



## Analytical Method Development and Validation for Estimation of Alpha Lipoic Acid in Bulk and Pharmaceutical Dosage Form by UV Spectrometric Method

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**ABSTRACT:** A rapid, specific and economic UV spectrophotometric method has been developed to determine the alpha lipoic acid content in bulk and pharmaceutical dosage formulations. At a pre-determined *absorption maxima* of 334 nm, it was proved linear in the range of 100-500µg/ml, and exhibited good correlation coefficient ( $R^2=0.998$ ) and excellent mean recovery (99.8% to 101.60%). This method was successfully applied to the determination of alpha lipoic acid and validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of alpha lipoic acid in bulks as well as in the commercial formulations.

**Keywords:** UV Spectroscopy; Method Development; Validation; Alpha Lipoic Acid and Regulatory Requirements for Drug Development.

**INTRODUCTION:** Alpha lipoic acid was first isolated by Reed and coworkers as an acetate replacing factor. It is slightly soluble in Water, and soluble in organic solvents. Alpha lipoic acid is a chiral molecule. Alpha lipoic acid is known by a variety of names, including thioctic acid; 1,2-diethylene-3 pentanoic acid; 1,2-diethylene-3 valeric acid; and 6,8-thioctic acid. Alpha lipoic acid found to be synthesized by animals and humans.<sup>1</sup> Alpha Lipoic acid (thioctic acid) is a potent anti-oxidant that has been widely used in food supplement preparations. Alpha lipoic acid has been used to alleviate peripheral pain in severe diabetic patients and its application in food preparations is getting popular. Alpha lipoic acid is usually present in the mitochondrial matrix in the cells of organisms where cells metabolisms and energy production take place. Alpha lipoic acid normally exists in the reduced form in living organisms.<sup>2</sup> Various beneficial effects of Alpha lipoic acid, e.g. skin whitening effect, inhibition of adipocytes production and growth promoting effect on Alpha lipoic acid ingredient for weight loss, cosmetics and anti-oxidative preparations muscle cells. Alpha lipoic acid is an antioxidant, an anti-diabetic drug which helps mainly to convert glucose (blood sugar) into energy.

In patients with type II Diabetes Mellitus, both acute and chronic administration of alpha lipoic acid improves insulin resistance, reduces plasma fructosa- mine levels.<sup>3</sup> Analysis is an important component in the formulation development of any drug molecule.<sup>4-8</sup> A suitable and validated method has to be available for the analysis of drug in the bulk, in drug delivery systems, from release dissolution studies and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples.<sup>9-12</sup> The efficient analytical method development and its validation are critical elements in the development of pharmaceuticals. An analytical method is selected on the basis of criteria such as accuracy, precision, sensitivity, selectivity, robustness, ruggedness, and the amount of available sample, the amount of analyte in the sample, time, cost, and availability of equipment.<sup>13-25</sup> Thus, present study was undertaken to develop and validate a simple sensitive, accurate, precise and reproducible UV method for Alpha Lipoic acid.

**MATERIAL AND METHODS:**

**Instruments:** The analysis was performed by using the analytical balance (Mettler), pH meter (Cyber scan), UV spectrophotometer (UV-Lambda 25, Perkin Elmer equipped with variable wavelength detector and data integration software).

**Reagents and solutions:** Alpha lipoic acid grade, Potassium dihydrogen phosphate & Sodium hydroxide analytical grade used.

**Preparation of solvent system:**

**Potassium dihydrogen phosphate ( $KH_2PO_4$ ):** 6.8 gm of dipotassium hydrogen phosphate was weighed accurately and transferred into a 1000 ml volumetric flask containing 900 ml of water and mixed well till clear solution obtained. pH of solution was adjusted up to 6.8 by using Sodium hydroxide. Finally volume make up to 1000 ml with water.

**Standard stock solution of alpha lipoic acid:** 100 mg of alpha lipoic acid weighted accurately and transferred into a 100 ml volumetric flask containing 60 ml water. Solution sonicated to dissolve alpha lipoic acid and cooled at room temperature then volume make up with water and mix well (Stock solution A). Pipette out 2 ml of standard stock solution, mixed well and diluted up to volume with 6.8 phosphate buffer solution. The resulting solution contains 0.2 mg/ml of alpha lipoic acid.

