

# Determination of Alpha-Lipoic Acid in a Nutritional Supplement Using High Performance Liquid Chromatography

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## Abstract

Alpha lipoic acid has the ability to react and neutralize reactive oxygen species (ROS) such as superoxide radicals, simple oxygen, hydroxyl radicals, hypochlorous acid and peroxy radicals. A rapid high-performance liquid chromatographic method for determination of lipoic acid in a nutritional supplement was developed. The method involved sample preparation and the mobile phase comprised of 50 mM disodium hydrogen phosphate buffer (pH 2.5 adjusted with 1 M H<sub>3</sub>PO<sub>4</sub>): acetonitrile in the ratio of 50:50. The separation was done using a C18 column (150 mm) and detection was carried out using UV detection at 201 nm. The assay was found to be linear in the range of 1.56 - 50 µg/mL with the correlation coefficient of 0.9997. Method precision was determined while LOD was 0.05 µg/mL and LOQ 0.15 µg/mL. The chromatographic peak LA retention time was 6 min.

## Keywords

Alpha-Lipoic Acid, HPLC, Antioxidant Activity, Nutritional Supplement

## 1. Introduction

Alpha lipoic acid is an organosulfur compound derived from octanoic acid and shows a strong antioxidant activity against various conditions related to oxidative stress. Its synthesis is found endogenously in the human body, specifically in the mitochondria [1]. Alpha-lipoic acid contains two sulfur (S) atoms at C6 and C8 carbons joined by a disulfide bond resulting in oxidation. The carbon atom (C6) is asymmetric and two enantiomers are found on it: (R)-(+)-Lipoic Acid (RLA) and (S)-(-)-Lipoic Acid (SLA) (Figure 1) [2].

It acts as a cofactor for a variety of mitochondrial enzymes, catalyzing the oxidative decarboxylation of pyruvic, acetoglutarate and branched-chain  $\alpha$ -keto acids. In both its reduced and oxidized form, it has the ability to react and neutralize reactive oxygen species (ROS) such as superoxide radicals, simple oxygen, hydroxyl radicals, hypochlorous acid and peroxy radicals [3]. Free radicals have a dual role in the body. On the one hand, they have beneficial properties necessary for the proper functioning of the organism since, within normal limits, they protect against viruses, parasites and microbes, shielding its defenses, while at the same time participating in signal transduction, redox homeostasis, gene expression, cell differentiation and cell proliferation. On the other hand, when free radicals are present in high concentrations in the body they tend to cause problems with damaging effects on biomolecules such as lipids, proteins and DNA in general. The consequence of this is both the onset of premature aging and a number of diseases. This condition is called oxidative stress and is what is caused when there is an excess of free radicals in the body. In biological systems, the disruption of the balance between the production of reactive oxygen species (ROS) and the availability and action of antioxidant systems constitutes a condition called oxidative stress [4].

The occurrence of oxidative stress is multifactorial and is influenced by UV radiation, tissue oxygen deficiency, chemotherapy, radiotherapy and aging factors [5] [6] [7]. In addition, it is responsible for the manifestation of pathophysiological diseases and skin conditions. Some of them are cancer, Parkinson's and Alzheimer's disease, rheumatoid arthritis, asthma, diabetes and arteriosclerosis, heart diseases and hypertension, premature aging, inflammatory diseases, phototoxicity, photo allergy, and immune system diseases [8] [9] [10] [11]. Concerning Diabetes mellitus, it is a multi-faceted metabolic disorder where there is increased oxidative stress that contributes to the pathogenesis of the disease. This has stimulated many investigations into the use of antioxidants as a complementary therapeutic approach. Alpha lipoic acid prevents beta cell destruction, improves glucose uptake, and its antioxidant effects may be helpful in reducing the development of diabetic complications such as diabetic neuropathy [11].

Alpha-lipoic acid is the only antioxidant substance with a dual nature as a partially water-soluble and fat-soluble antioxidant, with the consequence that its antioxidant action affects the whole body, inside and outside the cells. In particular, its solubility is low in water and oils but particularly high in ethyl alcohol [12] [13].

Alpha lipoic acid as an antioxidant:

- 1) It neutralizes oxygen free radicals and interacts with active radicals in all cells of the body which demonstrates it as a universal antioxidant [14].
- 2) Acts as a chelating agent and removes heavy metals responsible for oxidative stress [7].
- 3) Reduces blood glucose levels during metabolic activity.

4) Protects lipids, proteins and nucleic acids from oxidation [15].

