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Original Article

Alpha lipoic acid bioequivalence study redesigned: a candidate for highly variable drugs

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ABSTRACT

Background and Aims: Alpha lipoic acid 600 mg HR film tablet showed high intra-subject variabilities in bioequivalence studies. In this regard, this study aims to determine whether Alpha lipoic acid 600 mg HR film coated tablet is a highly variable drug.
Methods: First, a randomized, open-label, balanced, two-treatment, two-period, two-sequence, single-dose, two-way crossover oral bioequivalence study comparing the test product (Alpha lipoic acid HR film tablet - ILKO Pharmaceuticals, Turkey) with the reference product (Thioctacid®- Meda Pharma, Germany) was conducted in normal, healthy, adult human subjects under fasting conditions (Study 1). Secondly, a randomized, open-label, balanced, two-treatment, four-period, two-sequence, single-dose, fully replicate crossover oral bioequivalence study was conducted in normal, healthy, adult human subjects under fasting conditions (Study 2).
Results: Study 1 failed. It had a 90% confidence interval for LnC_{max} (ng/mL) value between 79.69% – 138.98% with a high intra-subject coefficient of variability (ISCV=57.5%). In study 2 a 90% confidence interval for LnC_{max} (ng/mL) was found between 88.40% – 129.81% while the ISCV value for LnC_{max} was 64.5%.
Conclusion: The findings suggest that bioequivalence study for Alpha lipoic acid HR film tablet should be redesigned since this is a highly variable drug. Therefore, conventional bioequivalence acceptable limits (80.0%-125.0%) should be adjusted to 69.84% – 143.19% for alpha lipoic acid.

Keywords: Alpha lipoic acid, bioequivalence, highly variable drugs, intra-subject variability, replicate design

INTRODUCTION

Alpha lipoic acid (ALA), also known as thioctic acid, serves as a cofactor of mitochondrial respiratory enzymes, catalyzing oxidative decarboxylation reactions. ALA has been shown to possess antioxidant, cardiovascular, cognitive, anti-aging, detoxifying, anti-inflammatory, anti-cancer, and neuroprotective pharmacological properties. At present, it is mostly used for its antioxidant function (Ghelani, Razmovski-Naumovski, & Nammi, 2017) and, in particular, it is widely used as a dietary supplement by the older adult population (Keith et al., 2012). ALA has two optical isomers, specifically R-ALA and S-ALA, and is commonly used in racemic mixture (R,S–ALA) (Mignini, Streccioni, Tomassoni, Traini, & Amenta, 2007). It is readily absorbed following oral administration and is rapidly converted to dihydrolipoic acid (DHLA), its primary metabolite. ALA and its primary metabolite DHLA can directly regenerate ascorbic acid from dehydroascorbic acid and indirectly regenerate vitamin E. ALA also increases intracellular glutathione and coenzyme Q10 levels (Amenta, Traini, Tomassoni, & Mignini, 2008).

In clinical trials alpha-lipoic acid has mainly been used in the treatment of symptomatic peripheral (sensorimotor) diabetic polyneuropathy. The reference product Thioctacid® (alpha lipoic acid) 600 mg HR (High Release) film coated tablet is manufactured by Meda

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Pharma, Germany. The pharmacokinetic parameters of the reference product evaluated in healthy volunteers under fasting condition are as follows: median time for peak absorption (T_{max}): 88.1 min; area under the curve from time zero to the time of last measurable concentration (AUC_t): 3270.9 ng x h/g; peak plasma concentration (C_{max}): 1266.2 ng/g (Amenta et al., 2008).

The United States Food and Drug Administration (FDA) defines bioequivalence as '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' (US FDA Code of Federal Regulations, 2019). For systemically available drug products, classical single-dose, two-period, two-sequence, and crossover RT/TR designs are used, wherein the reference product is denoted as R and the test product as T. In this design, subjects receive a single test product dose and a reference product dose at randomly assigned times (Kang & Vahl, 2017; Lohar et al., 2012). In some cases, the drugs studied can be highly variable according to their pharmacokinetic properties. As the subject is exposed to two doses of the same formulation at two different times in one study, the variability measured from the same subject is considered as intra-subject variability (Thota et al., 2013).

