Impact of Logarithmic Transformation on the Restoration of Normality in Bioequivalence Data

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Abstract: The Logarithmic Transformation is widely used to address the skewness and assumes the normality assumption of the bioequivalence data but this may not be true in all cases unless the underlying assumption is taken into account and verified that the randomly generated data is normally distributed in the BE studies. Instead of restoring the normality in the data, the Log-Transformation may introduce new problems like inducing skewness with an increase in variability, which are even more difficult to deal with, then the original problem of non-normal distribution of data. Pharmacokinetic parameters, derived from the real biodata of the bioequivalence study of Glimepiride 4mg tablet was statistically analyzed, with and without, Log-Transformation through ANOVA and the two were compared for normality assumption through the standard testing for normality like Shapiro-Wilk and Q-Q Plots. The comparison of the conclusive results from both approaches, linear and log-transformed data, does not conclude any significant difference. A further investigation is required to strengthen this notion and to identify the circumstances and situations where the deterministic parameters are ascertained to select a suitable model for the data analysis and conclusion. The alternative analytic methods that eliminate the need of transforming non-normal data distributions prior to analysis, like Wilcoxon-Mann-Whitney two one-sided test which has been recommended by Hauschke *et al*., Hodges-Lehmann estimator or the other newer analytic distribution-free methods, that are not dependent on the distribution of data like the generalized estimating equations (GEE) are recommended.

Keywords: Bioequivalence, Log-transformation, Normality, Normal Distribution, Log-Normal Distribution, Skewness, Confidence Interval, Hypothesis testing, Outliers.

INTRODUCTION

The randomly generated data in any bio-medical event like scientific experiment, survey or a clinical study, are mostly assumed and considered to be normally distributed. Normal Probability Distribution (NPD) is the most common amongst the continuous distributions and enjoys a prominent place in the bio-medical research. Yet the attributes in all bio-medical researches or events are not distributed normally and in many cases, instead of a bell-shaped normal curve, the data show skewness in the mechanisms of distributions. Many a times, such departure from normality can be corrected by applying a standard practices of data transformation, like the logarithmic or square root but all important variables cannot be normalized or transformed to normality this way.

The Logarithmic Transformation (LT) is commonly used in biodata to deal with its skewness in order to get the distribution closer to symmetric or Normality, prior to t-testing. The LT is used in evaluating the majority of random variables and concluding their statistical inferences but it's usefulness is based on the fundamental assumption that the generated biodata is distributed normally. Yet it is not guaranteed that LT will assume normality and will not induce a skewness which might worsen the situation as the LT may not only induce skewness but also increase the variability of the data. In addition, the results obtained in a standard statistical test performed on log-transformed data are sometimes not relevant to the original, non-transformed data.

The Bioequivalence Studies (BE) are conducted with an objective of evaluating the equivalence of Test to Reference drug products, mainly for the switchability and interchangeability of generic copies with the innovator's brands. The selected Pharmacokinetic (PK) parameters are computed from the biodata generated during the study and required to be Log.Transformed prior to the statistical analysis. It is generally acknowledged that the validity of such inferences can never be ascertained if the inter-subject or intra-subject variabilities of such data are not distributed normally. Such variabilities represent a case-in-point for BE data.

It has been conventionally assumed that since the decay of plasma drug levels of bio-data, or plasma concentration-time data is exponential and is linear on the logarithmic scale, hence the probability distribution of any PK metrics, extracted from such data will be normal on such scale. Accordingly, the distribution of the variability of main PK metrics such as the area

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under the plasma concentration-time curve (AUC) and the maximum plasma drug concentration level (C_{max}) is likely to be normal on such scale. LT of the bio-data is mandatorily required prior to the statistical analysis, by almost all regulatory guidances with the aim of assuming normality of the probability distribution [1-4].

OBJECTIVE

The aim of the present work is to assess the impact of log-transformation in assuming normality of data, generated during a Bioequivalence (BE) study. The main focus of this research is to examine whether the log-transformation removes the skewness of data and succeeds in the restoration of normality in a distribution. Another prime objective of this research is to demonstrate the intrusive nature of log-trans-formation and its impact on the dispersion of real data.

METHODOLOGY

The real data for a 2 x 2 BE study of Glimepiride (active pharmaceutical ingredient) 4mg tablet, were used in this research, with the generous permission of JPM [5]. Both products, Glitra (JPM) as Test and Amaryl (Aventis) as Reference product, satisfied the official requirements with regards to their pharmaceutical characteristics. The data, carrying unknown Probability Distribution, were assessed for the normality testing, in accordance with the officially approved statistical procedures using Biostat®, a software, similar to SAS programme. The results of originally generated and log-transformed data of the BE study were compared. The impact of LT on the several known normality indicators, Skewness and Kurtosis, as well as on the outcome of other test procedure and estimates like the width of shortest 90% Confidence Interval (CI) and the outcome of the two-one sided test (TOST) procedure were examined.

Study Design Features, Statistical Evaluation and Bioequivalence Conclusion

The data used in this research work was taken from Glitra (Glimepiride) BE study, which was conducted on thirty six, healthy, male volunteers, as per the protocol, approved by the Ethics Committee. After the drug administration, the blood samples were collected on pre-determined intervals and the plasma Glimepiride levels were determined by a fully validated analytical procedure.

For the BE evaluation, two procedures, recommended by the worldwide regulatory authorities, the 90% classical Confidence Interval (CI) and the two-one sided testing of hypothesis (TOST) were employed. These two procedures are generally operationally equivalent to one another since they are supposed to arrive to, more or less, the same decision with regards to concluding bioequivalence or bio-in-equivalence. Needless to say that both procedures have to be conducted on the Log-scale, despite the fact that the variance of all components of ANOVA was found insignificant on either scale.

