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HPLC/ELSD Determination and Validation of Alpha Lipoic Acid and Sucralose in Bulk and in Their Pharmaceutical Dosage Forms

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ABSTRACT

Accurate, sensitive, robust, and direct isocratic reversed phase-HPLC methods, using Evaporative Light Scattering Detector (ELSD), had been developed and fully validated for the detection and quantification of two non-chromophoric nutraceuticals; alpha-lipoic acid (ALA) and Sucralose (SUC). Chromatographic separation was achieved using C18 Zoprax, five µm, 15 cm column for both compounds. For ALA, the mobile phase was composed of acetonitrile: 0.1 M acetic acid (60:40, v/v), adjusted at pH 2.5 with 0.6 mL/min flow rate. For SUC, acetonitrile: deionized water (70:30, v/v) with a 1 mL/min flow rate was used. Temperatures of the drift tube, column, and spray chamber have been set to be 40°C and 30°C respectively during both assays. All varying chromatographic parameters were studied carefully. ALA and SUC had eluted at 4.81±0.02 min and 1.70±0.01 min, respectively. The obtained exponential ELSD responses for the two developed methods were linearly modeled using logarithmic transformation. For ALA and SUC estimation, good linearities were achieved over the concentration ranges of 100-750 ppm and 16-500 ppm. The suggested methods disclosed excellent precision and accuracy levels. All validation parameters were fulfilled according to the United States Pharmacopeia (USP) elements and International Council for Harmonisation (ICH) guidelines. Satisfactory percentages of recovery (>97%) resulted upon methods application for the assay of each dietary supplement in its pharmaceutical formulation. A comparative statistical study was conducted between the proposed HPLC/ELSD methods and the reported HPLC/UV method for ALA and the reported HPTLC method for SUC. Student's t-tests and F-variance ratios for both methods had resulted in satisfactory values.

Keywords: Alpha-lipoic acid, Evaporative light scattering detector, HPLC, Sucralose, Validation

1. INTRODUCTION

Alpha-lipoic acid (ALA) **(Figure 1)**, also known as thioctic acid, is official in British Pharmacopoeia (BP) and United States Pharmacopeia (USP). ALA is an organo-sulfur compound derived from caprylic acid (octanoic acid). It is also manufactured and available as a dietary supplement in some countries where it is marketed as an antioxidant. Meanwhile, it is known as a pharmaceutical drug in other countries. ALA is a naturally occurring micronutrient synthesized in small amounts by plants, animals, and humans with antioxidant and potential chemopreventive activities. ALA acts as a free radical scavenger and assists in repairing

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oxidative damage and regenerates endogenous antioxidants, including vitamins C, E, and glutathione. ALA also promotes glutathione synthesis. In addition, ALA exerts metal-chelating capacities and functions as a cofactor in various mitochondrial enzyme complexes involved in the decarboxylation of alpha-keto acids.[4, 5]

ALA possesses two sulfur atoms (at C6 and C8) connected by disulfide, as shown in Figure (1). IUPAC nomenclature of ALA is (R)-5-(1,2-Dithiolan-3-yl) pentanoic acid or 6,8-dithiooctanoic acid with a molecular formula of $C_8H_{14}O_2S_2$ and a molecular weight of 206.32 g/mol. ALA is yellow needle-like crystals with a melting point of 46 ºC, soluble in ethanol, DMSO, and acetonitrile, but very slightly soluble in water, with pKa (4.70 – 5.10) ±0.1.[3]

Sucralose (SUC) **(Figure 1)** is an official artificial and sugar substitute in USP.[14] Most ingested sucralose is not broken down by the body, so it is non-caloric. It is produced by the chlorination of sucrose. SUC is about 320 to 1,000 times sweeter than sucrose, three times as sweet as Aspartame, and twice as sweet as Sodium Saccharin. SUC is the no-calorie sweetener in regular SPLENDA® and NoCal® retail sweeteners. It is found in beverages and foods like chewing gum, dairy products, canned fruits, syrups because it is stable at high temperatures. SUC can be used in baked goods; only traces of sucralose are needed to achieve the sweetness of sugar.[15]

