ANALYTICAL METHODS OF ALPHA LIPOIC ACID FORMULATIONS

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ABSTRACT

Alpha lipoic acid is an antioxidant, used in the treatment of diabetic neuropathy and brain disorder. It is available in market either alone or in combination with various drugs. Various analytical methods have been developed for the estimation of alpha lipoic acid present either alone or in combination with other drugs. A review, of the reported analytical methods, is presented.

KEYWORDS: HPLC, alpha lipoic acid, UV spectroscopy, HPTLC, analytical method.

INTRODUCTION

Alpha lipoic acid (ALA), also known as α lipoic acid and thiotic acid, is an antioxidant which is an organosulphur compounds derived from octanoic acid. The alpha lipoic acid chemically called (R) – 5-(1, 2 –Dithiolane -3-yl) -pentatonic acid. Alpha lipoic consist of two sulfur atoms at C6 and C8 which is connected by a disulphide bond.[1]

It is used for the treatment of brain disorders, diabetic polyneuropathy, AIDS, diabetes, heavy metal poisoning, ischemia reperfusion injury and liver diseases.[2] It is available as over the counter nutritional supplements.[3]
Alpha lipoic acid is a yellow solid and it contains a terminal carboxylic acid and a dithiolane ring at the other terminal side. Its molecular formula is C₈H₁₄O₂S₂ with molecular mass of 206.33g/mol.[1,4]

Lipoic acid is involved in oxidative decarboxylation of keto acids and is presented as a growth factor for some organisms. The carbon atom at C6 is chiral and it exist as two enantiomers, the R & S enantiomer. The R enantiomer of an amino acid is biologically active and the S enantiomer is involved in the inhibition of R-enantiomer when a racemic mixture is given. Alpha lipoic acid is found to be soluble in organic solvents like methanol, ethanol, diethyl ether, chloroform and also in water. The logP value of alpha lipoic acid is 3.40.[3,5]

![Figure 1: α lipoic acid.](image)

**Source**

Vegetables and animal tissue are the main source of alpha lipoic acid. Some of the vegetables that contain alpha lipoic acid are spinach, broccoli, and tomatoes and its found in the form of lipoyllysine (attachment of LA to specific lysine residue). Spinach, broccoli, tomatoes contain 3.2, 0.9 and 0.6 x 10⁻³ g lipoyllysine /g dry mass respectively. In animal tissues, the high concentration of lipoyllysine is found in kidney, heart and liver, which contain 2.6, 1.5 and 0.9 x 10⁻³ g lipoyllisine /g dry mass respectively.[1,6]

**ANALYTICAL METHODS**

Literature revealed that most of the methods reported are HPLC methods with few spectrophotometric methods and one HPTLC method. Among the reported HPLC methods one method is stability indicating analytical method.

**High performance liquid chromatography**

Rajkumar et al.[2] have developed a HPLC method for the estimation of alpha lipoic acid and allopurinol in which the mobile phase used was acetonitrile: 0.02M ammonium acetate buffer in the ratio 50:50 v/v and the pH was adjusted to 4.6. Separation has been achieved on
reverse phase C18 column with a flow rate of 0.8ml/min and UV detector wavelength of 210 nm. This resulted in elution of alpha lipoic acid at 8.42min and allopurinol at 3.01min. The method was found to be linear in the concentration range of 50 µg /mL -175 µg /mL for both alpha lipoic acid and allopurinol with a correlation coefficient of 0.9996 for alpha lipoic acid and 0.9994 for allopurinol. This method has a longer elution time for alpha lipoic acid .The method is simple, precise, accurate and can be used for routine analysis.

Kothari et al.\[^7\] have reported a rapid and precise liquid chromatographic method for simultaneous determination of alpha lipoic acid and docetaxel in lipid based nanoformulation. In this method the mobile phase used was acetonitrile: sodium acetate buffer in the ratio 65:35 v/v at pH 3.5 with a flow rate of 1ml/min. It has a retention time of 4.84 min for alpha lipoic acid and 5.82 min for docetaxel. This method has been validated according the ICH guidelines. The linearity was found in the range of 1-15µg/ml for docetaxel and 2-30 µg/ml for alpha lipoic acid and with a correlation coefficient of 0.9999 for both alpha lipoic acid and docetaxel. This method has shorter elution time for alpha lipoic acid and the method is linear. The method is simple, precise, accurate and can be used for routine analysis.