5) Protects cell membranes by interacting with vitamin C, glutathione, vitamin E, while participating in their endogenous regeneration mechanisms [4] [16] [17]. Alpha lipoic acid restores other antioxidants in the body's antioxidant network, including coenzyme Q10, glutathione and vitamins C and E when they are in reduced amounts [18] [19].

This has the effect of maintaining the antioxidant capacity of the human body. Alpha-lipoic acid is added to various cosmetics because of its properties. It is added to anti-aging cosmetics as an active ingredient against aging, creams, emulsions both for its anti-wrinkle action and improvement of facial wrinkles as well as for its antioxidant action and protection of collagen [20]. In addition, alpha lipoic acid has been found to exhibit photoprotective activity and can be incorporated into sunscreen products [21] [22]. At the same time, it may protect the skin barrier due to its moisturizing properties [23]. Finally, it has an anti-inflammatory action with both a reduction of swelling and prevention of erythema [24] [25] [26].

HPLC (High Pressure Liquid Chromatography or High-Performance Liquid Chromatography) is considered to be the most commonly used chromatographic technique for quantitative analysis of complex mixtures of substances. High Performance Liquid Chromatography is a sophisticated form of column chromatography, where the mobile phase does not flow under gravity, but by means of a pump. HPLC is an analytical chromatographic technique which is an important tool both in research and in the cosmetics, pharmaceutical and food industries. HPLC is an extremely useful analytical tool due to its speed, accuracy, repeatability and wide range of application. Separation results from the combination of two immiscible phases of a static and a mobile phase and are based on the different distribution, adsorption, ion exchange, molecular blocking, or affinity of the substance to be analyzed between a static and a mobile phase.

## 2. Experimental

### 2.1. Reagents and Standards

Alpha-LA pure powder was purchased from Sigma Chemical Company, acetonitrile (ACN) (HPLC grade), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) were purchased from Chem-Lab NV. Deionized water was processed through a Milli-Q water purification system (Millipore, USA). The dietary supplement used was Nevalip 600 retard in tablets from Medical Pharma quality. The main ingredients included were: Alpha Lipoic Acid, Chromium Picolinate, Selenium,

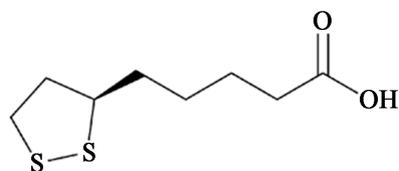


Figure 1. Structure of alpha-lipoic acid [2].

Zinc, Vitamins B1, B5, B6 and Biotin.

## 2.2. Instrumentation

Chromatographic analysis was performed using HPLC-UV Dionex, Model Ultimate™ 3000 HPLC System, consisting of a pump with a 20 µL inlet loop and a UV-visible detector, DAD UV/Vis detector. The analytical column was a C18, 250 × 4.6 mm ID, 5µ particle size (Hichrome, England). The mobile phase comprised of 50mM disodium hydrogen phosphate buffer (pH 2.5 adjusted with 1M H<sub>3</sub>PO<sub>4</sub>): acetonitrile in the ratio of 50:50. Prior to the preparation of mobile phase, the solvents were degassed separately using a Millipore vacuum pump with 0.45 µm filter paper. Sonication was done for 10 min to remove dissolved gas. The UV detector was set at 201 nm. The sample (20 µL) was injected into the HPLC system and the chromatogram was run for 10 minutes at a flow rate of 1 mL/min, with column oven temperature maintained at 40°C.

## 2.3. Preparation of Standard Solution

Stock standard solution of (1000 µg/mL) of LA was prepared by dissolving the pure powder in ACN. The concentrations of LA in the working standards were 1.56, 3.125, 6.25, 12.5, 25 and 50 µg/mL. The solutions were kept frozen at -20°C until analysis.

## 2.4. Sample Preparation

Weigh accurately 0.162 g of *α*-lipoic acid and dissolve with 100 mL ACN, followed by stirring in a vortex for 1 min, dissolving in an ultrasonic bath for 15 min and centrifugation for 12 min at 12500 rpm. A supernatant solution was analyzed chromatographically.

Solution A consisted of 20 µL of supernatant in 900 µL of ACN.

Solution B consists of 40 µL of supernatant in 900 µL of ACN.

## 2.5. Results

The detector at which the analyte showed optimal response was selected as the optimized range for the determination of LA. The UV detector at 201 nm showed an optimal response and it was used for measurement of LA content. This was performed using photodiode array (PDA), though LA can be detected using UV detector, as the detection wavelength falls within the range of UV detector. The chromatographic peak LA retention time was 6 min.

