Assessment of Bioequivalence
between a test drug and a reference drug

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1. Introduction

This report evaluates the bioequivalence of a test drug in comparison to its reference drug, following the bioequivalence standards set by the US Food and Drug Administration (FDA) in 1992. Bioequivalence is defined by 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[1]. The FDA's 1992 guidelines advocate for the application of two one-sided tests to analyze key pharmacokinetic (PK) parameters, such as the area under the curve (AUC) and peak concentration (C_{max}). Bioequivalence is confirmed when the 90% confidence interval of the geometric mean ratio for these parameters between the test and reference products falls within the acceptable range of 0.8 to 1.25[2]. Our study adheres to these criteria to ascertain if the test drug fulfills the established standards.

2. Methods

2.1 Study data

The study utilizes a crossover design to assess the bioequivalence of a test drug and a reference drug, with 28 subjects allocated to one of four sequences. This structure includes four periods, systematically administering both drugs to each participant in a specified sequence, designed to mitigate carryover effects, likely facilitated by implementing washout periods. The evaluation focuses on critical pharmacokinetic parameters including C_{max} (the maximum concentration of a drug in the bloodstream, cerebrospinal fluid, or target organ after a dose is given), AUC_{0-t} (the area under the curve of plasma drug concentration versus time over a specific period), and $AUC_{0-\infty}$ (the area under the curve of plasma drug concentration versus time extended to infinity)[3,4].

In the analysis, we excluded all observations that contain missing values. In the datasets, subject 20 has no recorded data for all three parameters during periods 3 and 4. Besides, $AUC_{0-\infty}$ is missing for subject 17 in period 3, subject 23 in period 4, subject 6 in periods 3 and 4 and subject 20 in period 1. The study's summary statistics, grouped by drug type, are displayed in Table 1.

Table 1. Summary Statistics of Study Data by Drug Type

Type	Parameter	Min	Max	Mean	GM [a]	SD [b]	CV [c]
Test	C_{max}	35.60	349.00	107.62	89.10	74.97	69.66
	AUC_{0-t}	355.13	3401.00	1217.47	1033.86	777.60	63.87
	$AUC_{0-\infty}$	402.05	3495.90	1397.00	1214.59	793.54	56.80
Reference	C_{max}	21.30	445.00	112.15	87.70	92.48	82.46
	AUC_{0-t}	293.49	2534.83	1183.77	997.47	694.74	58.69
	$AUC_{0-\infty}$	501.31	2670.03	1294.82	1158.27	642.88	49.65

[a] Geometric Mean of parameters.

[b] Standard deviation of parameters.

[c] coefficient of variation of parameters.

2.2 Hypothesis

The primary objective is to assess if the test drug is bioequivalent to its reference drug. The analysis specifically aims to verify that the 90% confidence intervals of the geometric mean ratios for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of two formulations fall within the FDA's accepted range of 0.8 to 1.25. Therefore, we set the hypothesis as below:

Null Hypothesis (H_0): $\mu_T - \mu_R > U$ or $\mu_T - \mu_R < L$ for any of the PK parameters.
Alternative Hypothesis (H_1): $L < \mu_T - \mu_R < U$ for all the PK parameters.

μ_T represents the geometric mean of the PK parameter for the test drug, μ_R represents the geometric mean of the PK parameter for reference drug, the upper bound (U) is defined as the natural logarithm of 1.25, the lower bound (L) is defined as the natural logarithm of 0.8.

If the condition $L < \mu_T - \mu_R < U$ holds true for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, we will reject the null hypothesis H_0 and accept the alternative hypothesis H_1, confirming bioequivalence. Otherwise, we will accept the null hypothesis and conclude that the drug fails to meet bioequivalence standards.

2.3 Statistical Methods

Our analysis employed a mixed-model Analysis of Variance (ANOVA), specifically designed to handle the complexities of crossover study designs. This approach allows for the assessment of both within-subject and between-subject variances. The statistical model can be expressed as follows:

$$Y_{jkm(i)} = \mu + Seq_i + Subj_{j(i)} + \tau_k + \pi_m + \varepsilon_{jkm(i)}$$

where $Y_{jkm(i)}$ is the log AUC value for the jth subject in the ith sequence receiving the kth treatment at the mth period. μ is the overall mean, Seq_i represents the ith sequence effect, $Subj_{j(i)}$ is the jth subject effect within i, τ_k is the kth treatment effect, for reference formulation or test formulation, π_m is the mth period effect, and $\varepsilon_{jkm(i)}$ represents the random error term.

