SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF α-LIPOIC ACID IN TABLET DOSAGE FORM

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ABSTRACT

Alpha lipoic acid (α-LPA), 1, 2-dithiolane-3-pentanoic acid, is a universal antioxidant present naturally in all prokaryotic and eukaryotic cells. Endogenously synthesized LPA functions as a cofactor for several important mitochondrial enzyme complexes. Due to its unique antioxidant properties, it has been administered in many oxidative stress related diseases and is also commercially available in the form of dietary supplements. The aim of the present research work was to develop a simple and rapid analytical method to determine LPA content in various pharmaceutical formulations. Few analytical methods like HPLC, GC have been established for the estimation of LPA in tablet dosage form; there was no mention of a method based on Spectrophotometric determination of LPA. Major problem for determination of LPA by spectrophotometry was the absence of conjugate double bond in its structure. But practically it was found that disulphide ring absorbs UV at 322nm (λmax of LPA). Absorption maxima in zero and first order derivative spectra was found to be 322nm (method-A) and 309nm (method-B) and area under curve was measured from 310-350nm (method-C) and 270-330nm (method-D), the drug followed a linear relationship in the range of 150-500 μg/ml while the correlation coefficient was at 0.999, 1.0, 0.999 and 0.999 respectively. The recovery was 99.52% ±0.56(method-A) and the coefficient of variance for intraday and interday was found to be less than 2%, LOD and LOQ for this method was found 1.64 μg/ml and 5.49 μg/ml(method-D) respectively. This method is found suitable for day to day analysis of α- LPA in tablet dosage form.

Keywords: Alpha lipoic acid (α-LPA), Zero order and first order Derivative spectroscopy, Area under curve (AUC) measurement, UV spectrophotometer

INTRODUCTION

Alpha lipoic acid is disulphide compound found naturally in a diverse group of micro-organisms and in a variety of plant and animal tissues. It is mainly present in mitochondria & plays a pivot role in metabolism. Alpha lipoic acid is eight-carbon fatty acid containing two sulphur atoms attached at carbon 6 & 8. Carbon 6 is asymmetric, so alpha lipoic acid exist as two enatiomers R (d) & S (l). Naturally occurring form is R (d). Most of pharmaceutical formulation contains racemic mixture of R & S enatiomers. Free LPA (non-protein bound) is a water and lipid soluble fatty acid. Its solubility in water is controlled by its carboxylate moiety. The octanoic backbone is hydrophobic. Limited water solubility and hydrophobicity of LPA affects its stability and utility in cell culture systems. Lipoic acid has gained considerable attention as an antioxidant. Lipoate, or its reduced form, dihydrolipoate, reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen. So, Administration of LPA has been shown to be beneficial in a number of oxidative stress models such as ischemia-reperfusion injury, diabetes, cataract formation, HIV activation, neurodegeneration, and radiation injury. The chemical structure of alpha lipoic acid is presented below (Figure 1)

Literature survey revealed that HPLC[1-3] & HPLC-ECD[4] have been established for the estimation of Alpha lipoic acid in bulk and tablet dosage form. Therefore, the objective of work is to develop and validate simple, precise, accurate "Spectrophotometric" methods for determination of Alpha lipoic acid in pharmaceutical formulation. Further the developed methods have to be validated as per ICH guidelines.
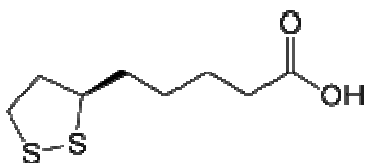

Fig. 1: It shows structure of alpha lipoic acid

MATERIALS AND CHEMICALS

Alpha lipoic acid was supplied by Centurion Laboratories (Vadodara) as a gift sample. Methanol AR grade was purchased from Merck Lab. & Tablets of Alpha lipoic acid were purchased from local market; each tablet was labelled to contain 300 mg of LPA.

Instrumentation

For all the Spectrophotometric methods, UV-Visible spectrophotometer (Shimadzu 1800 with UV Probe 2.21 software) and a pair of 1 cm matched quartz cells were used. Shimadzu AUX 220 weighing balance.

Selection of solvent

Alpha lipoic acid is slightly soluble in water but freely soluble in methanol. So, methanol is selected for solvent & used for preparation of stock solution & working standards.

