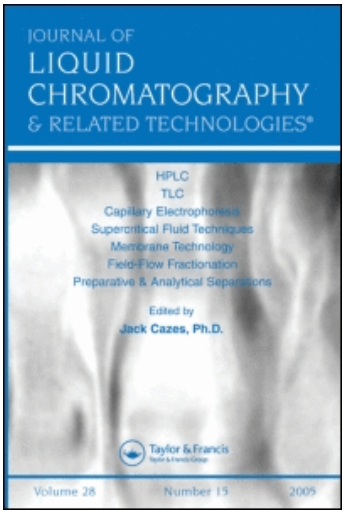

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Validated Method for Determination of α-Lipoic Acid in Dietary Supplement Tablets by Reversed Phase Liquid Chromatography

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ABSTRACT

A rapid high performance liquid chromatographic method was developed and validated for the determination of α-lipoic acid in pharmaceutical dosage forms. The analysis was performed using a reversed phase Supelcosil LC-18 (150 × 4 mm, 3 μm) column. The mobile phase consisted of acetonitrile : 0.05 M potassium mono-phosphate, pH 2.5 (45 : 55 v/v) at a flow rate of 0.8 mL/min. The UV-detector was set at 332 nm. The developed method showed a good linear relationship in the concentration range from 10–500 μg/mL with a correlation coefficient from 0.9999.

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The limit of detection and limit of quantification were 4.4 and 16.8 μg/mL, respectively. These values are high due to the low absorption coefficient of pure lipoic acid ($\varepsilon = 150$) at 332 nm. Statistical analysis proves that the method is reproducible.

Key Words: HPLC; α-Lipoic acid; Dietary supplement; Pharmaceutical analysis; High performance liquid chromatography.

INTRODUCTION

α-Lipoic acid is a natural, disulphide-containing compound and is chemically known as DL-6,8-thioctic acid, 6,8-dithiooctanoic acid, and 1,2-dithiolane-3-valeric acid (Fig. 1).

Lipoic acid is a cofactor that has been reported to be present in a diverse group of microorganisms and a variety of plant and animal tissues. α-Lipoic acid is an antioxidant and a coenzyme needed for the activity of enzyme complexes, such as those of pyruvate dehydrogenase and glycine decarboxylase. Exogenous thioctic acid is reduced (to dihydrolipoic acid) intracellular by two or more enzymes. The reduced form influences a number of cell processes by direct radical scavenging, recycling of other antioxidants, such as glutathione synthesis, and in turn, enhancing vitamin E. It also decreases the phagocytosis of myelin by macrophages.[1,2]

Furthermore, lipoic acid can regenerate vitamin C from its oxidized form, as well as regenerate other antioxidants.[3] The level of glutathione, which is a very important antioxidant increases after intake of lipoic acid. Lipoic acid is also used for the treatment of diabetes including diabetic neuropathy and diabetic nephropathy.[4] Treatment of diabetic patients with alpha lipoic acid reduces oxidative stress and urinary albumin excretion and slows the progression of endothelial cell damage. Furthermore, loss of taste and smell

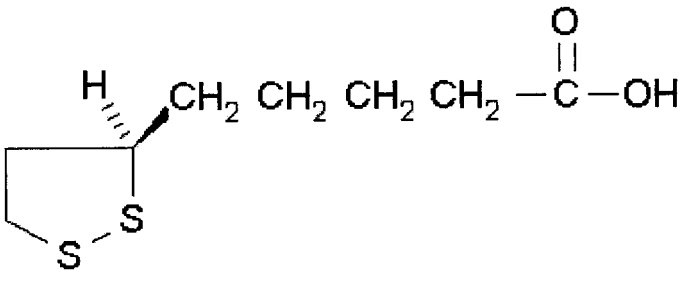

Figure 1. Chemical structure of α-lipoic acid.

could be regenerated by lipoic acid. The intake of this antioxidant gives a slight visual enhancement along with a mildly relaxed sense of well-being.[5] Several methods had been reported for the analysis of lipoic acid including microbiological assay,[6] enzyme immunoassay,[7] and spectrophotometric methods.[8,9] Kataoka[10] had published a comprehensive review on the chromatographic method of analysis of lipoic acid. A capillary electrophoretic method using electro kinetic chromatography has been reported by Panak et al.[11] Recently, Bernkop–Schnürch[12,13] described a sustained release dosage form for α-lipoic acid and evaluated the release of lipoic acid from the formulation in vitro and in human volunteers, by high performance liquid chromatography.