Highly variable drugs are commonly known to have an intra-subject (within-subject) coefficient of variability (ISCV) equal to or greater than 30% in terms of AUC or C_{max} (Kang & Vahl, 2017; Knahl, Lang, Fleischer, & Kieser, 2018). Intra-subject variability can be estimated from study designs with more than two periods (Knahl et al., 2018). High intra-subject variability makes it difficult to obtain 90% confidence interval (CI 90%) of the ratio between the test and reference products for log-transformed data in the acceptable bioequivalence interval (80.0%-125.0%) (Fagiolino, González, Vázquez, & Eiraldi, 2007; Kang & Vahl, 2017; Li et al., 2017). This may result in non-bioequivalence even with the same product due to the variability within it (Lohar et al., 2012). As a striking example, Siewert and coworkers (1990) could not demonstrate the bioequivalence of the product in a bioequivalence study conducted with 16 volunteers using the same product containing 80 mg immediate-release verapamil (Blume et al., 1994). According to David et al.'s review study of the FDA's Office of Generic Drugs from 2003 to 2005, 31% of the bioequivalence studies conducted with 180 drugs were highly variable (Lohar et al., 2012; Molins, Cobo, & Ocaña, 2017). At that time, the standard 2-way cross-over study designs could not be used for proving bioequivalence. Designs with more subjects and more periods, such as three- and four-period designs were needed to give an estimation of the relevant variability. Full-replicate designs such as TRTR/RTRT or partial-replicate designs such as TRR/RTR/RRT were developed (Knahl et al., 2018). The main requirement for developing replicate crossover designs in highly variable drugs was to enable subjects to receive at least one of the drug products more than once (Kang & Vahl, 2017).

In the literature, some technical limitations are mentioned for oral formulations of alpha lipoic acid because of low solubility, short blood half-life, elevated systemic elimination, and first-pass hepatic metabolism (Mignini, Nasuti, Gioventu, Napolioni, & Martino, 2012). The absolute bioavailability of alpha lipoic acid is around 30% (Brufani & Figliola, 2014). However, in addition to the lack of product-specific FDA and EMA guidelines including Alpha lipoic acid 600 mg HR tablet, there is no information available in the literature about the highly variable properties of this product. Therefore, researchers or generic drug development companies have to design a bioequivalence study for this product according to the general rules of the FDA and EMA bioequivalence guidance. If there is no special information about the highly variable properties, two-period, two-sequence, crossover RT/TR designs are generally used for classical single-dose products which is not suitable for this product. This study assesses bioequivalence of the test product (ILKO Pharmaceuticals) with the registered reference drug (Thioctacid® 600 mg HR film coated tablet) in order to determine whether alpha lipoic acid in high release tablet dosage form is a highly variable drug product.

MATERIALS AND METHODS

API grade alpha lipoic acid active substance was obtained from Olon SPA Company (Milano-Italy) in racemic form. Low-substituted hydroxypropyl cellulose (Shin-Etsu Chemical Co. – Japan), hydroxypropyl cellulose (Nippon Soda Co. – Japan), magnesium stearate (FACI SPA – Spain), and hypromellose based coating materials (Colorcon, Inc. – England) were used as inactive ingredients in formulations. Analytical grades of potassium dihydrogen phosphate (Merck, Germany), phosphoric acid (ortho-phosphoric acid 85%, Merck, Germany), methanol (J.T. Baker, Poland) and acetonitrile (J.T. Baker, Poland) were used in HPLC analysis. Quantitative stability indicating HPLC test methods were performed on Waters Alliance HPLC System equipped with the 2695 Separations Module (Waters, Milford, MA, USA) with variable wavelength UV-Detector and run with Empower-2 Software. Ultrapure deionized water was obtained from a Millipore water purification system (Millipore Corp., Bedford, MA, USA).

In all studies, the test product (T) alpha lipoic acid 600 mg HR film coated tablet manufactured by ILKO Pharmaceuticals, Turkey (Lot: 1305119001) and the reference product (R) Thioctacid® (racemic alpha lipoic acid) 600 mg HR film coated tablet manufactured by Meda Pharma, Germany (Lot: 3741051) were used (Table 1).