Preparation of standard solutions

Stock solution of 1000 μg/ml of Alpha lipoic acid was prepared in methanol. The standard solutions were prepared by dilution of the stock solution with methanol in a concentration range of 150-500 μg/ml with methanol for all methods.

METHODS[5-7]

Zero Order Spectrophotometry (Method A)

For the selection of analytical wavelength, standard solution of Alpha lipoic acid (200 μg/ml) was scanned in the spectrum mode from 800 nm to 200 nm. From the spectrum of drug, λ max of LPA, 322 nm was selected for the analysis.

First order derivative spectrophotometry (Method B)

For the selection of analytical wavelength, standard solution of Alpha lipoic acid (200 μg/ml) was scanned in the spectrum mode from 800 nm to 200 nm & scanned spectra are transformed into first order derivative spectra. From the first order derivative spectrum of drug, λ max of LPA, 309 nm was selected for the analysis.
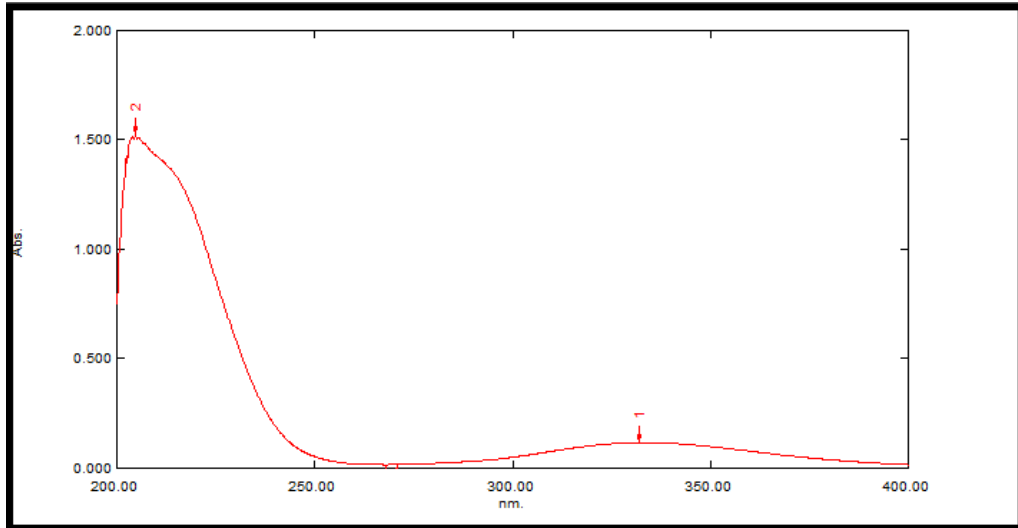
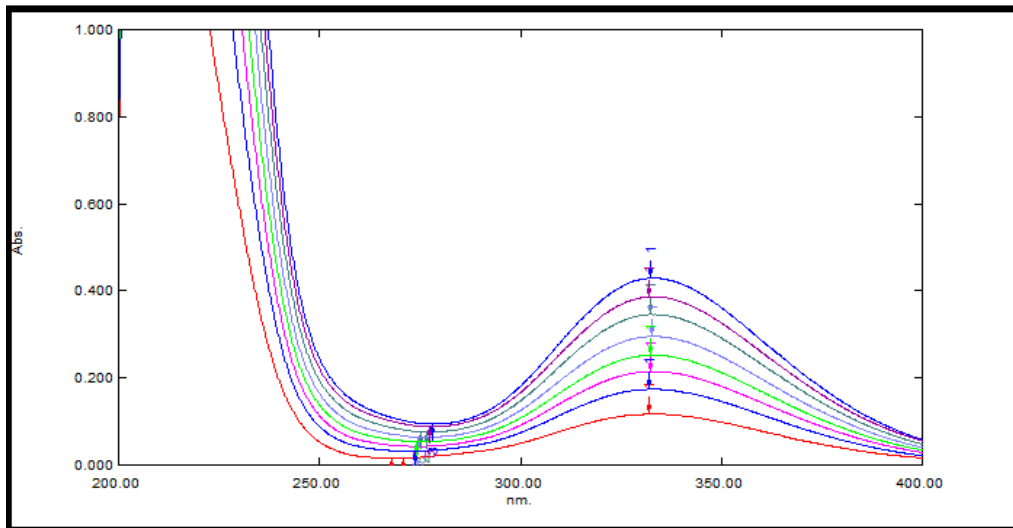Fig. 2: It shows spectra of alpha lipoic acid in methanol (zero order)