IUPAC nomenclature of SUC is (1→6)-dichloro-(1→6)-dideoxy-β-D-fructofuranosyl -4-chloro -4-deoxy -α-D-galactopyranoside or 1',4,6'-Trichlorogalactosucrose; Trichlorosucrose with a molecular formula of $C_{12}H_{19}Cl_3O_8$ and a molecular weight of 397.64 g/mol. It is odorless, off-white to white powder, soluble in water, with pKa 12.52±0.50. (14, 15)

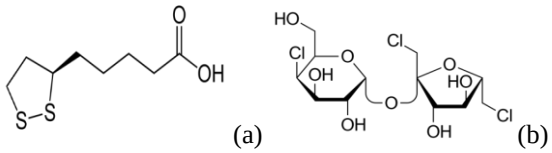

Figure 1: Chemical structures of alpha-lipoic acid (a) and Sucralose (b)

Literature review reveals the determination of ALA using Spectrophotometry[1,2] and differential scanning calorimetry (DSC), and infra-red (IR) Spectroscopy.[3] Different HPLC with various detectors is reported as UV,[4,5] liquid chromatography-electrospray ionization-mass spectrometry (LC–ESI-MS),[6] electrochemical (EC),[7,8] fluorimetry,[9,10] coulometric electrode array detector (CEAD), and an electrospray ionization mass spectrometer (ESI-MS),[11] DAD,[12] and capillary electrophoresis.[13] Literature review reveals several methodologies for SUC determination as Spectrophotometry,[14,15] TLC,[16] HPTLC,[17] DSC,[18] IR,[19] and FT-IR.[20] Many chromatographic methods for SUC determination with different detectors as UPLC coupled with DAD and CAD detectors[21] and HPLC coupled with (PAD-IC),[22] RID,[23] ELSD,[24] also Mass spectrometry applied as (SPE-LC-MS/MS),[25,26] triple quadrupole mass spectrometry[27] and liquid chromatography/time-of-flight mass spectrometry.[28] Quantitative analysis of SUC by (LC-ESI-MS),[29] GC coupled with MS, FID,[30] also Capillary electrophoresis,[31,32,33] chemometric,[34] and stability-indicating[35,36] methods of analysis were reported.

Evaporative light scattering detectors (ELSD) is a quasi-universal detector. It can detect almost all components that are less volatile than the mobile phase. Detection is based on a universal property of all analytes and does not require the presence of any chromophoric group or electro-active group. It is quite suitable for detecting and quantifying compounds that do not display sufficient UV absorption; many sugars, amino acids, and lipids have been determined without derivatization by HPLC/ELSD.[37] ELSD works extremely simple because the target components are converted to a fine spray by a nebulizer and heated so that only the mobile phase is evaporated. Light is directed at the remaining target substances, and the scattered light is detected.

One of the main limitations of ELSD is being a non-linear detector. After that, exponential responses are linearly modeled by logarithmic transformation,[38] concentrations of standard solutions are correlated with the obtained linear responses.

The aim of the present work is to develop rapid, simple, sensitive, specific, accurate, precise, and robust chromatographic methods for the detection and determination of ALA and SUC in bulk and pharmaceutical dosage forms. They are typical for routine and daily analysis in quality control laboratories of pharmaceutical companies. ALA in skin samples[7] and SUC[24] were previously determined by HPLC/ELSD. Meanwhile, the proposed methods are the first to estimate the drugs in pharmaceutical formulations. Besides, they disclose higher sensitivities of determination in a shorter run time. The present study typically applies a simple, rapid, and robust analytical methodology.

2. METHODS

2.1. Instrumentation

Chromatographic separation was performed on a Shimadzu HPLC system with YL 9181 - ELS Detector superior sensitivity, RP-C18 Zorpax column (250 x 4.6mm, 5 µm). A manually rheodyne injector 100 µL was used.

2.2. Chemicals and Reagents

Alpha-lipoic acid and sucralose pure were obtained as a sample from Borg Pharmaceutical Industry (Alexandria, Egypt). Dosage forms of ALA (Thiotacid® 300mg capsules) and SUC (NoCal sachets contain 0.9 mg SUC) were purchased from the community pharmacy. Acetonitrile was

obtained from Fisher Chemicals; glacial acetic acid was purchased from El-Nasr Chemical Company (Alexandria, Egypt) and deionized water. All chemicals used were HPLC grade.