Fixed effects include the sequence effect, the treatment effect, and the period effect. These are modeled to assess the impact of treatment order, the difference between test and reference formulations, and time-related changes. Random effects are represented by the subject effect within each sequence, capturing the natural variability in response among individuals.

The ANOVA model assumes the independence of observations, a normal distribution of data, and constant variance across treatment groups. To satisfy these requirements, specifically normality and homoscedasticity, all PK parameters are log-transformed.

This transformation ensures that the data adhere to a normal distribution and exhibit constant variance across groups, thereby fulfilling assumptions.

3. Results

$L < \mu_T - \mu_R < U$ was satisfied for all three parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$). This indicates that the 90% confidence intervals of the geometric mean ratios for PK parameters of two formulations fall within the FDA's accepted range of 0.8 to 1.25. Detailed results are provided in Table 2. Based on these results, we proceed to reject the null hypothesis H_0 and accept the alternative hypothesis H_1, affirming bioequivalence between the test and reference drugs as per the FDA's criteria.

Table 2. Geometric Mean Ratios for PK parameters of two formulations

Parameters	Mean Ratio (%)	90% Confidence Interval of Ratio (%)
C_{max}	101.60	85.16 ; 121.20
AUC_{0-t}	103.65	90.46 ; 118.77
$AUC_{0-\infty}$	104.86	92.38 ; 119.06

The results of ANOVA testing for the effect of sequence variable with subject within sequence as an error term are detailed in Table 3.

Table 3. ANOVA for the sequence effect with subject within sequence as an error term

Source	Parameter	*df* [a]	SS [b]	MS [c]	F value	P value
Sequence	C_{max}	3	3.79	1.26	1.90	0.16
	AUC_{0-t}	3	3.42	1.14	2.08	0.13
	$AUC_{0-\infty}$	3	1.85	0.62	1.60	0.22

[a] Degree of freedom.
[b] Sum of Squares.
[c] Mean Square.

4. Discussion and Conclusions

This study aimed to compare the bioequivalence of a test drug to a reference drug, focusing on PK parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. The criteria for bioequivalence are met if the geometric mean ratios of these parameters of two formulations are within the 0.8 to 1.25 range. The results showed that all parameters are within this range, indicating bioequivalence between the test drug and the reference drug.

The findings suggest that the test drug may act as a alternative to the reference drug, potentially offering comparable benefits to patients. Considering its confirmed bioequivalence, it is advisable to seek regulatory approval for the test drug.

Several limitations of the study are noteworthy. While bioequivalence suggests pharmacokinetic similarity between the test and reference drugs, it does not directly imply equivalent clinical efficacy and safety. Furthermore, the study does not

specifically address special populations, such as children and the elderly, who may exhibit different pharmacokinetic profiles. This omission limits the generalizability of the finding.

In light of these considerations, future research should aim to fill these gaps by including a broader range of participants and examining the clinical outcomes associated with the use of the test drug. Such studies could provide valuable insights into the drug's efficacy and safety across various patient demographics, enhancing our understanding of its potential clinical implications.

5. Reference

[1] U.S. Food and Drug Administration. 21 C.F.R. Sect. 314.3 (2023).

[2] U.S. Food and Drug Administration. Guidance on Statistical Procedures for Bioequivalence Studies Using a Standard Two-Treatment Crossover Design. Rockville, MD: Division of Bioequivalence, Office of Generic Drugs, Center for Drug Evaluation and Research, Food and Drug Administration; 1992.

[3] Clinicalinfo [Internet]. C_{max}; [cited 2024 Feb 12]. Available from: https://clinicalinfo.hiv.gov/en/glossary/cmax

[4] Scheff JD, Almon RR, Dubois DC, Jusko WJ, Androulakis IP. Assessment of pharmacologic area under the curve when baselines are variable. Pharm Res. 2011;28(5):1081-9.