For the purpose of concluding equivalence, statistical evaluation of the plasma drug concentration-time data was conducted on the Log-transformed data, included the analysis of the variance (ANOVA) for the area under the plasma conc.-time profile from time zero to the last measureable plasma levels ($AUC_{0\rightarrow t}$), from time zero to infinity ($AUC_{0\rightarrow\infty}$) and the maximum plasma concentration (C_{max}). The intra-subject residual component of ANOVA was used to construct the shortest classical 90% CI, as well as Schuirmann's Two One-Sided Test (TOST) procedure was adapted. Nevertheless, BE could only be concluded by the three statistical procedures, used for BE testing, as demonstrated in Tables and Figures, coming ahead.

Notwithstanding, since the prime objective of the present work was to assess the impact of Log-transformation on the results of biodata, the statistical evaluation of data was also performed on the linear scale so that a comparison between the two approaches is established.

RESULT AND DISCUSSION

The results, presented in this work provide preliminary evidence of the weaknesses and shortcoming of the statistical procedures that are presently used to assess BE data. Some suggestions may also be proposed to address this problem.

Details of the BE study including the features, data for individual volunteer, analytical results, main pharmacokinetic metrics and the graphic presentation of the plasma concentration-time profile for the test and reference products are presented in Tables **1** & **2**, and Figures **1** & **2** respectively. Detail of the ANOVA on linear-linear and linear-log scale is presented in Tables **3** & **4**, graphical presentation of the 90% CI is given in Figure **3**, assessment of the restoration of Normality by Shapiro-Wilk test and Q-Q plots of the studentized intra and inter- subject residuals is given in Table **5** & **6**, and the statistical inference of the results, according to the

Table 1: Glitra Bioequivalence Study – Sample Points, Sequence, Period and Blood Levels

