ION Nutritional Labs	Standard Operating Procedure **Determination of α-Lipoic Acid by HPLC/UV**	**SOP Number** **D-797**	**Revision** **1**
		Effective Date 06/13/23	**Page** **Page 1 of 7**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
SAS 06/12/23	06-12-23	06/12/23
Title: Analytical Development Scientist	**Title: Analytical Development Scientist**	**Title: Quality Control Director**

1.0 Purpose

This document describes the analytical procedure for the determination of α-Lipoic Acid in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of α-Lipoic Acid in raw materials and finished products. This method was validated under Protocol MV-LAB-19-125.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical chemists who have verified their ability to execute this procedure to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development Personnel to keep this procedure current with the associated monographs and laboratory practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **H3PO4** – Phosphoric Acid

4.3 **b** – Potassium Phosphate, Monobasic

4.4 **MeOH** – Methanol

4.5 **ACN** – Acetonitrile

4.6 **H_2O** – Deionized Water

4.7 **ALA** – α-Lipoic Acid

4.8 **HPLC** – High Performance Liquid Chromatography

4.9 **UV/Vis** – Ultraviolet & Visible Electromagnetic Spectra

5.0 References

5.1 MV-LAB-19-125 – α-Lipoic Acid Determination Using HPLC with UV/Vis Spectroscopy

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better

6.1.1 H_2O

6.1.2 MeOH

6.1.3 ACN

6.1.4 KPhos Mono

6.1.5 H_3PO_4

6.1.6 ALA Reference Standard

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 Volumetric glassware and/or adjustable pipettes and tips

6.2.3 Weigh paper or funnels

6.2.4 10ml Syringes with 0.45u Nylon Syringe Filters

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Sonicator bath

6.3.4 Wrist Action Shaker

7.0 Procedure

7.1 Mobile Phase, Extraction Solvent & Diluent Preparation

7.1.1 Buffer – 0.68 g/L KPhos Mono

7.1.1.1 Combine 0.68g of KPhos Mono with 1000 mL of H_2O. Mix well and filter/degas at 0.45µ.

7.1.2 8.3% H_3PO_4 Solution

7.1.2.1 Dilute 8.3 mL of H_3PO_4 to 100 mL with H_2O. Mix well.

7.1.3 Mobile Phase – 58:46:9 MeOH/ 0.68g per L KPhos Mono/ ACN, pH 3.0 – 3.1

7.1.3.1 Combine 580 mL MeOH, 460 mL Buffer, and 90 mL ACN. Mix well.

7.1.3.2 pH adjust mobile phase to 3.0 – 3.1 using the 8.3% H_3PO_4.

7.1.4 Extraction Solvent - MeOH

7.1.5 Diluent – 60:40 MeOH / H_2O

7.1.6 Preparations may be scaled as necessary

7.2 Standard Prep

7.2.1 Accurately weigh and transfer about 25 mg of ALA reference standard into a 50-mL volumetric flask. Add 25mL of Extraction Solvent. Sonicate for 5min, equilibrate to room temperature, then QS to volume with Extraction Solvent.

7.2.2 Dilute 1:5 w/ Diluent for a Working Standard concentration of 100 µg/ml.

7.3 Sample Preparation

7.3.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below, maintaining concentration within the linear range listed below.

7.3.2 The validated range for the analytical method is 25 – 500 mcg/mL.

7.3.3 For raw materials: weigh no less than 20 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate an analyte concentration that is within the validated linearity range. Fill the flask to about 50% of the calculated volume with Extraction Solvent and shake mechanically for 10 minutes. Sonicate for 5 minutes, equilibrate to room temperature and bring up to volume with Extraction Solvent.

7.3.4 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight (capsules), serving size (powders and liquids) or tablet weight per dose, weigh no less than 100 mg of the pooled dosages into a suitably sized volumetric flask of no less than 25 mL to generate an analyte concentration that is within the validated linear range. Fill the flask to about 50% of the calculated volume with Extraction Solvent and shake mechanically for 10 minutes. Sonicate for 5 minutes, equilibrate to room temperature and bring up to volume with Extraction Solvent.