EXPERIMENTAL

Chemicals and Reagents

Lipoic acid was obtained from Serva Feinbiochemical Heidelberg, Germany (Art. Nr. 28000), HPLC-grade methanol, acetonitrile, potassium phosphate (monobasic), and *o*-phosphoric acid were purchased from Fluka, Buchs, Switzerland. Capsules of lipoic acid (50 mg of alpha lipoic acid per capsule) were obtained from General Nutrition Corp., Pittsburgh, PA.

Instrumentation

The chromatographic system consisted of a Waters pump, Model 510, Milford, MA, equipped with a Waters Lambda Max Model 481, UV-detector was set at 332 nm. The column was a Supelcosil LC-18 (150 × 4 mm, 3 μm) and the integrator a Waters 746, Data Module. Injections were carried out using a 20 μL loop at room temperature. The pH-meter used was Orion Research (Model 611), Orion Research Inc., USA. The Millipore Milli-Q Plus System (Bedford, MA) was used for deionized water. The mobile phase was filtered through a Millipore membrane filter (0.2 μm) from Nihon, Millipore (Yonezawa, Japan) and degassed before use.

Preparation of Standard Stock Solution

A stock solution of α-lipoic acid was prepared by accurately weighing 100 mg of the analyte and dissolving it in acetonitrile to 100 mL volumetric

flasks. Serial dilutions were carried out to obtain the concentration ranges of 10–500 μg/mL.

Preparation of Standard Solution of α-Lipoic Acid Capsules

One capsule was weighed and its content was extracted with methanol. This solution was sonicated (30 min) and after centrifugation the clear supernatant was injected.

Quantitation and Linearity

Equal concentrations of the standard solution and sample solution were injected into the chromatograph and the chromatograms were recorded (Fig. 2). A calibration curve was constructed. The data were treated by linear least square regression analysis,[14] and the slope, intercept, and correlation coefficient data were calculated.

Selectivity

The selectivity of the proposed method was investigated by observing any interference encountered from excipients present in the formulations. No interference from the tablets excipients was observed using the proposed method.

Validation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by using the calibrating curves[14] along with the correlation coefficient, slope, and intercept. The good linearity of the calibration curve and the negligible scatter of experimental points are clearly evident by the values of the correlation coefficient and standard deviation.

Chromatographic Conditions

Few reports have been cited on the determination of lipoic acid and related compounds by HPLC.[11,15,16] Howard and McCormick[17] reported that lipoic acid and some of its analogues could be separated by reversed phase (RP) HPLC on a μ-Bondapak C18 column (30 × 3.9 cm I.D.) using UV-detection