Elapsed Time:				0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	9.0	12.0	16.0	24.0	30.0
Per	Seq	Tre	Subj	C-01	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
I	I	R	1	0.0	10.4	39.8	58.7	70.3	76.3	127.3	119.4	161.6	180.9	133.2	91.8	60.3	61.0	46.0	52.5	42.5	19.9	10.3	6.1
I	I	R	2	0.0	11.6	36.3	63.6	69.9	66.0	73.7	71.3	112.4	148.4	97.2	73.8	64.5	60.4	61.5	77.1	86.4	40.8	12.7	11.3
I	I	R	5	0.0	12.4	41.2	43.0	38.1	24.5	27.9	29.3	32.5	63.4	59.2	48.8	34.3	26.3	16.2	11.6	11.2	8.8	5.5	
I	I	R	6	0.0	0.0	14.1	34.6	42.7	46.1	50.9	56.9	60.2	91.8	96.1	79.4	70.2	59.5	48.9	34.5	25.2	10.2		
I	I	R	7	0.0	13.9	48.6	68.3	71.0	60.6	51.1	45.9	44.7	164.1	246.9	242.4	203.1	185.0	180.7	89.7	52.7	42.5	13.2	9.8
I	I	R	8	0.0	12.6	34.4	43.4	55.5	44.9	47.8	54.8	73.9	182.5	174.1	157.7	116.4	81.3	62.9	25.5	9.0			
I	I	R	11	0.0	8.3	25.0	64.0	59.0	65.7	68.8	76.5	88.2	131.9	142.6	116.6	93.8	78.0	71.1	44.1	22.8	12.4	7.6	6.4
I	I	R	12	0.0	8.4	34.9	41.0	46.9	43.8	62.9	104.3	176.2	209.5	142.1	124.0	98.3	78.3	61.0	44.9	34.1	8.6	8.8	
I	I	R	14	0.0	27.3	82.9	77.7	106.8	96.8	158.7	312.3	--	331.5	235.5	176.3	153.1	116.4	123.7	71.1	31.7	18.7	8.8	6.0
I	I	R	15	0.0	79.0	158.1	148.6	101.8	97.1	96.0	64.1	52.1	49.1	37.7	36.9	36.7	38.0	35.7	37.1	56.2	14.5	6.9	5.9
I	I	R	16	0.0	15.2	50.0	67.5	83.6	89.6	109.0	99.7	126.7	164.7	170.6	124.3	102.2	81.5	76.1	29.9	26.5	15.2	7.7	5.3
I	I	R	17	0.0	29.9	121.3	159.4	113.6	85.3	91.0	60.7	81.8	56.0	78.7	76.0	70.1	64.1	98.0	109.2	82.4	41.2	10.2	7.1
I	I	R	19	0.0	10.3	26.4	23.9	22.8	21.4	22.1	22.4	31.9	96.8	184.5	192.6	161.3	135.9	132.1	58.9	23.8	11.5	6.3	
I	I	R	21	0.0	0.0	40.9	35.1	20.0	21.5	83.1	104.1	125.9	155.7	82.3	47.8	37.5	25.7	22.6	9.1				
I	I	R	23	0.0	37.2	97.1	99.1	71.8	39.2	52.2	61.0	156.7	118.1	68.0	67.0	27.2	21.7	17.8	9.8	5.4			
I	I	R	28	0.0	12.5	43.7	67.6	53.3	50.3	39.0	42.3	48.8	44.4	34.2	29.6	26.8	23.1	24.5	21.5	32.2	136.8	118.6	30.2
I	I	R	31	0.0	0.0	27.2	46.6	48.6	37.6	38.5	49.7	62.5	66.1	135.0	161.6	140.9	127.6	87.4	32.5	19.0	9.1		
I	I	R	35	0.0	12.8	83.3	122.7	138.2	114.5	80.1	63.1	57.1	85.0	122.8	123.7	124.7	128.2	150.5	107.3	54.5	19.2	7.4	
I	II	T	3	0.0	7.4	46.6	116.6	128.3	152.3	255.7	306.5	341.7	319.5	233.2	199.6	170.6	144.2	134.1	72.8	43.4	27.9	10.6	7.3
I	II	T	4	0.0	5.3	8.1	12.4	19.6	20.9	24.9	25.0	25.1	36.8	51.2	75.8	65.5	86.0	102.7	41.1	26.3	17.0	11.6	7.0
I	II	T	9	0.0	0.0	0.0	6.0	5.0	6.7	19.5	58.3	147.2	196.0	163.5	126.5	100.0	72.9	52.1	24.9				
I	II	T	10	0.0	0.0	14.6	18.0	15.6	15.2	17.9	52.8	121.9	239.0	151.4	102.6	71.8	60.1	64.4	44.5	27.2	13.9		
I	II	T	13	0.0	6.2	13.4	13.7	15.5	13.0	13.9	18.5	32.9	77.7	141.5	210.5	184.5	192.5	147.5	95.5	74.9	104.0	12.3	6.9
I	II	T	18	0.0	0.0	20.7	22.0	20.4	27.9	23.8	11.3	54.1	72.6	144.1	125.6	98.3	79.3	82.2	44.7	30.5	12.6	5.9	
I	II	T	20	0.0	0.0	13.1	16.9	17.8	21.4	34.1	44.9	77.2	65.6	49.2	84.2	105.6	77.3	65.5	32.8	22.7	13.8	5.3	
I	II	T	22	0.0	11.3	30.0	37.1	36.0	36.0	31.3	29.4	36.8	73.1	90.0	81.4	73.8	69.4	81.0	82.0	117.5	80.6	39.9	16.4
I	II	T	24	0.0	0.0	13.0	15.1	14.0	12.9	9.8	12.9	14.0	18.0	115.1	101.7	70.8	61.6	57.4	34.2	30.0	28.0	8.3	
I	II	T	25	0.0	5.4	21.8	32.0	48.4	115.9	199.7	263.3	378.8	472.4	410.2	363.3	355.7	327.4	361.6	268.5	203.6	123.5	51.3	42.1
I	II	T	26	0.0	14.2	62.0	89.9	71.3	72.7	73.1	48.7	249.8	324.0	153.0	125.5	123.6	113.2	125.6	76.1	36.9	26.6	11.8	8.7
I	II	T	27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1	45.3	95.0	113.3	117.8	110.0	89.9	31.2	6.4			
I	II	T	29	0.0	0.0	5.8	8.9	10.4	13.0	14.3	28.6	46.0	38.5	47.8	37.9	32.2	28.1	23.6	28.4	84.4	49.1	7.2	
I	II	T	30	0.0	7.2	25.2	37.2	48.0	66.9	46.4	56.5	54.2	49.5	72.3	106.5	102.6	91.6	70.6	43.0	90.3	32.9		
I	II	T	32	0.0	0.0	0.0	7.5	5.0	10.3	16.4	39.5	79.1	58.4	54.4	106.9	88.5	56.5	45.4	17.6	37.9	26.6	13.2	5.9
I	II	T	33	0.0	0.0	0.0	9.2	14.1	14.3	11.8	11.4	8.4	10.7	24.0	22.4	24.2	21.2	16.3	14.0	47.0	20.3		
I	II	T	34	0.0	0.0	0.0	7.7	7.7	8.8	6.8	7.3	5.3	5.5	4.5	6.4	11.1	12.3	12.7	20.2	133.5	95.0	35.6	12.9