Padmaja et al.\[^8\] have described a stability indicating reverse –phase high performance liquid chromatography method for simultaneous estimation of methylcobalamin, alpha-lipoic acid , pyridoxine HCL, and folic acid in combined dosage form. The chromatographic separation was achieved on a C18 column with a mobile phase of hexane -1- sulphonicacid: acetonitrile in the ratio 10:90 v/v, the flow rate was adjusted to 1ml/min using 285nm as the UV detector wavelength. This resulted in the elution of alpha lipoic acid, methylcobalmin, pyridoxine hydrochloride and folic acid at 6.7 min, 3.5 min, 8.5 min and 9.3min respectively. The method was found to be linear in the range 0-2130 µg/ml for methylcobalamin , 0-142.5 µg/ml for alpha lipoic acid, 0-4.54µg/ml for pyridoxine hydrochloride and 0-2µg/ml for folic acid. The correlation coefficient for each drug was found to be 0.999. This method has a shorter retention time for alpha lipoic acid. The method is simple, precise, accurate and can be used for routine analysis.

Patel et al.\[^9\] have developed and validated a RP HPLC method for simultaneous estimation of alpha lipoic acid and metformin in their tablet dosage form. The mobile phase used was phosphate buffer: acetonitrile 60:40 v/v. The flow rate was adjusted to 1.2 ml/min. This resulted in the elution of alpha lipoic acid at 8.983 min and metformin at 2.003 min. The method was precise, specific and selective. The linearity was found to be between 2-12 µg/ml
and 150-500µg/ml for alpha lipoic acid and metformin HCL respectively. The correlation coefficient was found to be 0.9984 for alpha lipoic acid and 0.9989 for metformin HCL. This method has taken a longer retention time for alpha lipoic acid. The method is simple, precise, Accurate and can be used for routine analysis.

Nandini et al.\cite{10} have developed a simple and validated RP HPLC method for the estimation of methylcobalamin and alpha lipoic acid in soft gelatin capsule dosage form. The mobile phase used was 0.02M phosphate buffer: acetonitrilein the ratio 60: 40. Detection was carried out at 240 nm and 266 nm for alpha lipoic acid and methylcobalamin respectively. The method developed is linear 80-120% for alpha lipoic acid and75 -200% for methylcobalamin and the correlation coefficient was found to be 0.99995 and 0.99941 for methycobalamin and alpha lipoic acid respectively. The method is simple, precise, accurate and can be used for routine analysis.

Anil et al.\cite{11} reported an RP-HPLC method development and validation for the simultaneous quantitative estimation of pregabalin, mecobalamin and alpha lipoic acid in capsules, the mobile phase used in this study was potassium dihydrogen orthophosphate buffer, methanol and acetonitrile which is in the ratio 75:10:15, the flow rate was set as 1.2ml/min and the detection wavelength was 210nm using UV detector. The retention time obtain for alpha lipoic acid, pregabalin and mecobalamin are 11.3min, 2.6 min and 6.4 min respectively. The developed method is linear in the range 187.5-750µg/ml, 1.87-7.5µg/ml ,250-1000µg/ml for pregabalin, mecobalamin and alpha lipoic acid respectively. The method is simple, precise, accurate and can be used for routine analysis.

Poongothhal et al.\cite{12} reported a simultaneous and accurate determination of vitamin B1, B6, B12 and alpha lipoic acid in multivitamin capsule by reverse – phase high performance liquid chromatography. The mobile phase used in this method is phosphate buffer and acetonitrile in the ratio 50:50. The detection wavelength was selected in the range of 200-600nm. The method is linear in the range of 489.9-1469.6µg/ml,499.4-1498.1µg/ml,4.6-13.9µg/ml and 497.7-1469.6µg/ml for vitamin B1, B6, B12 and alpha lipoic acid respectively and the R² value is 0.9997, 0.9990, 0.0995, 0.9998 for vitamin B1, B6, B12 and alpha lipoic acid respectively. The method is simple, precise, accurate and can be used for routine analysis.

Khan et al.\cite{13} developed a simultaneous determination of the endogenous free alpha lipoic acid and dihydrolipoic acid in human plasma and erythrocytes by RP-HPLC with
electrochemical detection. The mobile phase used in this is acetonitrile and phosphate buffer at pH of 2.5. The analytes got separated at less than 7 min. The method is linear has a correlation coefficient at 0.999. The method is linear in the range of 0.1-500 ng / ml for alpha lipoic acid and 0.25 -1,000 ng / ml for dihydrolipoic acid respectively. This method is sensitive, precise and accurate.