EXPERIMENTAL

Analytical methods

Dissolved alpha lipoic acid content at *in vitro* condition was determined spectrophotometrically by a validated HPLC method at 215 nm using a Waters 2695 separation module (Waters, Milford, MA, USA). Separation was achieved on a C18 ACE 5 µm column (4.6 mm × 250 mm) using a mobile phase of buffer: methanol : acetonitrile (350:350:300). The buffer was prepared by dissolving 680 mg of potassium dihydrogen phosphate in 1 L of deionized water and adjusted to pH 3.0 with phosphoric acid. The flow rate was 1.2 mL/min, and the signal was monitored at a wavelength of 214 nm. The analytical method

Table 1. Active substance and excipients of test product (T) Alpha lipoic acid 600 mg HR film tablet and reference product (R) Thioctacid® 600 mg HR film coated tablet.

	Test Product (T) Alpha Lipoic Acid 600 mg HR Film Tablet	Reference Product (R) Thioctacid® 600 mg HR Film Coated Tablet
Active substance	- Alpha lipoic acid 600 mg	- Alpha lipoic acid 600 mg
Excipients	Core Tablet - Poly (O-2-hydroxypropyl) cellulose - Magnesium stearate - Hydroxypropyl cellulose - HPMC based film coating agents	Core Tablet - Poly (O-2-hydroxypropyl) cellulose - Magnesium stearate - Hydroxypropyl cellulose - HPMC based film coating agents

of alpha lipoic acid was validated for specificity, selectivity, sensitivity, linearity, recovery, accuracy and precision parameters.

All the plasma samples from all subjects of *in vivo* bioequivalence study were assayed as per protocol criteria for alpha lipoic acid using a validated LC-MS/MS method. Nineteen (19) blood samples were collected from each subject during each period. The venous blood samples (0.5 mL per sample) were withdrawn at pre-dose [(0.00) (within 2.00 hours prior to dosing)] and at 0.083, 0.167, 0.25, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00 and 8.00 hours post-dose. Samples from those subjects who completed at least two clinical study periods and who received test and reference products at least once were assayed. Plasma samples were assayed for alpha lipoic acid using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method.

The method was developed and validated in-house with Guidance on Bioanalytical Method Validation by EMEA. This method for alpha lipoic acid was validated by solid phase extraction method for its selectivity, sensitivity, accuracy, precision and other parameters. Calibration curve standards were prepared by spiking known concentration of analyte into screened and pooled biological matrix.

In vitro dissolution study

Before the *in vivo* study, an *in vitro* dissolution study was conducted comparing the dissolution behavior of the test product (T) and reference product (R) to verify the similarity of the products. *In vitro* dissolution testing was performed using USP type II paddle apparatus at 75 rpm at 10, 15, 20, 30, 45 and 60 min using 900 mL of deionized water, 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer dissolution media. The conditional release profiles of the test product and reference product were plotted as the cumulative percent of drug dissolved vs. time.

The dissolution profiles were compared; the dissolution profiles obtained were evaluated by similarity factor (f_2) (Helmy & Bedaiwy, 2013). According to the EMEA and FDA Guidelines, dissolution similarity may be determined using the f_2 statistic as follows:

$$f2 = 50.\log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}[R(t)-T(t)]^2}{n}}}\right] \quad \text{Eq.1}$$

In this equation *(Eq.1)* f_2 is the similarity factor, n is the number of time points, R(t) is the mean percent reference drug dissolved at time t after initiation of the study; T(t) is the mean percent test drug dissolved at time t after initiation of the study. For both the reference and test formulations, percent dissolution should be determined. An *f2* value between 50 and 100 suggests that the two dissolution profiles are similar (EMEA Guideline on the Investigation of Bioequivalence, 2010).

In vivo bioequivalence study

Initially, a randomized, two-period, single-dose, two-way crossover oral bioequivalence study comparing the test product (T) and reference product (R) was conducted in normal, healthy, adult human subjects under fasting conditions (indicated below as Study 1). According to the results, this study failed with a high ISCV value, and then it was decided to repeat the study with a full replicate study design proposed for highly variable products (indicated below as Study 2). The details of the two studies are presented below.