I	II	T	36	0.0	7.5	42.6	69.1	80.8	79.5	76.2	89.3	186.6	198.2	204.7	167.3	156.8	165.2	182.5	135.9	155.8	80.5	35.2	10.4
II	I	T	1	0.0	7.5	22.5	36.1	38.1	46.9	61.5	98.7	108.1	133.0	84.7	63.8	51.4	46.6	41.6	41.4	31.8	25.3	14.1	9.7
II	I	T	2	0.0	13.1	44.7	58.5	69.7	68.1	63.2	58.0	69.4	75.1	114.3	105.3	112.6	90.4	104.7	149.6	76.2	36.6	12.0	10.0
II	I	T	5	0.0	9.1	36.8	45.8	41.7	56.1	51.2	81.8	147.0	175.7	126.7	77.4	57.6	42.9	43.6	19.2	14.4	9.0	5.6	
II	I	T	6	0.0	0.0	8.3	13.5	19.4	21.0	21.2	30.2	67.3	64.5	35.3	25.0	21.7	18.9	18.2	12.5	10.2	5.7		
II	I	T	7	0.0	10.7	23.4	26.2	28.9	36.7	82.3	122.6	125.7	207.1	176.6	161.4	160.2	146.9	113.1	77.7	40.9	23.9	12.3	10.5
II	I	T	8	0.0	0.0	13.3	19.0	23.2	19.7	17.3	18.3	24.8	104.4	174.8	136.9	107.2	87.0	86.6	35.2	24.3	8.1		
II	I	T	11	0.0	18.0	51.4	60.9	43.5	45.1	44.5	38.9	35.6	36.0	28.9	24.6	23.5	22.7	19.9	15.8	15.9	15.2	8.3	7.2
II	I	T	12	0.0	0.0	11.5	18.6	31.9	35.8	66.6	102.2	109.9	105.0	110.1	90.9	93.7	66.3	69.7	58.0	105.0	33.8	17.9	6.2
II	I	T	14	0.0	5.2	13.8	26.5	33.0	24.7	31.5	21.7	39.4	129.7	176.7	141.9	135.2	88.7	94.6	49.0	41.2	34.8	19.4	15.4
II	I	T	15	0.0	17.5	40.7	45.3	43.5	43.3	41.7	33.5	37.4	52.2	42.3	45.2	49.7	52.1	63.5	46.1	46.6	18.9	7.0	6.1
II	I	T	16	0.0	0.0	0.0	7.7	22.8	11.7	9.8	17.0	8.8	14.9	14.3	24.5	44.0	134.9	177.2	124.5	75.6	23.5	8.2	
II	I	T	17	0.0	14.4	72.4	118.0	127.4	102.7	71.3	52.9	50.1	39.0	32.1	29.5	31.0	39.7	47.6	17.3	68.7	21.5	6.2	5.1
II	I	T	19	0.0	5.5	10.2	11.4	10.2	9.9	7.9	15.5	17.1	37.5	66.9	79.6	100.2	101.9	92.5	69.8	31.9	13.7	6.0	
II	I	T	21	0.0	6.6	16.0	32.3	28.8	34.0	37.5	50.5	28.9	20.8	28.8	13.6	14.2	13.3	13.4	22.2	15.7	7.2		
II	I	T	23	0.0	0.0	9.1	17.9	17.9	18.6	24.3	29.4	39.8	72.6	111.5	95.7	62.8	45.4	34.5	13.2	11.2	7.4		
II	I	T	28	0.0	0.0	13.9	24.9	43.0	54.1	54.9	46.9	48.5	58.5	47.4	59.3	97.6	127.7	160.9	109.6	128.9	87.4	22.4	19.8
II	I	T	31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	13.0	117.2	165.0	134.8	100.2	95.5	76.3	34.4	17.0	12.6	7.0	
II	I	T	35	0.0	10.2	30.0	42.5	65.2	63.6	61.3	61.5	56.2	51.1	71.7	227.4	240.6	180.6	156.4	61.3	32.9	14.3	7.7	
II	II	R	3	0.0	15.7	58.5	79.2	91.9	104.0	119.4	151.4	214.4	275.8	207.3	171.9	150.2	120.2	95.8	57.6	32.3	15.7	6.7	
II	II	R	4	0.0	16.0	50.7	77.5	82.6	75.4	74.8	63.0	45.4	57.1	46.2	106.4	115.8	106.0	96.2	34.2	18.5	12.3	6.9	
II	II	R	9	0.0	6.6	24.4	26.7	38.8	44.3	78.8	131.9	164.9	--	173.8	136.0	97.0	64.5	40.5	11.7				
II	II	R	10	0.0	130.8	185.0	196.5	173.7	140.4	99.0	92.3	78.8	62.1	41.1	27.3	25.9	20.1	20.2	17.7	19.4	12.3		
II	II	R	13	0.0	5.1	7.2	8.0	8.6	8.0	10.8	10.5	9.4	38.3	181.4	185.5	172.6	136.2	136.3	54.6	155.7	107.4	18.5	10.6
II	II	R	18	0.0	21.3	32.4	46.7	53.8	53.6	51.7	30.8	40.1	66.2	116.2	92.9	71.0	60.8	53.7	49.5	34.0	18.8	5.8	
II	II	R	20	0.0	0.0	12.1	20.1	28.8	24.2	23.8	16.7	18.4	21.3	15.3	18.1	38.4	96.6	140.3	108.3	55.7	16.7		
II	II	R	22	0.0	6.8	37.7	54.2	45.5	46.4	44.2	50.1	59.8	104.2	90.2	67.5	67.4	88.7	92.7	76.7	95.5	73.2	33.5	21.6
II	II	R	24	0.0	8.2	21.6	21.5	19.2	16.2	10.8	9.3	8.9	56.4	121.4	110.1	103.7	74.5	59.7	28.5	92.0	23.5		
II	II	R	25	0.0	8.2	35.9	47.2	64.8	64.1	74.3	63.3	67.0	76.1	87.9	155.0	216.8	256.8	275.1	256.3	218.2	99.7	38.3	26.8
II	II	R	26	0.0	5.6	12.0	12.4	10.9	9.3	8.7	8.4	12.4	15.5	12.2	12.9	45.7	59.7	74.9	92.7	260.2	108.0	17.4	10.9
II	II	R	27	0.0	0.0	12.8	32.1	35.8	28.9	21.5	19.0	19.2	16.6	28.8	66.8	105.3	109.0	89.8	34.1	7.0			
II	II	R	29	0.0	5.7	16.2	23.1	19.4	17.0	16.5	19.8	98.3	228.4	209.3	171.8	133.3	111.8	103.2	64.8	23.9	11.9	5.9	
II	II	R	30	0.0	0.0	28.6	43.2	41.9	34.5	41.8	52.9	59.7	56.0	45.8	103.5	85.6	71.7	54.5	35.7	40.2	56.7	5.8	
II	II	R	32	0.0	5.6	9.7	17.7	12.4	14.8	15.0	14.1	14.8	28.9	61.8	36.1	34.8	22.2	19.9	8.5	9.6			
II	II	R	33	0.0	0.0	17.8	29.8	27.5	24.7	20.3	20.0	41.4	52.4	47.8	47.5	42.9	41.9	39.2	22.5	16.5	5.5		
II	II	R	34	0.0	0.0	10.1	16.2	15.6	15.1	14.3	11.7	12.7	21.3	72.8	79.9	88.0	67.7	69.3	89.6	109.3	86.8	16.4	5.8
II	II	R	36	0.0	0.0	19.0	50.4	51.5	50.8	49.7	71.8	243.5	228.8	279.9	278.8	207.6	172.1	139.1	89.4	70.8	35.8	16.9	