Fig. 3: It shows linearity curve for zero order derivative spectroscopy of alpha lipoic acid
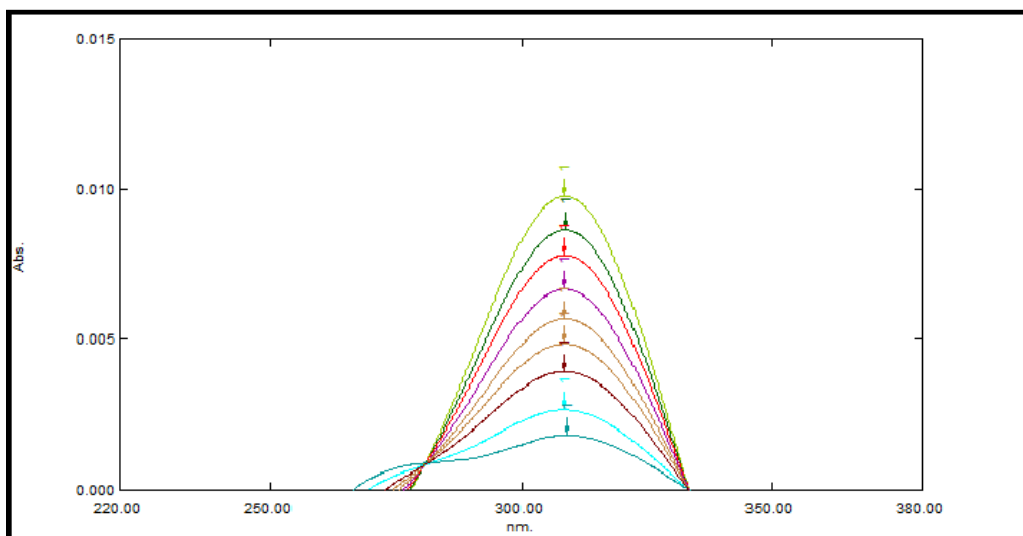

Fig. 4: It shows linearity curve for first order derivative spectroscopy of alpha lipoic acid

Spectrophotometry method using AUC technique- zero order derivative spectra (Method C)

It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. From the spectrum of drug, AUC in the wavelength range of 310.0 – 350.0 nm was selected for the analysis.

Spectrophotometry method using AUC technique-first order derivative spectra (Method D)

It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ3 and λ4. From the spectrum of drug, AUC in the wavelength range of 270.0 – 330.0 nm was selected for the analysis.
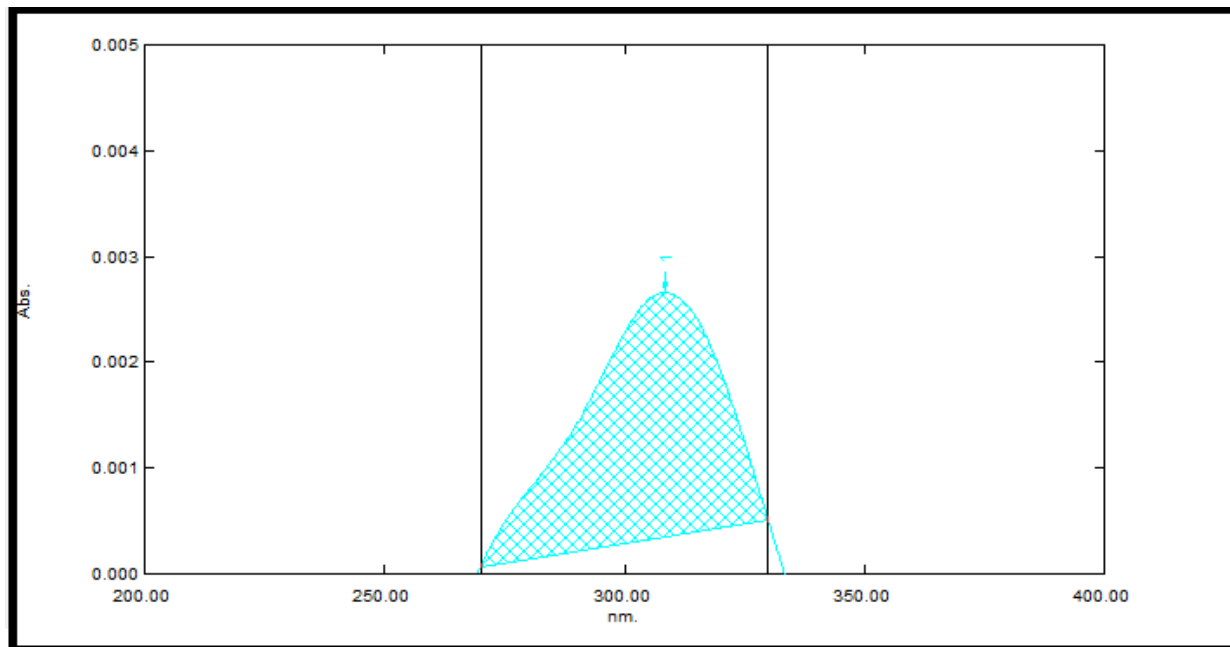