Ezhilarasi et al.\textsuperscript{[14]} developed a simple and specific method for estimation of lipoic acid in human plasma by high performance liquid chromatography. The mobile phase used in this method is disodium hydrogen phosphate: acetonitrile: methanol which is in the ratio of 50:30:20. The analyte got separated in 3.5 min. The method is valid and linear. The linearity range is 0.78 -50µg / ml and the correlation coefficient was found to be 0.9998. The method is found to be simple, precise and accurate and can be used for basic research studies for the determination of alpha lipoic acid.

**Spectrophotometric methods**

Pratik et al.\textsuperscript{[15]} have reported the development and validation of ratio–derivative spectrophotometric method for simultaneous estimation of gabapentin, methylcobalamin and alpha lipoic acid in tablet formulation, in this method the spectra of alpha lipoic acid, gabapentin, methylcobalamin are well resolved by first–derivative of the ratios of their direct absorption spectra. The derivative ratio absorbance of gabapentin, methylcobalamin and alpha lipoic acid were measured at 731.10nm, 768.53nm and 242.21nm for their quantification. This method has been validated for accuracy, precision, linearity, robustness and sensitivity. Gabapentin, methylcobalamin and alpha lipoic acid have shown linearity in the concentration range of 100-500 µg/ml, 0.5- 2.5 µg/ml and 100 -500 µg/ml . The correlation coefficient was 0.9996, 0.9998 and 0.9999 for gabapentin, methylcobalamin and alpha lipoic acid respectively.

Pratik et al.\textsuperscript{[16]} have developed spectrophotometric methods for the estimation of α- lipoic acid in tablet dosage form, which include zero order spectrophotometry, first order derivative spectrophotometry, spectrophotometric method using AUC technique of zero order derivative spectra and spectrophotometry method using AUC technique of first order derivative. The method which use zero order spectrophotometry has a spectrum mode of 800 nm – 200 nm. The absorption maxima was found to be 322nm. For the first order derivatives the absorption maxima was found to be 309nm. The spectrophotometry method using AUC technique – zero order derivative, the wavelength range was found to be 310.0 -350.0 nm. The
spectrophotometry method using AUC technique – first order derivative spectra which has wavelength range of 270.0 -330.0nm. It is found that all the method was found to be precise, accurate, simple and sensitive.

Mayur et al.\textsuperscript{[17]} have reported a novel analytical method for simultaneous estimation of metformin and alpha lipoic acid in pharmaceutical dosage form by UV spectroscopic method using phosphate buffer of pH 8 and measuring absorption at 232 nm and 334 nm. The method has been validated as per the ICH guidelines. The accuracy of the method was found between 99.1% - 100.45% for metformin hydrochloride and 99.8%-101.60% for alpha lipoic acid. This method showed linearity in the concentration range of 2-12µg/ml for metformin and 100-500µg/ml for alpha lipoic acid. The correlation coefficient found to be 0.993 for metformin HCL and 0.998 for alpha lipoic acid.

**High performance thin layer chromatography**

Patel et al.\textsuperscript{[18]} have developed a method for the simultaneous estimation of metformin hydrochloride and alpha lipoic acid in tablet dosage form by HPTLC method, in this method the mobile phase used was toluene, ammonium acetate, ethyl acetate in the ratio 5:4:1 v/v/v. The TLC plate was coated with silica gel. The Rf value of metformin and alpha lipoic acid was 0.28 and 0.65 respectively. The method was linear in the range 1500-7500ng/band for metformin hydrochloride and 600-3000ng/band for alpha lipoic acid and the correlation coefficient was found to be 0.9972 for metformin hydrochloride and 0.9981 for alpha lipoic acid.

**LC -MS / MS method**

Ravi et al.\textsuperscript{[19]} reported the determination of lipoic acid in rat plasma by LC – MS/ MS with electrospray ionization: assay development, validation and application to a pharmacokinetic study. The mobile phase used was formic acid: acetonitrile in the ratio 40:60 v/v. The method was linear over the range 5-1000ng/ ml. The $R^2$ was > 0.99.

**CONCLUSION**

Literature revealed that only a few analytical methods have been reported for the estimation of alpha lipoic acid either alone or in combination which are based on HPLC method, UV spectrophotometric methods or HPTC method. The mobile phase used in all the reported methods are acetonitrile with different buffers and the retention time of alpha lipoic acid was more than six minutes. In the case of spectrophotometric methods mostly derivative
spectrophotometric methods have been used. The HPLC method is found to be more valid than spectrophotometry and HPTLC.

**REFERENCE**