7.3.5 For chewable gels (gummies), homogenize at least 10 dosage units according to the procedure outlined in D-793 Cryogenic Grinding of Chewable Gels. Quickly weigh no less than 400 mg of the pooled and homogenized dosages into a beaker. Use several small portions of Extraction Solvent to completely transfer the sample into a suitably sized volumetric flask to generate an analyte concentration that is within the validated linear range. Fill the flask to about 50% of the calculated volume with Extraction Solvent and shake mechanically for 10 minutes. Sonicate for 5 minutes, equilibrate to room temperature and bring up to volume with Extraction Solvent.

7.3.6 To manage large volumes, the sample can be initially prepared at a higher concentration and further diluted into the linear range using Diluent. Dilutions can be made using volumetric glassware and/or adjustable pipettes. Dilutions can be prepared in HPLC vials

7.3.7 Centrifuge an aliquot of the final sample at 10,000 rpm for 5 min to remove particulates. Alternatively, the sample may be filtered through a 0.45 µm membrane discarding the first 3 – 4 mL before collecting a portion for analysis.

7.4 HPLC Parameters

7.4.1 Column: Agilent InfinityLab Poroshell 120 EC-C18, 4.6 x 100mm, 2.7u

7.4.2 Column Temperature: 40°C

7.4.3 Flow rate: 0.9 mL/min

7.4.4 Wavelength: 215 nm

7.4.5 Injection Volume: 5 µL

7.4.6 Run Time: 10 minutes.

7.4.7 Recommended 3-D Spectral Range (for Identification) - 200nm to 350nm

7.4.8 Mobile Phase Gradient - Isocratic

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the Diluent.

7.5.2 Make five (5) injections of Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

7.5.4 Make a single injection of the Working Standard after every ten (10) sample injections and at the end of a run.

7.6 System Suitability Requirements

7.6.1 The %RSD of the first five (5) standard injections is NMT 2.0%

7.6.2 The %RSD of all standard injections is NMT 3.0%.

7.6.3 If present, any interference in the diluent should be subtracted out of the sample and standard peak areas.

7.7 Example calculations for determining finished product % label or raw material % purity

$$\% \text{ Analyte} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{SS}{SA} \times \frac{V_{spl}}{LA} \times 100$$

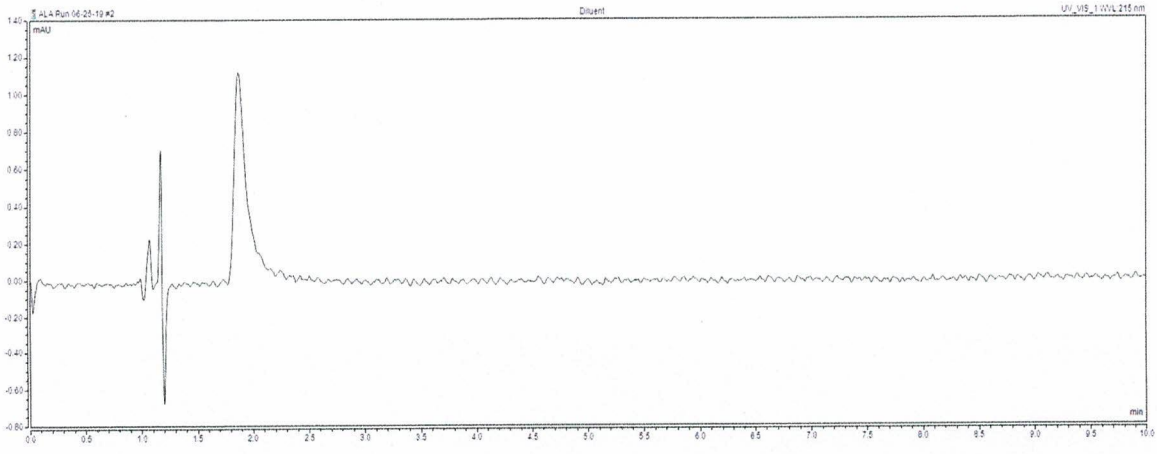
R_u	Sample peak area
R_s	Mean (n=5) standard peak area
Wt_{std}	Weight of the reference standard in mg (corr. for water if applicable)
V_{std}	Volume of the standard preparation accounting for dilutions in mL
P	Purity of the reference standard in decimal format
SA	Sample amount in mg
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
SS	Serving size: Weight of a single dosage unit in mg (use 1 for raw materials)
LA	Label amount in mg of analyte (use 1 for raw materials)

