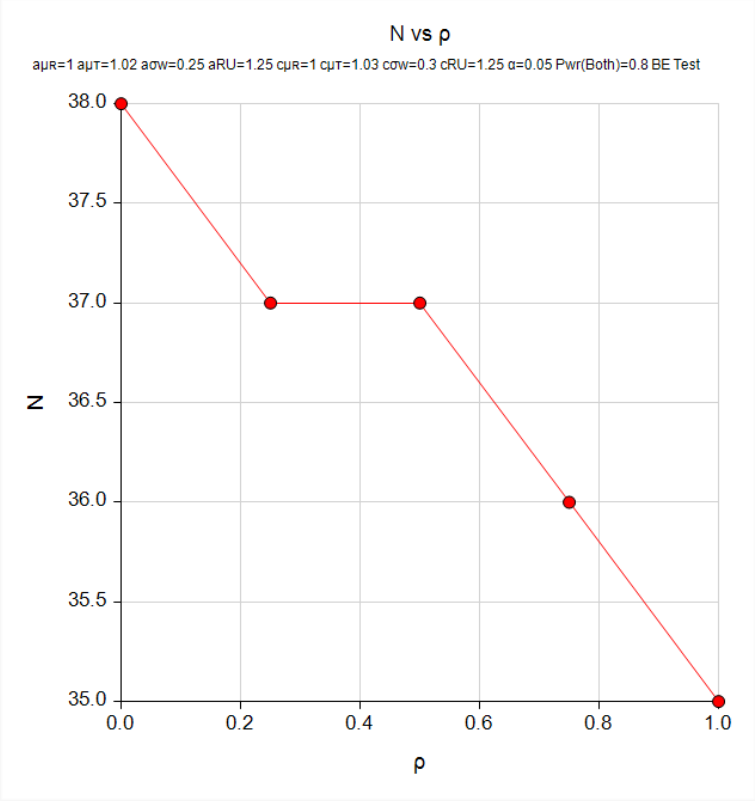
Patterson, S.D., Jones, B. 2017. Bioequivalence and Statistics in Clinical Pharmacology. Second Edition. Chapman & Hall/CRC Press. New York.
Senn, S. 2002. Cross-over Trials in Clinical Research. Second Edition. John Wiley & Sons. New York.

This report shows the sample size for the indicated scenarios.

Plots Section

Plots



This plot shows the correlation versus the sample size.

Example 2 – Validation using Julious (2012)

Julious (2012) slide 55 presents a table of sample size inflations for various parameter values. We will use these values to validate this procedure. The power is 0.90 and the significance level is 0.05. The COV is 0.3 which corresponds to $\sigma_W = 0.2935604$. The bioequivalence boundaries are 0.8 and 1.25. On the first row of the table, the true ratio is set to 0.85. The sample size inflations are 1.0 for $\rho = 1$, 1.211 for $\rho = 0.5$, and 1.253 for $\rho = 0$.

Setup

If the procedure window is not already open, use the PASS Home window to open it. The parameters for this example are listed below and are stored in the **Example 2** settings file. To load these settings to the procedure window, click **Open Example Settings File** in the Help Center or File menu.

Design Tab	
Solve For	**Sample Size**
Power	**0.9**
Alpha	**0.05**
AUC_RU (Upper Equivalence Limit)	**1.25**
AUC_RL (Lower Equivalence Limit)	**1/RU**
AUC_μT (Treatment Geometric Mean)	**0.85**
AUC_μR (Reference Geometric Mean)	**1.0**
AUC_σw (Within Standard Deviation)	**0.2935604**
Cmax_RU (Upper Equivalence Limit)	**1.25**
Cmax_RL (Lower Equivalence Limit)	**1/RU**
Cmax_μT (Treatment Geometric Mean)	**0.85**
Cmax_μR (Reference Geometric Mean)	**1.0**
Cmax_σw (Within Standard Deviation)	**0.2935604**
ρ (Correlation Between AUC and Cmax)	**0 0.5 1**

Output

Click the Calculate button to perform the calculations and generate the following output.

Numeric Results for Testing Equivalence

Solve For: Sample Size
Ratio: R = Treatment Mean / Reference Mean
Hypotheses: H0: R ≤ RL or R ≥ RU vs. H1: RL < R < RU for both AUC and Cmax

Variable	Power	Total Sample Size N	Means: Treatment μT	Means: Reference μR	Means: Ratio R	Equivalence Limits: Lower RL	Equivalence Limits: Upper RU	Within Standard Deviation σw	Correlation Between AUC and Cmax ρ	Alpha
AUC	0.94869	505	0.85	1	0.85	0.8	1.25	0.29356	0.0	0.05
Cmax	0.94869	505	0.85	1	0.85	0.8	1.25	0.29356	0.0	0.05
Both	0.90029	505								
AUC	0.94255	488	0.85	1	0.85	0.8	1.25	0.29356	0.5	0.05
Cmax	0.94255	488	0.85	1	0.85	0.8	1.25	0.29356	0.5	0.05
Both	0.90017	488								
AUC	0.90002	403	0.85	1	0.85	0.8	1.25	0.29356	1.0	0.05
Cmax	0.90002	403	0.85	1	0.85	0.8	1.25	0.29356	1.0	0.05
Both	0.90022	403								

We must manually calculate the three inflation ratios. The sample size inflations are 403/403 = 1.0 for $\rho = 1$, 488/403 = 1.211 for $\rho = 0.5$, and 505/403 = 1.253 for $\rho = 0$. Since these ratios match, the procedure is validated.

Example 3 – Validation using Another PASS Procedure

We will use results from the previously validated **Equivalence Tests for the Ratio of Two Means in a 2x2 Cross-Over Design (Log-Normal Data)** procedure to further validate the results of Example 2 above.

When $\rho = 0$ was used in Example 2, the sample size was 505 for both AUC and Cmax. The power was 0.94869 for each variable. We will duplicate these results.

In the other procedure, set Solve For to Power, N to 505, RU to 1.25, RL to 1/RU, R1 to 0.85, and COV to 0.3. The results of using these values will be shown below.

The **Equivalence Tests for the Ratio of Two Means in a 2x2 Cross-Over Design (Log-Normal Data)** procedure is set up as follows.

Design Tab

Solve For .....**Power**
Alpha.....**0.05**
N (Total Sample Size).....**505**
RU (Upper Equivalence Limit) **1.25**
RL (Lower Equivalence Limit).....**1/RU**
R1 (Actual Ratio) **0.85**
COV (Coefficient of Variation).....**0.3**

This set of options generates the following report.

Numeric Results for Testing Equivalence

Solve For: Power
Ratio: R = Treatment Mean / Reference Mean
Hypotheses: H0: R ≤ EL or R ≥ EU vs. H1: RL < R < RU

Power	Total Sample Size N	Lower Equiv Limit RL	Upper Equiv Limit RU	Actual Ratio R1	COV	Alpha	Beta
0.94869	505	0.8	1.25	0.85	0.3	0.05	0.05131

Note that the power is again found to be 0.94869 which matches the result above.

One further note, since the correlation is 0, the joint power is the product of the individual powers. That is, $0.94869^2 = 0.9000$. This matches the joint power of 0.90029 found in Example 2 to within rounding.