Ethics statement

The study protocol was approved by the Kavach Ethics Committee (ethics committee registration no: ECR/96/Indt/AP/2013) from Drugs Control General of India (DCGI) on December 12, 2014 (approval number: T-BE-5341/14). The study was conducted in compliance with the approved protocols, ethical principles laid down in the Declaration of Helsinki, and Good Practice Guidelines issued under the applicable regulations. A written informed consent of volunteers was obtained following a detailed explanation of the procedures that they may undergo.

Study subjects

Adult, healthy, male volunteers between 22 to 45 years of age with a body mass index (BMI) between 18.5–30.0 kg/m^2 and a mean body weight of 45 kg were enrolled as the study subjects. Before the study, medical and surgical histories of the volunteers were determined by general clinical examinations and laboratory tests. The clinical phase of the study lasted 20 days.

The subjects maintained 10.00 hours of overnight fasting before the scheduled dosing time. According to the randomization schedule, each subject was administered either a single dose of the test product (T) or reference product (R) with 240±5 mL of water in a standing position at ambient temperature (23±4°C). The subjects were instructed not to chew or crush the tablet but to consume it as a whole. They were

instructed to maintain an upright posture (sitting) for the first two hours after dosing in each period except when a change of posture was clinically indicated or necessary.

Study design

Study 1: A randomized, open-label, balanced, two-treatment, two-period, two-sequence, single-dose, two-way crossover oral bioequivalence study comparing the test product (T) and reference product (R) in 24 normal, healthy, adult human subjects under fasting conditions.

Study 2: A randomized, open-label, balanced, two-treatment, four-period, two-sequence, single-dose, fully replicate crossover oral bioequivalence study comparing the test product (T) and reference product (R) in 28 normal, healthy, adult human subjects under fasting conditions.

The randomization for the bioequivalence studies was generated using statistical software SAS® Version 9.4. Eighteen blood samples of 0.5 mL each were collected in vacutainers containing K_2EDTA from each subject during each period at pre-dose [(0.00) within 2.00 hours prior to dosing] and at 0.083, 0.167, 0.25, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 5.00 and 6.00 hours post-dose. Plasma samples taken from the subjects who completed all clinical phases were analyzed. Quantification was performed with LC-MS/MS using solid-phase extraction method.

Pharmacokinetic analysis

Based on the plasma concentrations of alpha lipoic acid, pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el}, and $t_{½}$) were calculated using "Non-compartmental model" for test and reference treatments. All pharmacokinetic analyses were carried out using WinNonlin Professional Software Version 5.4 (Pharsight Corporation, USA).

Pharmacokinetic parameters are summarized as follows: C_{max} [ng/mL] is the observed maximum concentration in ng/ml, AUC_{0-t} [ngxh/mL] is the area under the plasma concentration vs. time curve in ngxh/ml, $AUC_{0-\infty}$ [ngxh/mL] is the area under the plasma concentration vs time curve, T_{max} [h] is the time observed to reach C_{max}, and $t_{½}$ (λ) [h] is the terminal half-life calculated from λ according to $t_{1/2} = \ln(2)/\lambda$.

Statistical analysis

Descriptive statistics (such as mean, minimum, maximum, standard deviation, standard error, median, CV% geometric mean and coefficient of variation) were calculated for plasma concentrations of alpha lipoic acid at several time points as well as for the pharmacokinetic parameters C_{max} and AUC_{0-t} of the test and reference treatments.

Statistical analyses were performed on the pharmacokinetic parameters using the SAS Statistical Software Version 9.4 or higher, SAS Institute. Inc., CARY, USA. ANOVA, and two one-sided t-tests, 90% confidence intervals, ratio analysis for Ln transformed C_{max} and AUC_{0-t} were calculated for the test and reference formulations.

If the intra-subject variability of the reference is ≤ 30% for C_{max}, then the 90% confidence intervals for the difference between treatments and least-squares means will be calculated for Ln-transformed C_{max} and AUC_{0-t}.

If the intra-subject variability of the reference is > 30% for C_{max} (not resulting from outliers), then the 90% CI will be calculated according to the formula [U, L] = exp [±k·SWR], where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and SWR is the within-subject standard deviation of the log-transformed values of C_{max} of the reference product.

The test and reference products showed similar relative bioavailability if the difference between compared parameters was found to be statistically insignificant ($p>0.05$) and the 90% CI for these parameters was found to be within 80% to 125%. The acceptance range for C_{max} may be wider than that for AUC, particularly for drugs having highly variable peak concentrations; in such situations, the recommended range for C_{max} is 69.84% to 143.19% (Helmy & Bedaiwy, 2013).