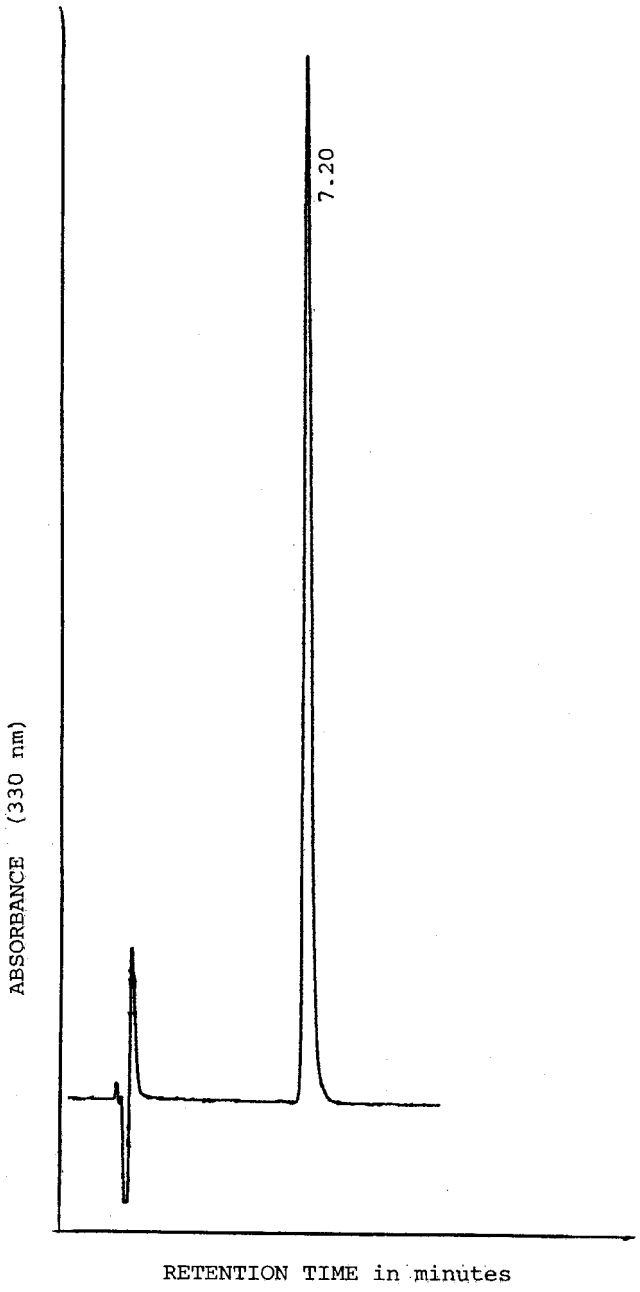

Figure 2. Chromatogram of standard α-lipoic acid.

at 330 nm (absorption maximum of the dithionate ring), with or without a gradient elution system. Due to the low absorbance coefficient of α-lipoic acid it was not easy to find a simple, rapid, accurate, and reproducible method to examine the lipoic acid content in the dietary supplement capsules (Table 1). An ideal chromatographic method should determine the drug content and also be able to resolve the drug from its contaminants. Hence, an attempt is made to develop an accurate, rapid, and reproducible method for the determination of α-lipoic acid in pharmaceutical supplement dosage forms (Fig. 3) (Table 2).

The mobile phase used in this study was acetonitrile : potassium monophosphate [0.05 M] pH2.5 (45 : 55 v/v). A stock solution of α-lipoic acid (1 mg/mL) was prepared in acetonitrile. A series of standard curves were prepared over a concentration range of 10–500 μg/mL. The polynomial regression data for the calibration plots ($n = 3$) showed an excellent linear relationship over a concentration range of 10–500 μg/mL. The correlation coefficient was 0.999 (SD = 1.56). The data were treated by linear least square regression analysis.[15]

Precision and Accuracy

The precision and accuracy was tested by injecting 10 times the same concentration of analyte, the area showed a SD from 1519 with a mean of 56,000, RSD of 2.71% (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and the LOQ were, respectively, 4.4 and 16.8 μg/mL. These concentrations are high due to the low absorption coefficient of pure lipoic acid ($\varepsilon = 150$) at 332 nm. Statistical analysis proves that the method is reproducible.

Table 1. Accuracy and precision data for the analysis from lipoic acid in dietary supplement capsules.

	Concentration (mg/L)	Measured concentration (mg/L)	Recovery (%)	Error (%)	RSD (%)
$n = 6$	1.000	0.670	67	33	1.34
$n = 3$	0.100	0.066	66	34	5.41