Table 2: Estimates of the Pharmacokinetic Metric of Glimepiride BE Study

Subj	Seq	Per	tmt	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}	T_{max}	λ_Z	$t_{0.5}$	$MRT_{0-\infty}$
1	1	1	R	1,142.9	1,201.9	180.9	4.5	0.104	6.658	10.172
2	1	1	R	1,414.5	1,519.1	148.4	4.5	0.108	6.428	12.463
5	1	1	R	403.2	496.1	63.4	4.5	0.059	11.784	13.995
6	1	1	R	607.2	665.5	96.1	5.0	0.174	3.975	8.046
7	1	1	R	1,733.6	1,825.0	246.9	5.0	0.107	6.458	10.912
8	1	1	R	695.5	717.1	182.5	4.5	0.417	1.661	5.536
11	1	1	R	935.0	1,009.4	142.6	5.0	0.087	8.009	10.735
12	1	1	R	980.2	1,051.4	209.5	4.5	0.124	5.591	8.969
14	1	1	R	1,733.6	1,788.2	331.5	4.5	0.111	6.267	8.042
15	1	1	R	985.2	1,041.2	158.1	1.0	0.106	6.546	9.746
16	1	1	R	1,066.0	1,127.5	170.6	5.0	0.086	8.025	9.597
17	1	1	R	1,567.4	1,617.7	159.4	1.5	0.140	4.943	10.328
19	1	1	R	966.1	1,002.7	192.6	5.5	0.171	4.042	8.613
21	1	1	R	427.2	446.8	155.7	4.5	0.467	1.485	4.553
23	1	1	R	513.1	533.6	156.7	4.0	0.264	2.628	4.351
28	1	1	R	2,196.4	2,329.0	136.8	16.0	0.228	3.042	18.561
31	1	1	R	745.9	780.2	161.6	5.5	0.266	2.610	6.975
35	1	1	R	1,420.3	1,461.7	150.5	7.0	0.178	3.900	8.344
3	2	2	R	1,378.1	1,422.0	275.8	4.5	0.154	4.508	7.473
4	2	2	R	830.4	880.9	115.8	6.0	0.137	5.046	8.649
9	2	2	R	640.8	657.6	173.8	5.0	0.695	0.997	4.764
10	2	2	R	798.6	1,068.6	196.5	1.5	0.046	15.216	12.712
13	2	2	R	2,048.5	2,143.0	185.5	5.5	0.112	6.198	13.583
18	2	2	R	814.3	856.6	116.2	5.0	0.136	5.096	9.557
20	2	2	R	841.2	903.3	140.3	7.0	0.269	2.578	9.419
22	2	2	R	1,762.1	2,070.7	104.2	4.5	0.070	9.917	17.580
24	2	2	R	805.9	1,119.8	121.4	5.0	0.075	9.251	14.643
25	2	2	R	3,303.0	3,536.2	275.1	7.0	0.115	6.028	13.469
26	2	2	R	2,150.7	2,215.5	260.2	12.0	0.168	4.138	13.895
27	2	2	R	455.9	469.6	109.0	6.5	0.511	1.358	6.475
29	2	2	R	1,004.8	1,039.7	228.4	4.5	0.168	4.117	8.381
30	2	2	R	994.1	1,044.6	103.5	5.5	0.115	6.043	11.528
32	2	2	R	204.6	251.9	61.8	5.0	0.204	3.398	7.785
33	2	2	R	381.0	407.3	52.4	4.5	0.207	3.342	7.700
34	2	2	R	1,558.9	1,589.1	109.3	12.0	0.194	3.580	13.175
36	2	2	R	1,779.0	1,917.6	279.9	5.0	0.122	5.690	10.289
1	1	2	T	945.8	1,087.7	133.0	4.5	0.0685	10.1130	14.9293
2	1	2	T	1,576.3	1,662.7	149.6	9.0	0.1154	6.0083	11.5388
5	1	2	T	703.8	755.1	175.7	4.5	0.1085	6.3905	8.5211
6	1	2	T	274.0	320.1	67.3	4.0	0.1235	5.6118	8.9575
7	1	2	T	1,394.2	1,508.3	207.1	4.5	0.0922	7.5188	11.5570
8	1	2	T	670.2	702.7	174.8	5.0	0.2483	2.7916	7.5517
11	1	2	T	527.5	693.5	60.9	1.5	0.0433	16.0227	20.5603
12	1	2	T	1,367.9	1,419.9	110.1	5.0	0.1188	5.8340	11.9652
14	1	2	T	1,210.0	1,479.5	176.7	5.0	0.0572	12.1198	17.9250
15	1	2	T	810.3	865.5	63.5	7.0	0.1097	6.3213	11.8568
16	1	2	T	1,126.6	1,171.2	177.2	7.0	0.1846	3.7547	10.8420
17	1	2	T	1,306.0	1,341.6	171.3	9.0	0.1431	4.8442	9.8317
19	1	2	T	744.9	780.9	101.9	6.5	0.1659	4.1770	10.1229
21	1	2	T	304.5	420.9	50.5	3.5	0.0623	11.1317	13.7547
23	1	2	T	402.4	443.4	111.5	5.0	0.1797	3.8564	7.7943
28	1	2	T	2,005.5	2,186.4	160.9	7.0	0.1095	6.3298	14.5577
31	1	2	T	660.4	715.5	165.0	5.0	0.1273	5.4448	10.4668
35	1	2	T	1,161.3	1,207.4	240.6	6.0	0.1669	4.1526	8.5788
3	2	1	T	1,976.1	2,042.1	341.7	4.0	0.1099	6.3044	8.4194
4	2	1	T	755.5	845.8	102.7	7.0	0.0780	8.8893	14.2932
9	2	1	T	540.8	594.2	196.0	4.5	0.4662	1.4867	5.8105
10	2	1	T	755.3	841.9	239.0	4.5	0.1609	4.3079	8.4402
13	2	1	T	1,883.0	1,932.8	210.5	5.5	0.1383	5.0120	12.1930
18	2	1	T	770.6	809.4	144.1	5.0	0.1519	4.5617	9.4333
20	2	1	T	650.6	689.0	105.6	6.0	0.1380	5.0225	9.7406
22	2	1	T	1,847.2	1,992.8	117.5	12.0	0.1124	6.1642	15.2641
24	2	1	T	693.0	774.7	115.1	5.0	0.1016	6.8221	12.8009
25	2	1	T	4,559.6	5,011.2	472.4	4.5	0.0932	7.4372	13.4473
26	2	1	T	1,504.9	1,593.9	324.0	4.5	0.0982	7.0554	10.2931
27	2	1	T	445.7	458.0	117.8	6.0	0.5213	1.3298	6.8929
29	2	1	T	874.9	905.0	84.4	12.0	0.2396	2.8927	12.5293
30	2	1	T	959.7	1,422.4	106.5	5.5	0.0711	9.7422	15.4526
32	2	1	T	764.8	852.6	106.9	5.5	0.0672	10.3179	15.1606
33	2	1	T	346.2	443.1	47.0	12.0	0.2096	3.3074	12.3515
34	2	1	T	1,433.1	1,524.4	133.5	12.0	0.1415	4.8993	17.0483
36	2	1	T	2,635.9	2,718.2	204.7	5.0	0.1260	5.5015	11.8550