Safety assessment

The safety assessments included monitoring of adverse events comprising adverse drug reactions, periodic physical examination, vital signs at regular predetermined intervals and those determined by the principal investigator. Pre-study 12-lead ECG, chest X-ray, urine analysis, and serology were conducted for screening of volunteers. Pre-study hematology and serum chemistry assessments were done to select participants with baseline values within reference ranges or clinically non-significant values if outside the reference range. These were repeated in post-study stage to determine any clinically significant abnormality.

Urine drug screening and alcohol breath test were done during the enrollment period of the study to detect participants for any recent substance abuse. A clinical assessment, which includes general and systemic examination, was conducted initially during the pre-study screening and finally during the post-study examination. Blood glucose monitoring was done at 01.00 and 03.00 hours post-dose (within±30 minutes of scheduled time) in each period or whenever the physician felt necessary during the conduct of the study.

RESULTS AND DISCUSSION

Validation of analytical methods

Analytical method for estimation of dissolved alpha lipoic acid in *in vitro* analysis was developed and validated using HPLC. Calibration curve for alpha lipoic acid ranged from 0.135 mg/mL to 0.812 mg/mL; correlation coefficient between concentrations and areas was higher than 0.99 ($r^2>0.99$); recovery of analyte was 100.4%.

Analytical method for estimation of alpha lipoic acid in human plasma was developed and validated using LC-MS/MS. The validated analytical method was used for analysis of plasma samples. Calibration curve for alpha lipoic acid ranged from 20.006 to 16004.569 ng/mL; linear relationship between concentration and signal intensity were obtained ($r^2>0.99$); the limit of quantitation (LOQ) was 20.006 ng/mL; precision values were 2.5%, 3.9%, 3.2% and 4.0% at 9653.792 ng/mL, 6564.578

ng/mL, 1641.145 ng/mL and 50.875 ng/mL concentrations, respectively; accuracy values were 90.0%, 92.6%, 96.0% and 95.0% at 9653.792 ng/mL, 6564.578 ng/mL, 1641.145 ng/mL and 50.875 ng/mL concentrations, respectively; recovery of analyte was 97.37%.

In vitro dissolution study results

The results obtained confirmed that there were acceptable similarities between the test and reference products for various dissolution media (deionized water, 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer) under comparison; as a result, *f2* values of all dissolution media are higher than 50. The results of *in vitro* tests confirm acceptable similarity between the test and reference products at different dissolution media such as deionized water, 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer (Figure 1) having similarity factors (f_2) 60.1, 60.0, 64.6 and 70.7, respectively.

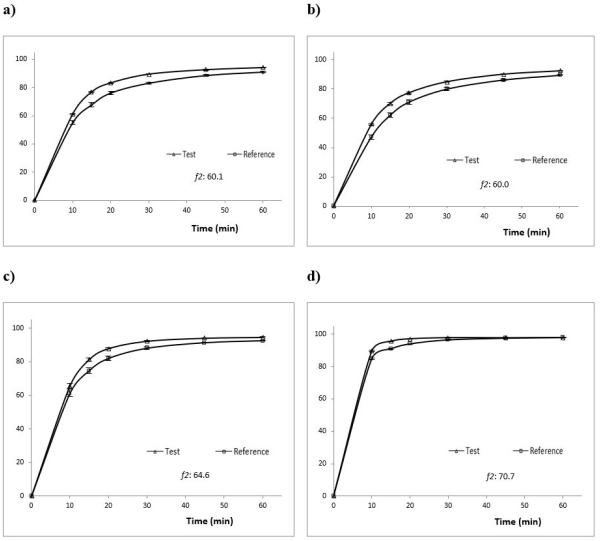

Figure 1. *In vitro* % released alpha lipoic acid vs. time profiles from the test and reference products in four different conditions. a) deionized water; b) 0.1 N HCl; c) pH 4.5 acetate buffer; d) pH 6.8 phosphate buffer. The data represent mean±standard error (n=12).

Safety results

As for these bioequivalence studies, the drugs were well tolerated upon single-dose administration to normal, healthy, adult, human subjects. No serious adverse events occurred during the conduct of these studies.