Appendix

6.1 SAS Code

```
* DRUG #2, TRT 1= Test, TRT 2= Reference ;

data dta01;
    INPUT SUBJ SEQ PERIOD DRUG REPL CMAX AUCT AUCINF;
    cards;
1    1    1    1    1    135.0    2194.6    2259.17
2    2    4    1    2    113.0    1038.73    1143.25
3    4    3    1    2    111.0    1213.00    1278.08
5    2    4    1    2    150.0    2525.43    2898.41
6    1    1    1    1    83.90    865.43    1144.60
7    3    2    1    1    108.0    1164.36    1239.18
8    4    3    1    2    73.10    660.75    703.02
9    4    3    1    2    37.30    569.33    763.30
10   3    2    1    1    349.0    2919.61    3030.59
11   2    4    1    2    52.10    582.22    674.18
12   1    1    1    1    120.0    1037.14    1098.31
13   1    1    1    1    38.10    775.52    1049.15
14   2    4    1    2    102.0    885.21    1144.47
15   4    3    1    2    108.0    877.45    982.47
16   3    2    1    1    43.30    919.45    1134.72
```

17	1	1	1	1	103.0	2048.9	.
20	4	3	1	2	.	.	.
21	4	3	1	2	66.20	464.11	528.98
22	3	2	1	1	133.0	1020.85	1184.53
23	2	4	1	2	20.70	305.87	.
24	1	1	1	1	100.0	1068.27	1203.59
25	2	4	1	2	78.30	2031.97	2124.94
26	1	1	1	1	49.40	897.80	1027.45
27	3	2	1	1	142.0	1196.63	1397.46
28	4	3	1	2	97.50	1138.85	1228.17
1	1	3	1	2	68.80	1478.33	1613.09
2	2	1	1	1	111.0	1131.28	1194.96
3	4	2	1	1	247.0	1632.98	1723.13
5	2	1	1	1	125.0	2282.23	2563.82
6	1	3	1	2	51.30	1266.15	.
7	3	4	1	2	124.0	992.0	1114.25
8	4	2	1	1	50.60	355.13	402.05
9	4	2	1	1	42.70	554.83	817.25
10	3	4	1	2	286.0	3221.0	3379.61
11	2	1	1	1	56.20	553.16	652.78
12	1	3	1	2	121.0	1016.41	1099.57
13	1	3	1	2	76.70	900.15	1242.31
14	2	1	1	1	47.50	697.50	1750.56
15	4	2	1	1	57.00	610.22	726.15
16	3	4	1	2	36.30	743.05	1116.96
17	1	3	1	2	87.00	1109.53	.
20	4	2	1	1	83.50	1153.58	2233.71
21	4	2	1	1	112.0	566.22	604.34
22	3	4	1	2	228.0	1349.15	1554.16
23	2	1	1	1	75.80	548.64	612.97
24	1	3	1	2	226.0	1843.11	1895.79
25	2	1	1	1	242.0	3401.0	3495.90
26	1	3	1	2	108.0	905.60	977.03
27	3	4	1	2	182.0	1516.29	1582.60
28	4	2	1	1	35.60	841.48	981.71
1	1	2	2	1	62.70	976.87	1093.57
2	2	3	2	2	99.70	1007.60	1100.48
3	4	4	2	2	128.0	1098.58	1174.05
5	2	3	2	2	222.0	2822.96	2924.07
6	1	2	2	1	191.0	1303.33	1383.17
7	3	1	2	1	147.0	1478.15	1581.18
8	4	4	2	2	49.40	525.72	574.36
9	4	4	2	2	71.80	503.70	547.45
10	3	1	2	1	445.0	2491.44	2581.36

```
11  2  3  2  2  61.90  526.61  591.28
12  1  2  2  1  126.0  1027.63  1114.23
13  1  2  2  1  66.60  819.95  983.89
14  2  3  2  2  121.0  1691.56  1772.25
15  4  4  2  2  71.40  735.45  868.92
16  3  1  2  1  31.60  653.82  922.92
17  1  2  2  1  95.90  2266.53  2331.91
20  4  4  2  2  .  .  .
21  4  4  2  2  114.0  607.81  642.09