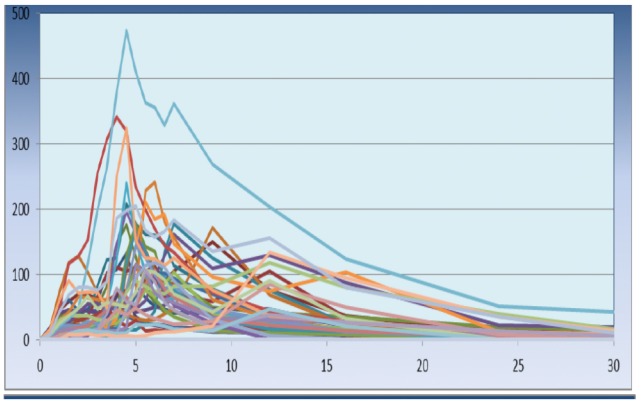


Figure 1: Plasma Concentration -Time Profiles of Glimepiride Test Product (Glitra).

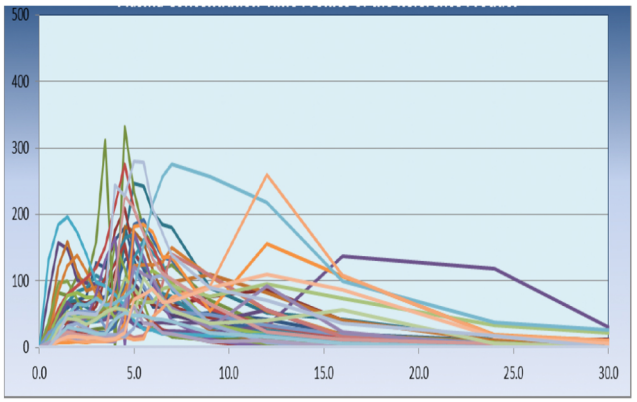


Figure 2: Plasma Concentration-Time profile of Glimepiride Reference Product (Amaryl).

Table 3: Analysis of the Variance (ANOVA) on Linear and Linear Scale (Glitra)

$AUC_{0\rightarrow t}$						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	35131490	1003757		0.9995	0.5013 **
Sequence	1	985648	985648	4946883	0.9814	0.3288 **
Subj(Sequence)	34	34145842	1004289	1004289	15.7089	0.0000
Intra-Subject						
Treatment	1	6736	6736	1219277	0.1054	0.7475 **
Period	1	220756	220756	284688	3.4530	0.0718 **
Error	34	2173668	63931			
Total	71	37532650				
% CV =	**22.24 (ISV)**				**** = Not Significant**	

$AUC_{0\rightarrow \infty}$						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	39750468	1135728		1.0042	0.4957 **
Sequence	1	1298515	1298515	6324999	1.1482	0.2915 **
Subj(Sequence)	34	38451953	1130940	1130940	15.6480	0.0000
Intra-Subject						
Treatment	1	0	0	1369287	0.0000	0.9981 **
Period	1	191104	191104	263377	2.6442	0.1132 **
Error	34	2457310	72274			
Total	71	42398882				
% CV =	**21.89 (ISV)**				**** = Not Significant**	

C_{max}						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	328044	9373		0.9834	0.5201 **
Sequence	1	4007	4007	25558	0.4204	0.5211 **
Subj(Sequence)	34	324037	9530	9530	4.0881	0.0000
Intra-Subject						
Treatment	1	1132	1132	11731	0.4857	0.4906 **
Period	1	9037	9037	11368	3.8765	0.0572 **
Error	34	79264	2331			
Total	71	417477				
% CV =	**29.92 (ISV)**				**** = Not Significant**	

Table 4: Analysis of the Variance (ANOVA) on the Linear and Log Scale (Glitra)

$AUC_{0\rightarrow t}$						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	23.27929	0.66512		0.9816	0.5223 **
Sequence	1	0.24020	0.24020	1.63844	0.3545	0.5555 **
Subj(Sequence)	34	23.03909	0.67762	0.67762	11.6334	0.0000
Intra-Subject						
Treatment	1	0.05066	0.05066	0.56973	0.8697	0.3576 **
Period	1	0.17929	0.17929	0.23754	3.0781	0.0884 **
Error	34	1.98043	0.05825			
Total	71	25.48967				
% CV =	**24.49 (ISV)**				**** = Not Significant**	

$AUC_{0\rightarrow \infty}$						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	21.70115	0.62003		0.9847	0.5186 **
Sequence	1	0.29227	0.29227	1.79877	0.4642	0.5003 **
Subj(Sequence)	34	21.40887	0.62967	0.62967	12.6743	0.0000
Intra-Subject						
Treatment	1	0.01524	0.01524	0.49065	0.3067	0.5834 **
Period	1	0.11565	0.11565	0.16533	2.3278	0.1363 **
Error	34	1.68916	0.04968			
Total	71	23.52119				
% CV =	**22.57 (ISV)**				**** = Not Significant**	

C_{max}						
	df	SS	MS=SS/df	E(MS)	F-value	p-value
Inter-Subject						
Subject	35	12.09435	0.34555		0.9731	0.5323 **
Sequence	1	0.02137	0.02137	0.44055	0.0602	0.8077 **
Subj(Sequence)	34	12.07299	0.35509	0.35509	3.5821	0.0002
Intra-Subject						
Treatment	1	0.17003	0.17003	0.41106	1.7152	0.1991 **
Period	1	0.34214	0.34214	0.44127	3.4514	0.0719 **
Error	34	3.37041	0.09913			
Total	71	15.97693				
% CV =	**32.28 (ISV)**				**** = Not Significant**	