Pharmacokinetic and statistical analysis results

Pharmacokinetic parameters and statistical analyses of the test and reference products after administration to healthy volunteers are summarized in Tables 2, 3 and 4, for both Study 1 and Study 2. For Study 1, the plan was for twenty-four healthy, adult, human subjects to take part, but only twenty-two completed the study. On the other hand, for Study 2, twenty-eight

Table 2. Pharmacokinetic parameters of alpha lipoic acid with the test (T) and reference (R) product for Study 1.

	Pharmacokinetic Parameters		Test (T)	Reference (R)
Study 1	C_{max} (ng/mL)	Mean	5852.0	5049.6
		Min – Max	1085.7 - 19504.2	1680.3 - 13827.8
		Median	4514.2	3493.6
		SD	4722.7	3819.1
		CV%	80.7	75.6
	AUC_{0-t} (ng . h/mL)	Mean	3933.9	3815.6
		Min – Max	1604.1 – 7903.2	1819.1 – 8139.7
		Median	3769.8	3493.6
		SD	1685.0	1473.6
		CV%	42.8	38.6
	$AUC_{0-\infty}$ (ng . h/mL)	Mean	3998.3	3845.0
		Min – Max	1691.5 – 7934.8	1857.3 – 8156.2
		Median	3795.6	3531.1
		SD	1689.5	1473.9
		CV%	42.3	38.3
	T_{max} (h)	Mean	0.98	0.82
		Median	0.50	0.59
		SD	0.77	0.68
		CV%	78.4	83.5
	K_{el} (1/h)	Mean	1.3	1.7
		SD	0.59	0.54
		CV%	44.5	32.5
	$t_{1/2}$ (h)	Mean	0.66	0.47
		SD	0.36	0.17
		CV%	54.2	36.8

Table 3. Pharmacokinetic parameters of alpha lipoic acid with the test (T) and reference (R) product for Study 2 - replicate design.

	Pharmacokinetic Parameters		Test (T)	Reference (R)
Study 2	C_{max} (ng/mL)	Mean	5215.8	4873.7
		Min – Max	592.4 - 19039.3	803.1 - 11791.0
		Median	4021.0	3655.0
		SD	3669.7	3131.6
		CV%	70.4	64.3
	AUC_{0-t} (ng . h/mL)	Mean	3648.7	3383.6
		Min – Max	1263.4 – 9755.7	803.1 – 11791.0
		Median	3244.8	3232.0
		SD	1745.7	1406.9
		CV%	47.8	41.6
	$AUC_{0-\infty}$ (ng . h/mL)	Mean	3710.6	3413.5
		Min – Max	1275.0 – 9786.7	1301.2 – 7455.6
		Median	3307.2	3285.8
		SD	1746.7	1398.7
		CV%	47.1	41.0
	T_{max} (h)	Mean	0.96	0.98
		Median	0.59	0.67
		SD	0.80	0.69
		CV%	83.7	70.2
	K_{el} (1/h)	Mean	1.76	1.82
		SD	0.54	0.62
		CV%	30.8	33.8
	$t_{1/2}$ (h)	Mean	0.44	0.45
		SD	0.20	0.23
		CV%	45.6	50.9

Table 4. Statistical analysis for Log transformed C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ data for test (T) and reference (R) product.

	Pharmacokinetic Parameters	Statistical Analysis						
		Least Square Geometric Mean		T/R	90% C.I.		ISCV** %	Power
		Reference	Test		Lower	Upper		
Study 1	LnC_{max} (ng/mL)	4216.5	4006.6	105.2%	79.7%	139.0%	57.5%	37.1%
	$LnAUC_{0-t}$ (ng · h/mL)	3602.0	3580.8	100.6%	89.7%	112.8%	22.3%	94.2%
	$LnAUC_{0-\infty}$ (ng · h/mL)	3668.3	3612.0	101.6%	91.1%	113.2%	21.1%	95.8%
Study 2	LnC_{max} (ng/mL)	4056.1	3786.4	107.1%	88.4%	129.8%	64.5%	60.6%
	$LnAUC_{0-t}$ (ng · h/mL)	3266.2	364.2	106.6%	99.5%	114.7%	22.2%	100.0%
	$LnAUC_{0-\infty}$ (ng · h/mL)	3312.3	3102.0	106.8%	101.3%	116.2%	21.2%	100.0%

* C.I: Confidence Interval; ** ISCV: Intra-Subject Coefficient of Variability

healthy, adult, human subjects were enrolled and initially dosed at the beginning of the study. Twenty-two subjects completed four periods of the study, and twenty-eight subjects who completed at least two periods dosed with T and R were considered for pharmacokinetic and statistical analysis for alpha lipoic acid.