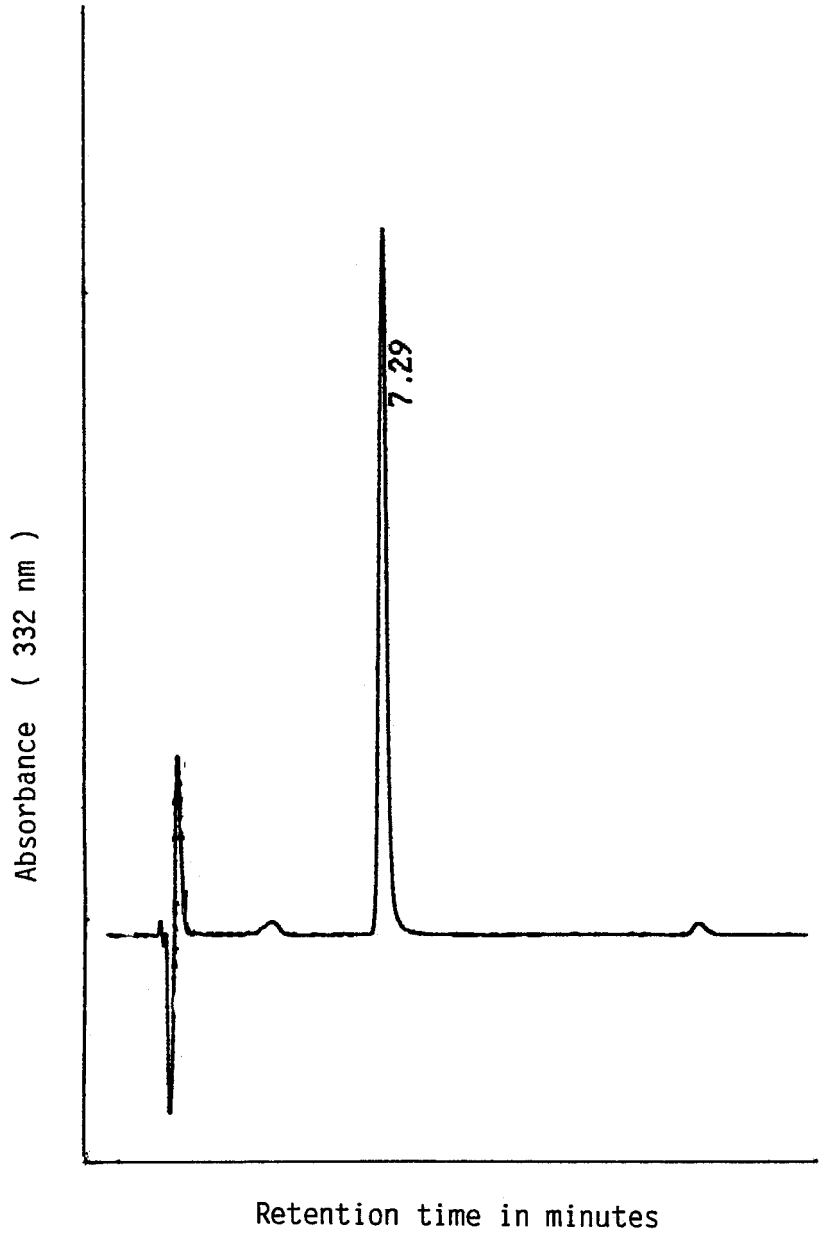

Figure 3. Chromatogram of α-lipoic acid capsule.

Table 2. Validation parameters for the determination of α-lipoic acid in dietary supplement capsules.

Parameter	Values
Concentration range (μg/mL)	10–500
Intercept (a)	744
Slope	−1,099
Correlation coefficient	0.9999
LOD (μg/mL)[a]	4.4
LOQ (μg/mL)	16.8

[a]$S/N = 5$.

Recovery

The recovery was measured after testing the solubility of lipoic acid in several different solvents. Methanol and acetonitrile as solvent, had a recovery of 95–100%, respectively ($n = 5$) with a relative standard deviation of 0.26%. The recovery of the lipoic acid in the sample was 98.8% (Table 4).

CONCLUSION

A precise, accurate, and validated HPLC method has been developed for the determination of lipoic acid in dietary supplement capsules. The method utilizes a Supelcosil C18 column under reversed phase mode with UV detection. There are no interferences by other excipients in the tablet. This method can be used for routine determination and chromatographic purity of lipoic acid in bulk material and in dosage forms for quality control purposes.

Table 3. Inter- and Intra day precision of α-lipoic acid.

	Concentration in mg/L	SD	Average	RSD (%)
Intra-day $n = 6$	0.100	965	55,161	1.7
Inter-day[a] $n = 3$	0.100	971	72,399	1.3

[a]Determined for 7 days.

Table 4. Recovery with spiking the sample (+50%, +100%, +150%).

Recovery (%)					
Nominal	Measured	Diff. (%)	Mean	SD	RSD (%)
150.00	149.32	0.68	5,53,961	777	0.14
200.00	198.20	1.80	7,35,308	779	0.11
250.00	247.97	2.03	9,19,936	729	0.08
$n = 5$					
Drug content		98.80%	7,34,642	1,933	0.26

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