2.3. Selection of mobile phase

Based on sample solubility, stability, and checking miscibility chart, various mobile phase compositions were tried to get good resolution and sharp peaks. The standard solution was run in different mobile phases. Acetonitrile: 0.1 M acetic acid (60:40, v/v) with adjusted pH= 2.5 and acetonitrile : deionized water (70:30, v/v) were from various mobile phases chosen for ALA and SUC, respectively since it gave sharp peaks with good symmetry.

2.4. Preparation of 0.1 M Acetic Acid

An accurate volume of 1.5 mL glacial acetic acid was dissolved in 250 mL distilled water. The solution was sonicated for 5 min, and then pH was adjusted to be (2.5±0.1), using 0.1 M NaOH.

2.5. Preparation of Mobile Phase

The composition of the mobile phase was prepared from a filtered and degassed mixture of acetonitrile: 0.1 M acetic Acid with ratio (60:40 v/v) and acetonitrile: deionized water (70:30 v/v) for ALA and SUC, respectively.

2.6. Chromatographic Conditions

The selected chromatographic parameters for the proposed HPLC/ELSD method are mentioned in Table (1)

2.7. Preparation of standard solutions

Accurate weighs of 100 mg of each of ALA and SUC pure standard powders were carefully and quantitatively transferred into two 100-mL volumetric flasks. Portions of (acetonitrile) about 30 mL were added and sonicated for 5 min to dissolve them completely. The volumes were made up to the mark with deionized water (stock solution-1). From the above solutions, volumes of 0.63, 1, 1.25, 2.5, 5 and 7.5 mL ALA were pipetted into 10-mL individual volumetric flasks and similarily, volumes of 0.02 , 0.04 , 0.08 , 0.16 , 0.3 , 0.6 , 1.25 , 2.5 , 5, and 7.5 mL SUC were pipetted into 10-mL individual volumetric flasks. Diluent was added up to the mark to give (63 , 100 , 125 , 250 , 500 , 750 ppm) and (2 , 4 , 8 , 16 , 30 , 60 , 125 , 250 , 500 , 750 ppm) concentrations for ALA and SUC respectively.

Table 1: Optimization of chromatographic parameters

	ALA	SUC
Stationary phase	RP-C18 Zorpax column 15 cm (250 x 4.6mm, 5 µm).	
Mobile phase Mobile Phase ratio	ACN / 0.1 M acetic acid (60:40, v/v)	ACN / deionized H_2O (70:30, v/v)
pH of mobile phase	2.5±0.1	7.0±0.1
N_2 gas pressure	47.6 ±0.05 psi	47.9±0.05 psi
N_2 Gas flow rate	2 L/min	
Injection volume	100 µL	
Flow rate	0.6 mL/ min	1 mL / min
Column temperature	40 ±2˚C	
Drift Tube temperature	40 ±2˚C	
Spray chamber temperature	30 ±2˚C	
Mode of operation	Isocratic Elution	
Run time	≤ 7 min	≤ 3 min

ALA: Alpha-lipoic acid, SUC: Sucralose

2.8. Construction of calibration curves

The prepared standard solutions mentioned previously in part (2.7 preparation of standard solutions) in the range of 100-750 µg/mL and 16-500 µg/mL for ALA and SUC respectively were injected into isocratic 100-µL rheudyne autosampler, and the peak area for each concentration has been recorded.

2.9. Preparation of sample solutions

2.9.1. Alpha-lipoic acid: (Thiotacid comp® 300mg capsules)

Ten capsules of ALA 300 mg were weighed and powdered. The stock solution was prepared by dissolving (equivalent weight to 300 mg ALA) an insufficient volume of acetonitrile in a 100-mL volumetric flask. The solution was sonicated for 30 min, filtered into another 100-mL volumetric flask. The final volume was completed to the mark by the diluent. From the previous stock solution, three volumes (5, 2.5 and 1 mL) were pipetted out into 10-mL individual volumetric flasks to prepare (0.5, 0.25, and 0.1 mg/mL) sample solution concentrations.

2.9.2. Sucralose (NoCal® sachets)

Nocal® packet contains 0.9 mg SUC. A portion of 0.05 g was weighed from Nocal packet and dissolved in a sufficient volume of diluent in a 50 mL volumetric flask to prepare a 1 mg/mL stock solution. The solution was sonicated for 20 min and filtered into another 50 mL volumetric flask. Then the volume was made up to the mark by the diluent.

From this stock solution volumes of (5, 2.5 and 1 mL) were pipetted out into 10 mL individual volumetric flask to give (0.5, 0.25, and 0.1 mg/mL) sample solution concentrations.