The peak for LA was observed linear at different concentrations. The calibration curve of LA standard solutions constructed using six concentrations shows good linearity in the range 1.56 to 50.0 µg/mL as shown in **Figure 2** (correlation coefficient (*r*) of 0.9997). The peak area is measured into mAu/min.

Precision is the most used quality attribute of an analytical method. Precision was attributed through repeatability. Repeatability is presented in **Figure 2**, where standard solutions 1.56, 12.5, 50 µg/mL were measured three times and

coefficient of variation, CV was calculated (Table 1).

The assessment of detectability was made by calculating the limit of Detection (LOD) and the Limit of Quantification (LOQ). Therefore, it was measured a series of samples (after dilution the standard solution 1.56 µg/mL 1/2) (n=10) and the standard deviation was calculated (Table 2).

The Limit of Detection LOD, was  $LOD = 3 \times SD = 0.05 \mu\text{g/mL}$  and the Limit

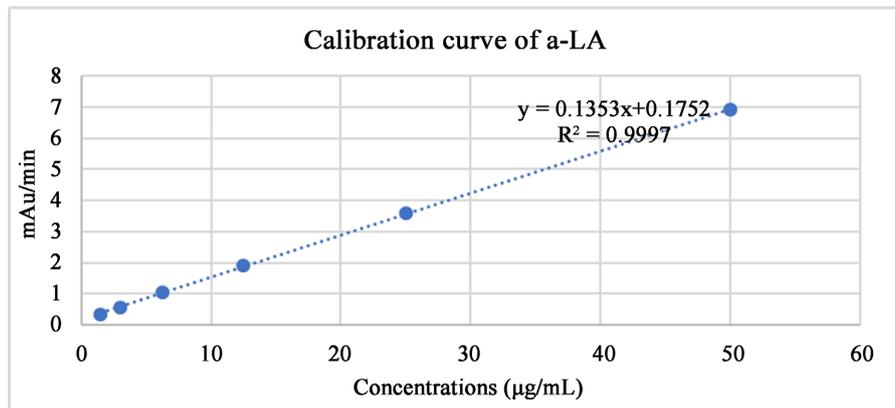


Figure 2. Calibration curve of alpha-LA.

Table 1. Method repeatability.

LA	mAu/min			Average	SD	CV%
Concentration µg/mL	1	2	3			
1.56	0.3300	0.4301	0.3507	0.370267	0.052841	14.0
12.5	1.8956	1.9678	2.001	1.9548	0.053889	2.7
50	6.9094	7.0987	6.9987	7.002267	0.0947	1.3

Table 2. Measurements of a series of samples (after dilution the standard solution 1.56 µg/mL 1/2) (n = 10).

A/A	Peak area mAu/min
1	0.150
2	0.165
3	0.124
4	0.130
5	0.153
6	0.143
7	0.129
8	0.167
9	0.148
10	0.156
SD	0.014931

**Table 3.** Measurements of solutions A and B.

LA	mAu/min	mAu/min	mAu/min				
	1	2	3	Average	SD	CV%	Concentration µg/mL
A	2.3359	2.1868	1.7658	2.096167	0.241405	13.6	14.2
B	3.8083	4.2200	3.9765	4.0016	0.16901	4.2	28.3

of Quantification LOQ, was  $LOQ = 10 \times SD = 0.15 \mu\text{g/mL}$ . In **Table 3**, the measurements of solutions A and B and the estimated concentrations of them, were calculated. The calculated concentrations were equivalent to those reported in the specific nutritional supplement used.

### 3. Conclusion

A method HPLC-UV was developed for the quantification of alpha-lipoic acid in a nutritional supplement. The method involved sample preparation and the mobile phase comprised of 50 mM disodium hydrogen phosphate buffer (pH 2.5 adjusted with 1 M  $\text{H}_3\text{PO}_4$ ): acetonitrile in the ratio of 50:50. The separation was done using a C18 column (150 mm) and detection was carried out using UV detection at 201 nm. The assay was found to be linear in the range of 1.56 - 50 µg/mL. LOD was 0.05 µg/mL and LOQ 0.15 µg/mL. The chromatographic peak LA retention time was 6 min. The validated HPLC-UV method may be applied to the measurement of LA levels in cosmetology analysis. Further the LA concentrations can be confirmed by LC-MS quantification using different nutritional supplements.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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