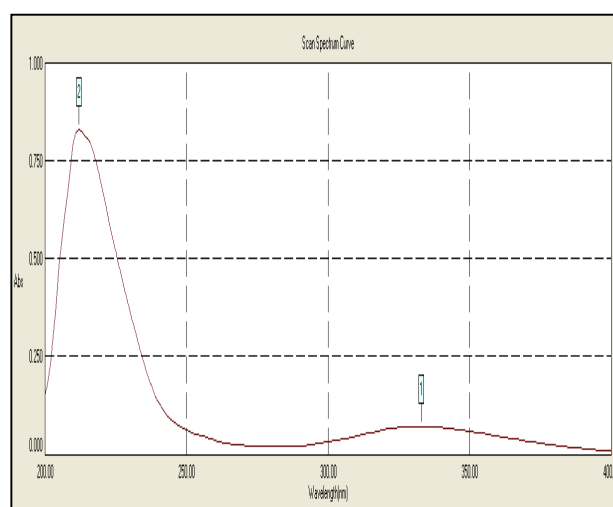
**Sample Stock Solution:** Average weight of the tablets was determined and fine powder made with the help of mortar and pestle. Transferred accurately equivalent to one tablet weight into a 500 ml volumetric flask containing 250 ml water and sonicated till clear solution, finally cooled at room temperature. Final volume made with water and mixed well. Prepared solution then centrifuge at 3500 rpm for 5 minutes and used as standard test solution (Stock solution C). 2 ml of clear supernatant sample stock solution were transferred into a 100 ml volumetric flask and dilute to volume with 6.8 phosphate buffer. The resulting solution contains 0.2 mg/ml of Alpha Lipoic acid.

**Spectral study:** The final stock solution scanned in UV spectrophotometer over the range 200-400nm (Figure-1).

**RESULTS AND DISCUSSION:** The methods discuss in the present work provide a convenient, precise and accurate way for alpha lipoic acid pharmaceutical dosage form. An absorption maximum of alpha lipoic

acid was selected at 334 nm for the analysis. Regression analysis shows linearity over the concentration range of 100- 500 $\mu$ g/ml for correlation coefficients of 0.998 (Figure 2). The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by re-covery studies and the results are expressed as percentage recovery. Percentage recovery for alpha lipoic acid was found within the range of 99.8 % and 101.61%.

The % RSD value for alpha lipoic acid was found to be less than 2%. In this study the alpha lipoic acid was carried out by UV Spectroscopy method satisfactorily. The result of developed method and validation was given in table 1.



**Figure 1: UV Spectra of alpha lipoic acid.**

**Table 1: Result of method development and validation.**

S. No.	Parameter		Result
1	SPECIFICITY (Interference of peaks)		No interference observed
2	PRECISION	System Precision	0.02%. RSD
3		Precision of Method	1.12% RSD
4			
5	LINEARITY		0.998
6	ACCURACY		99.8% to 101.60%
7	RUGGEDNESS		2.1% RSD
8	ROBUSTNESS		Complies

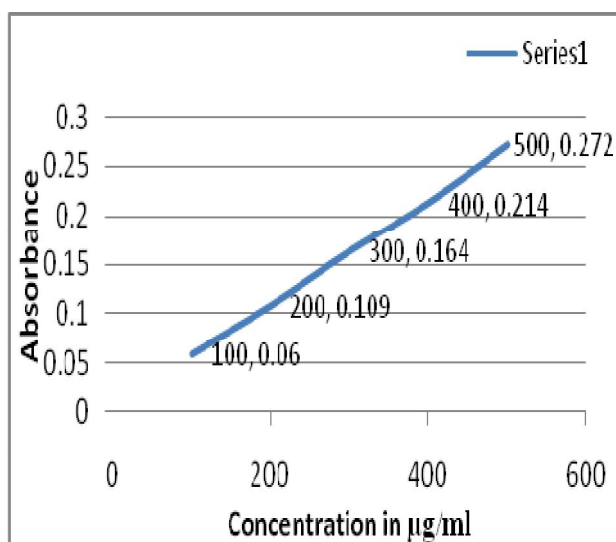


Figure 2: Calibration curve of alpha lipoic acid.

**CONCLUSION:** The analytical method for determination of alpha lipoic acid has been validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for determination of alpha lipoic acid in bulk as well as tablet formulation.

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