7.8 Column Wash and Storage

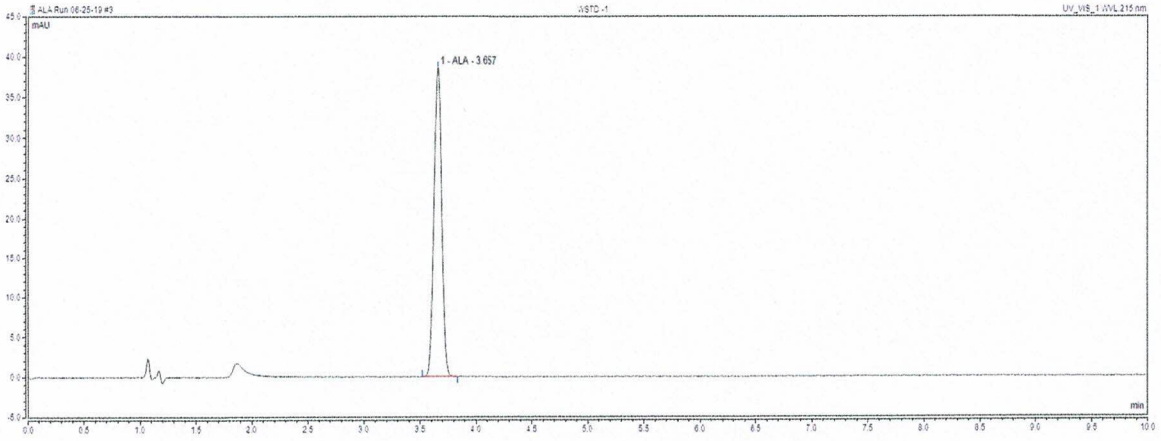
7.8.1 Wash and store the column in 75:25 MeOH / Milli-Q Water.

8.0 Example Chromatograms

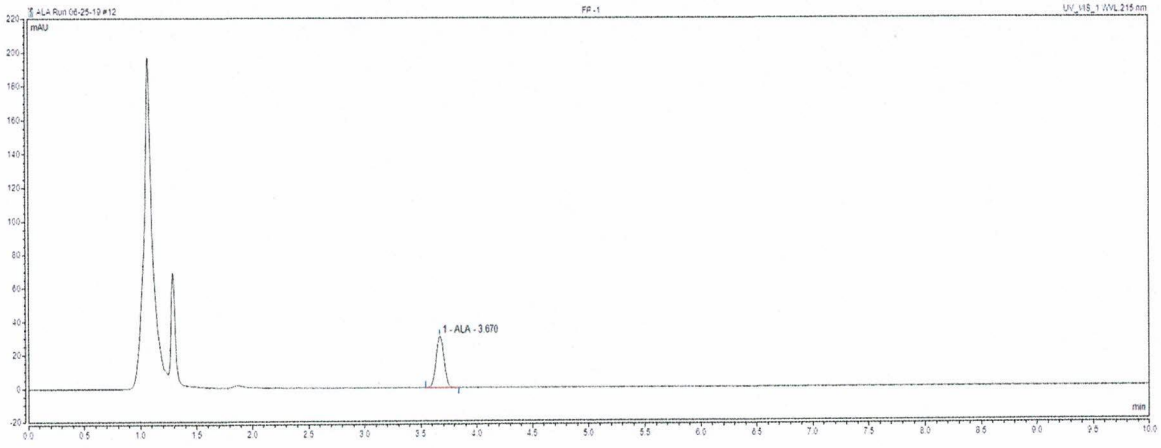
8.1 Typical Diluent Chromatogram



8.2 Typical Working Standard Chromatogram



8.3 Typical Sample Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	08/26/20	New	N/A	C. Perry
1	06/06/23	Minor edits for consistency with current methods. Simplified mobile phase preparation. Added instruction to follow sample preparation test details. Added specific sample prep instructions for different dosage forms. Updated format and logo.	CC-23-0275	S. Sassman