22  3  1  2  1  134.0  1089.31  1294.44
23  2  3  2  2  223.0  1164.57  1213.66
24  1  2  2  1  68.90  1294.22  1348.28
25  2  3  2  2  271.0  2161.10  2261.51
26  1  2  2  1  101.0  875.94  982.97
27  3  1  2  1  309.0  1825.60  1901.51
28  4  4  2  2  96.90  1062.46  1129.17
1  1  4  2  2  144.0  2052.72  2119.83
2  2  2  2  1  112.0  879.40  952.72
3  4  1  2  1  88.50  1130.78  1175.88
5  2  2  2  1  146.0  2534.83  2670.03
6  1  4  2  2  43.80  980.31  .
7  3  3  2  2  143.0  1070.43  1262.65
8  4  1  2  1  34.10  417.03  985.61
9  4  1  2  1  44.80  562.61  737.77
10  3  3  2  2  331.0  3069.13  3186.74
11  2  2  2  1  41.10  435.48  501.31
12  1  4  2  2  95.80  910.18  993.46
13  1  4  2  2  31.70  579.48  1406.66
14  2  2  2  1  63.60  772.59  1237.15
15  4  1  2  1  41.20  441.05  540.58
16  3  3  2  2  35.20  721.24  1096.44
17  1  4  2  2  73.00  1724.79  1807.58
20  4  1  2  1  125.0  2100.41  .
21  4  1  2  1  85.10  539.03  576.69
22  3  3  2  2  32.90  252.65  398.53
23  2  2  2  1  126.0  1039.93  1134.28
24  1  4  2  2  130.0  1712.04  1775.59
25  2  2  2  1  96.40  2344.80  2426.03
26  1  4  2  2  144.0  1105.30  1195.52
27  3  3  2  2  85.70  1047.60  1228.97
28  4  1  2  1  21.30  293.49  618.23
;

proc sort;
```

```
    by subj drug period;

data dta02;
    set dta01;
    by subj drug period;

    if first.drug;
    keep subj drug period seq cmax auct aucinf lauct laucinf lcmax;

    if repl=1;
    lauct=log(auct);
    laucinf=log(aucinf);
    lcmax=log(cmax);

proc means data=dta02 noprint;
    class drug;
    var cmax auct aucinf lauct laucinf lcmax;
    output out=SummaryStats(drop=_TYPE_ _FREQ_)
min=Min_cmax Min_auct Min_aucinf
    max=Max_cmax Max_auct Max_aucinf
mean=Mean_cmax Mean_auct Mean_aucinf Mean_lauct Mean_laucinf Mean_lcmax
std=StdDev_cmax StdDev_auct StdDev_aucinf
    cv=CoeffVar_cmax CoeffVar_auct CoeffVar_aucinf;
run;

data GeoMeans;
    set SummaryStats;
    GeoMean_cmax=exp(Mean_lcmax);
    GeoMean_auct=exp(Mean_lauct);
    GeoMean_aucinf=exp(Mean_laucinf);
    keep drug GeoMean_cmax GeoMean_auct GeoMean_aucinf;
run;

PROC GLM data=work.dta02;
    CLASS SUBJ SEQ PERIOD DRUG;
    MODEL lcmax=SEQ SUBJ(SEQ) DRUG PERIOD / SS3;
    TEST H=SEQ E=SUBJ(SEQ);
    MEANS DRUG / T CLDIFF ALPHA=0.1;
    RUN;

PROC GLM data=work.dta02;
    CLASS SUBJ SEQ PERIOD DRUG;
    MODEL LAUCT=SEQ SUBJ(SEQ) DRUG PERIOD / SS3;
    TEST H=SEQ E=SUBJ(SEQ);
```

```
    MEANS DRUG / T CLDIFF ALPHA=0.1;
    RUN;

PROC GLM data=work.dta02;
    CLASS SUBJ SEQ PERIOD DRUG;
    MODEL laucinf=SEQ SUBJ(SEQ) DRUG PERIOD / SS3;
    TEST H=SEQ E=SUBJ(SEQ);
    MEANS DRUG / T CLDIFF ALPHA=0.1;
    RUN;
```