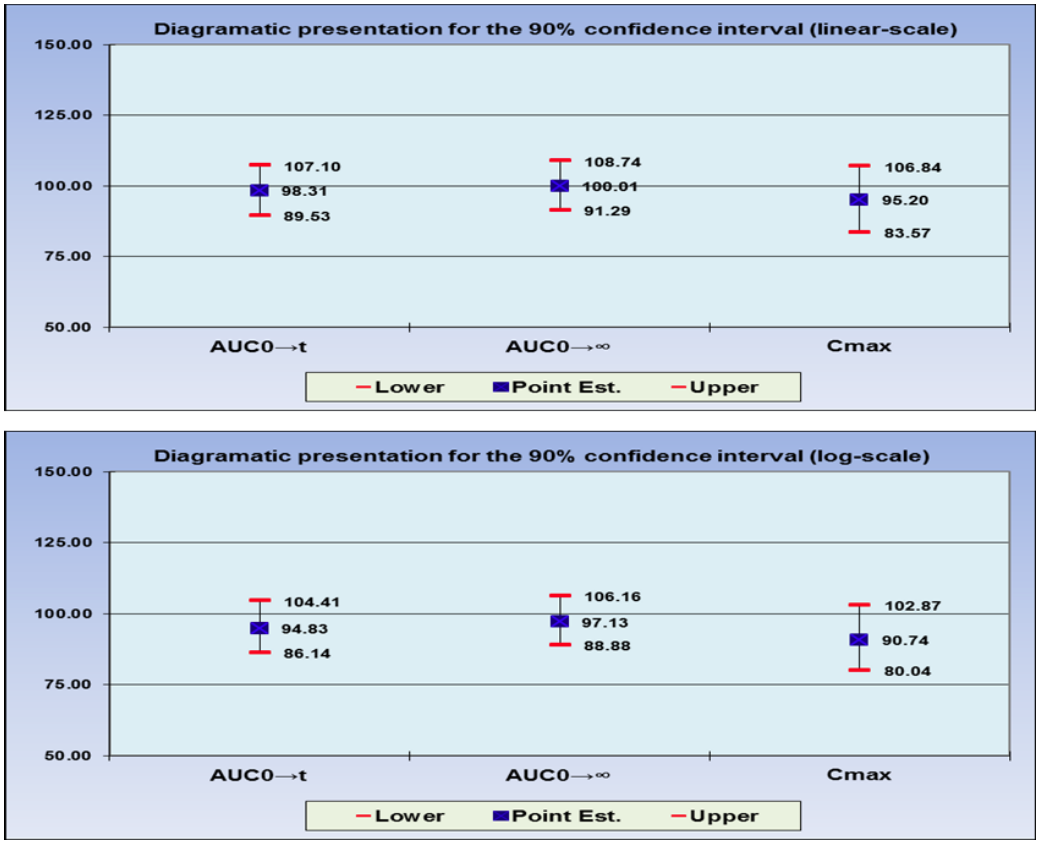
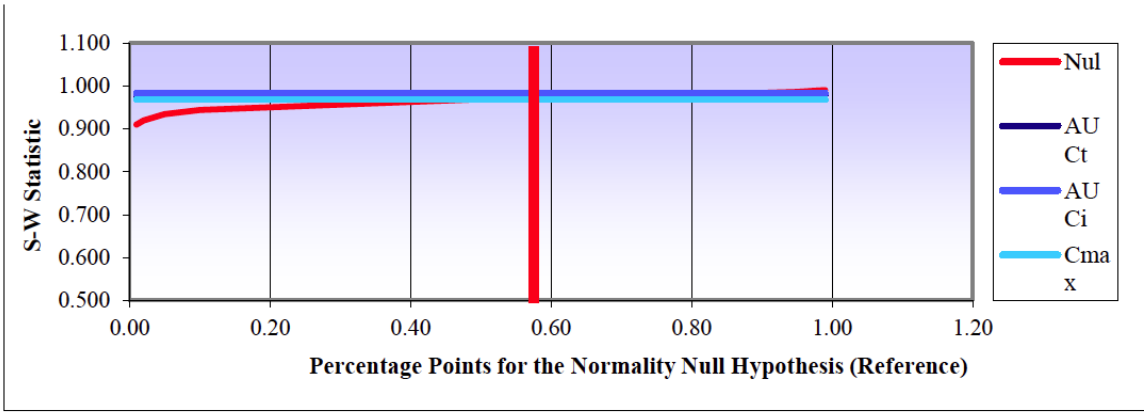


Figure 3: Point estimators and the upper and lower 90% Confidence Intervals on Linear and Log scale (Glitra BE study).

Table 5: Assessment of the restoration of Normality by Shapiro-Wilk Test (Reference & Test Products)



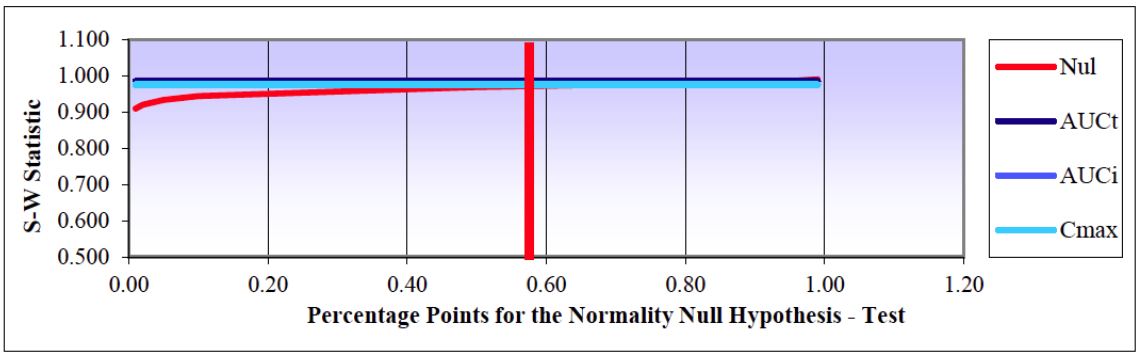


Table 6: Q-Q Plots for AUC (Reference and Test Products)