Fig. 5: It shows wavelength selection for area under curve method

Validation Parameter[8-10]

Linearity

In the experimental studies, the graph obtained for zero order, first order and area under the curve spectrum showed linear relationship. Regression analysis was done for the slope, intercept and correlation coefficient values.

Method precision (repeatability)

The precision of the methods was assessed by repeated scanning and measurement of the absorbance of solutions (n = 6) of LPA (250 µg/ml) without changing the parameters for the methods. The repeatability was expressed in terms of relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intra-day and inter-day precision of the proposed method was done by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of LPA (200, 250 and 300 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% recovery)

The accuracy of the method was performed by calculating % recovery of LPA by the standard addition method. Known amounts of standard solutions of LPA were added at 80, 100 and 120% levels to pre-quantified sample solutions of LPA (200 µg/ml). At each level of the amount three determinations were performed. The amount of LPA was estimated by applying obtained values to regression equation.[6, 7]

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were obtained from intercept.

Ruggedness

Ruggedness of the developed methods was determined by analysis of aliquots from homogenous slot by two analysts keeping same operational and environmental conditions. Results were interpreted by calculating the % RSD value and found to be within range.

Analysis of Tablet Formulation

For analysis of commercial formulation; twenty tablets were weighed, average weight determined and crushed into finely powder. An accurately weighed quantity of powder equivalent to 100 mg of LPA was transferred into 100 mL volumetric flask containing 50 mL methanol, shaken manually for 2 min; volume was adjusted to mark with same solvent and filtered through Whatmann filter paper if necessary. From above solution 2.0 mL was transferred to 10 mL volumetric flask, volume was adjusted to the mark and used for estimation of LPA. The analysis was repeated for six times. There was no interference from the excipients commonly present in the tablets. The low % R.S.D. value indicated the suitability of this method for routine analysis of LPA in tablet dosage form.

RESULTS AND DISCUSSION

The methods discussed in present work provide a convenient and accurate way for analysis of Alpha lipoic acid in its pharmaceutical dosage form. For 'Method A', absorbance maxima was selected at 322 nm; 'Method B', absorbance maxima was selected at 309 nm; in 'Method C', AUC was included between wavelengths 310.0 – 350.0 nm; in 'Method D', AUC was included between wavelengths 270.0 – 330.0 nm. Results for linearity, repeatability, reproducibility, sensitivity and Ruggedness were discussed in table 1. Results of accuracy study and analysis of marketed formulation were discussed in table 2 & 3 respectively.

Table 1: It shows optical and regression characteristics and validation parameters

Parameters	Method-A	Method-B	Method-C	Method-D
Beer's Law Limit (µg/ml)	150-500µg/mL	150-500µg/mL	150-500µg/mL	150-500µg/Ml
Molar Absorptivity (1 mole^{-1} cm^{-1})	147.4228	4.1266	1028.555	138.6331
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	6.78× 10^{-6}	2.42 × 10^{-4}	9.72 × 10^{-7}	7.21× 10^{-6}
Regression equation (y* = a+bc)	7.145	0.2	49.85	6.719
Slope (b)	-0.002	-0.001	0.010	- 0.004
Intercept (a)				
Correlation Coefficient (r^2)	0.999	1.00	0.999	0.999
LOD (µg/ml)	44.16	26.51	13.02	1.64
LOQ (µg/ml)	147.21	88.38	19.38	5.49
Precision				
Intra-day (n=6) (% CV)	0.80-1.77	0.34-0.76	1.02-1.25	0.98-1.21
Inter-day (n=6) (% CV)	1.69-1.87	0.55-0.94	1.11-1.34	0.95-1.33
Ruggedness (% CV)				
Analyst-1	0.449	0.548	0.129	0.239
Analyst-2	0.371	0.623	0.095	0.152