According to the results of Study 1, two-way crossover design study, the test product could not be considered to be bioequivalent to the reference product as the 90% confidence interval for LnC_{max} (ng/mL) was 79.69% – 138.98% (ISCV=57.5%). However, according to Study 2, a fully replicate design study, the test product was bioequivalent in terms of $LnAUC_{0-t}$ (ng · h/mL) and $LnAUC_{0-\infty}$ (ng · h/mL), but for LnC_{max} (ng/mL) the 90% confidence intervals (88.40% – 129.81%) were slightly higher than acceptable limits (80.0%-125.0%). When the intra-subject variations were considered, a moderately variable $LnAUC_{0-t}$ and $LnAUC_{0-\infty}$ having ISCV 22.2% and 21.2% respectively, and a highly variable LnC_{max} (ISCV=64.5%) which is much higher than 30% were found. Therefore, intra-subject variabilities showed that alpha lipoic acid is a highly variable drug.

Mean plasma concentration vs. time profiles from 0 to 6 h obtained after administration of the test product and the reference product are shown in Figure 2. The curves after administration of the test or reference products are similar for alpha lipoic acid, especially for Study 2, a full replicate design study.

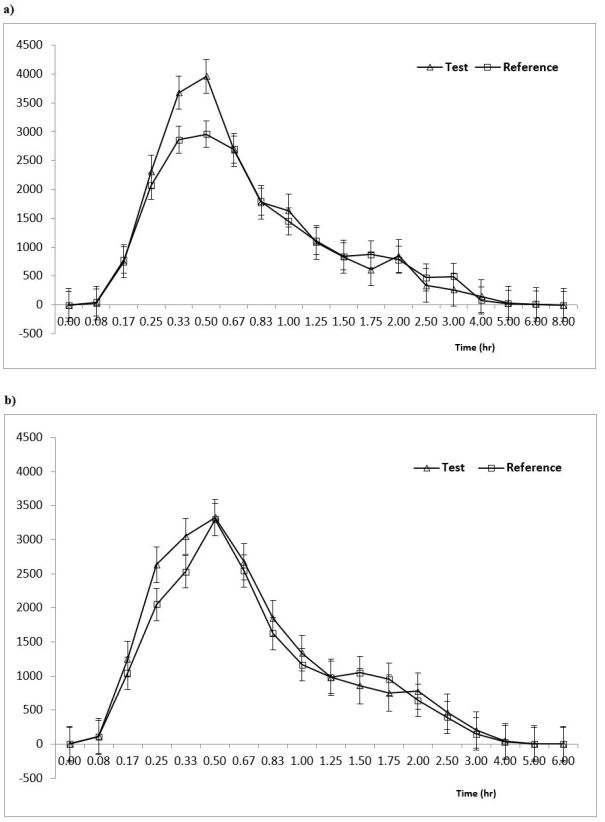

Figure 2. Mean±standard error (SE) plasma concentration vs. time profile of alpha lipoic acid following single oral dose administration of test (T) and reference (R) product in healthy, adult, male volunteers under fasting condition. **(a)** Study 1 (n=22); **(b)** Study 2 Replicate design study (n=28).

CONCLUSION

Alpha lipoic acid meets the criteria for a highly variable drug with respect to replicate design bioequivalence study results (Study 2). Alpha lipoic acid 600 mg HR film tablet, which does not have product-specific FDA and EMA bioequivalence guidance, has been shown to have high intra-subject variabilities. Therefore, conventional bioequivalence acceptable limits (80.0%-125.0%) should be adjusted to 69.84%-143.19% for alpha-lipoic acid. This study will contribute greatly to the literature and especially to pharmaceutical companies that develop generic products while designing the bioequivalence study.

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