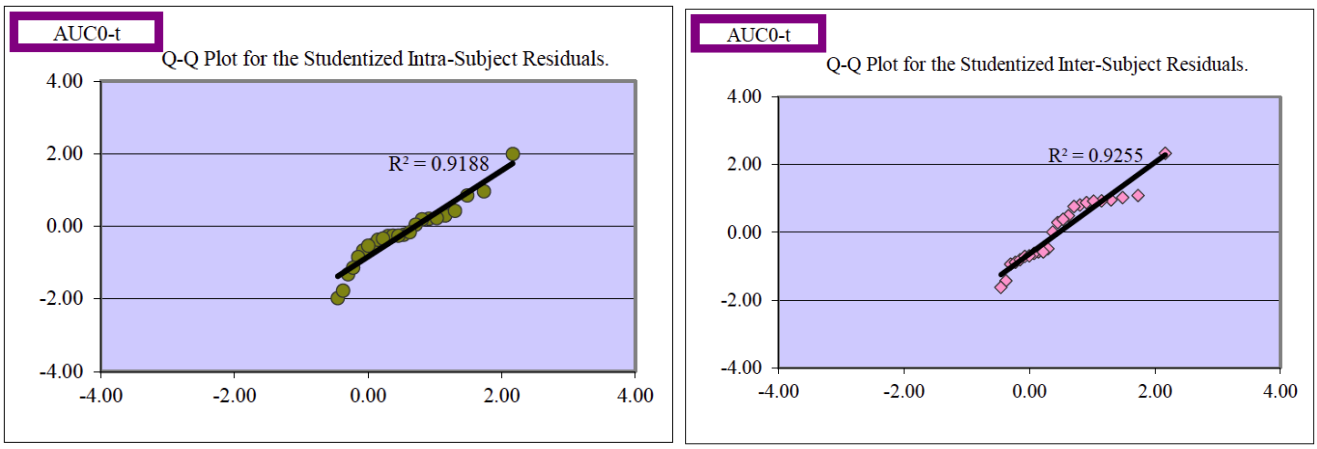


Table 7: Hypothesis Testing According to Schuirmann and Anderson Hauck Procedures

Schuirmann's Two One-sided Test

Metric	t-values		Prob of t-Value		Decision	
	T_L	T_U	Lower	Upper	T_L	T_U
AUC_{0-t}	3.524	-4.173	0.0014	0.0004	√	√
$AUC_{0-\infty}$	3.878	-3.873	0.0007	0.0007	√	√
C_{max}	0.397	-0.647	0.3484	0.2634	X	X

Anderson & Hauck's Test

Metric	T_{AH}	Non-Centrality Parameter	p-value	Decision
AUC_{0-t}	-0.325	3.849	0.00052	√
$AUC_{0-\infty}$	0.002	3.876	0.000003	√
C_{max}	-0.697	2.906	0.01651	√

TOST or Anderson & Hauck's test methods are presented in Table **7**.

Log-Transformation and Normality Assumptions

Detail of the impact of Log-transformation on the normality indicators for both, Test and Reference products of Glitra data, is presented in Table **5**. The contents demonstrate that for PD approximated normality for both products, according to Shapiro-Wilk (SW) test procedure, despite the fact that the PD of C_{max} data did not attain normality according to SW test. However, in spite of the fact that SW test is based on the intra-subject variability, the studentized Q-Q plots of this variability (studentized intra-subject residual) contradicted the outcome of SW test procedures in most of the examined metrics of Glitra study.

A tentative conclusion, may be drawn from these observations is that LT is likely to produce inconsistent outcome with regard to the restoration normality. Whilst the impact of LT on the PD is construed as the restoration of normality, an increase in the values of TOST is indicative of failure to reject the bio-in-equivalence hypothesis which favors the conclusion that both products are bioequivalent.

Shapiro-Wilk Statistics and Normality Evaluation

SW test is considered by many scholars as the most robust procedure for the assessment of departure from normality, or the proximity, of probability distribution for any set of random variables. Hence, it was adopted in this work, together with other test procedure for this purpose. As per this test, 50% of the data in question should have a value of SW statistic that is higher than the null hypothesis cut-of value. This situation is exemplified in the Table **5**.

CONCLUSION

Though Log-Transformation is assumed to restore or improve normality in the biodata yet it may not

achieve the intended purpose as it is not guaranteed that log transformation will assume normality and will not induce a skewness and variability to deteriorate the situation. Using log-transformation may be somewhat problematic and if used at all, its limitations should be carefully considered, particularly when interpreting the relevance of the analysis of transformed data for the hypothesis of interest about the original data.

If the basic assumptions of normality restoration are not observed, in many circumstances, the log transformation does not restore normality or reduce the variability but introduce skewness in the data. Moreover, the inferences concluded from log-transformed data may not usually characterize the original data, since it does not share much with the original data.

It is also concluded that if the data can be reasonably modeled by a parametric distribution such as the normal distribution, it is preferable to use the classic statistical methods because they usually provide more efficient inference but if log-transformation is inevitable and used at all, it must be applied very cautiously. On contrary, in case of skewed data, instead of finding an appropriate statistical distribution or transformation to model the observed data, it may be more appropriate to switch to the other distribution-free methods like Wilcoxon-Mann-Whitney two one-sided test [6] which has been recommended by Hauschke *et al*. [7], Hodges-Lehmann estimator [8] or the other newer analytic distribution-free methods, that are not dependent on the data distribution like the generalized estimating equations (GEE) [9, 10]. The GEE approach ignores the distribution assumption and provide valid inference irrespective of the probability distribution of data, nevertheless, it is applicable only in case of the skewed data.

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