Table 2: It shows recovery studies

Amt. of sample taken (µg/ml)	Amt. of std. added (µg/ml)	Total amt. (µg/ml)	Total amount found (µg/ml)±SD				% Recovery			
			Methods				Methods			
			A	B	C	D	A	B	C	D
200	160	360	358.25 ±0.56	361.56 ±0.52	362.78 ±0.69	360.60 ±0.38	99.52	100.43	100.77	100.17
200	200	400	402.19 ±0.25	401.21 ±0.16	399.96 ±0.19	398.60 ±0.24	100.55	100.30	99.99	99.65
200	240	440	440.12 ±0.12	441.87 ±0.24	438.98 ±0.09	439.97 ±0.18	100.03	100.43	99.79	99.99

Table 3: It shows analysis of marketed formulation

Formulation	%Amount found ±SD using zero order derivative UV (Method A)	%Amount found ±SD using first order derivative UV (Method B)	%Amount found ±SD using zero order derivative AUC method (Method C)	%Amount found ±SD using first order derivative AUC method (Method D)
BRAND-1 (300mg)	300.12±0.22	298.66±0.52	297.98±0.79	296.13±0.77
BRAND-2 (300mg)	298.26±0.46	299.22±0.45	295.78±0.67	297.65±0.68

CONCLUSION

All methods that were developed for the determination of α-LPA are based on different analytical techniques like Zero order Spectrophotometric, first order Spectrophotometric and AUC method for zero & first order spectra. All the methods were validated and found to be simple, sensitive, accurate, and precise. Hence, above all methods can be used successfully for routine analysis of tablet dosage forms of LPA.

REFERENCES

1. Poongothai S, Ilavarasan R, Karrunakaran C. Simultaneous And Accurate Determination Of Vitamins B1, B6, B12 And Alphalipoic Acid In Multivitamin Capsule By Reverse–Phase High Performance Liquid Chromatographic Method. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(4):0975-1491.
2. Teichert J, Preiss R. High-Performance Liquid Chromatographic Assay for Alpha-Lipoic Acid and Five of Its Metabolites in Human Plasma and Urine. J Chromatogr: B Analyt Technol Biomed Life Sci 2002; 769(2):269-81.
3. Perricone N, Nagy K, Horvath F, Dajko G, Uray I. Alpha Lipoic Acid (ALA) Protects Proteins against the Hydroxyl Free Radical-Induced Alterations: Rationale for Its Geriatric Topical Application 1999; 29(1):45-56.
4. Khan A, Khan M, Iqbal Z, Ahmad L, Shah Y, Watson D. Determination of Lipoic Acid in Human Plasma by HPLC-ECD Using Liquid-Liquid and Solid-Phase Extraction: Method Development, Validation and Optimization of Experimental Parameters 2010; 878(28):2782-8.
5. Loni B, Ghante R. Spectrophotometric Estimation of Cinacalcet Hydrochloride in Bulk and Tablet Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(3):513-515.
6. Ganesan M, Solairaj P. A Simple Spectrophotometric Method for the Estimation of Mecobalamin in Injections. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(3):559-562.
7. Moitra S, Ghatak S. Derivative Spectrophotometric Determination of Rosiglitazone Maleate in Bulk Drug and Pharmaceutical Formulation. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(2):201-204.
8. ICH Q2 R1. Text on Validation of Analytical Procedures, International Conference on Harmonization Tripartite Guidelines 1995.
9. ICH, Q2A. Harmonised Tripartite Guideline, Test On Validation Of Analytical Procedures, IFPMA, In: Proceedings Of The International Conference On Harmonization, Geneva 1994.
10. ICH, Q2B. Harmonised Tripartite Guideline, Validation Of Analytical Procedure Methodology, IFPMA, In: Proceedings Of The International Conference On Harmonization, Geneva 1999.