3. RESULTS & DISCUSSION

3.1. Methods development

The proposed HPLC/ELSD methods have achieved accurate determinations of ALA and SUC, which eluted at 4.80±0.02 min and 1.71±0.01 min, respectively, under the selected chromatographic conditions. Figure (2) and Figure (3) show the typical chromatograms of ALA and SUC, respectively.

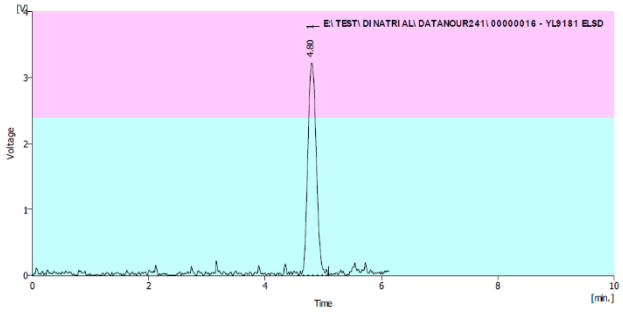

Figure 2: Typical HPLC Chromatogram for 750 ppm of alpha-lipoic acid (ALA)

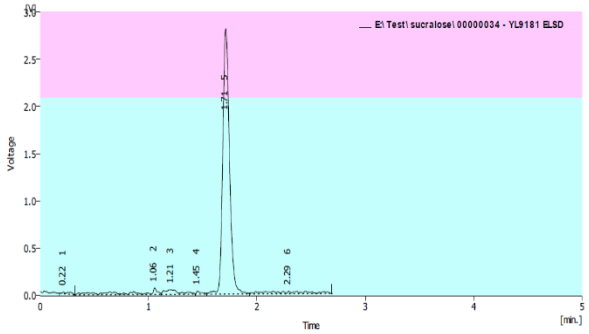

Figure 3: Typical HPLC Chromatogram for 600 ppm of sucralose (SUC)

3.2.Methods optimization

3.2.1. Drift tube (DT) temperature study

DT temperature mainly affects the sensitivity of detected responses (peak areas). Setting DT at 30°C and 35 ºC did not enable complete drugs evaporation. A portion of each injected drug retained on the column, resulting in decreased peak areas. Increasing DT temperature above 40ºC resulted in an exponential decrease in area count due to rapid evaporation. High DT temperature caused the degradation of drug molecules. Consequently, only small amounts were only detected, and a relative decrease in peak areas. Setting DT at 40ºC disclosed the optimum, as shown in Figure (4A) and Figure (5A) for ALA and SUC, respectively.

3.2.2. Mobile phase ratio study

For ALA: Increasing the acetonitrile ratio led to good sensitivity and adjusted peak sharpness. Meanwhile, the acetic acid ratio controlled the pH variation, which in turn affected drug stability. The mobile phase of acetonitrile and 0.1 M acetic acid 60:40 v/v respectively was chosen as illustrated in Figure (4B).

For SUC: Similarly, increasing the acetonitrile ratio resulted in high sensitivity and sharpened drug peaks. Deionized water in the mobile phase ensured good solubility and compatibility of SUC during elution of its peaks. Regarding sensitivity ACN: deionized H_2O in the ratio of 70:30 v/v respectively was the optimum ratio as shown in Figure (5B).

3.2.3. Flow rate study

Flow rate variation affected not only sensitivity but also the retention time of the peaks. Low flow rates delayed peak appearance making the process time-consuming. A high flow rate ensured a rapid process but with bad resolution. So, owing to sensitivity, 0.6 mL/min and 1 mL/min showed optimum flow rates for ALA and SUC elution, respectively, as shown in Figures (4C) and (5C).

3.2.4. pH study

The pKa of ALA ranges from (4.70 – 5.10).[40] Keeping the drug unionized at pH <pKa by two units is initially considered. The pH of the mobile phase has been changed in the acidic range (2, 2.5, 3, and 3.5). Regarding drug sensitivity, pH 2.5 is the optimum choice, as shown in Figure (4D).

3.3.Analytical validation

Both HPLC methods for ALA and SUC determination were validated as per the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures (Q2R1).[42]

3.3.1. Linearity and range

The linearity of the proposed procedures was evaluated by analyzing a series of different concentrations for ALA and

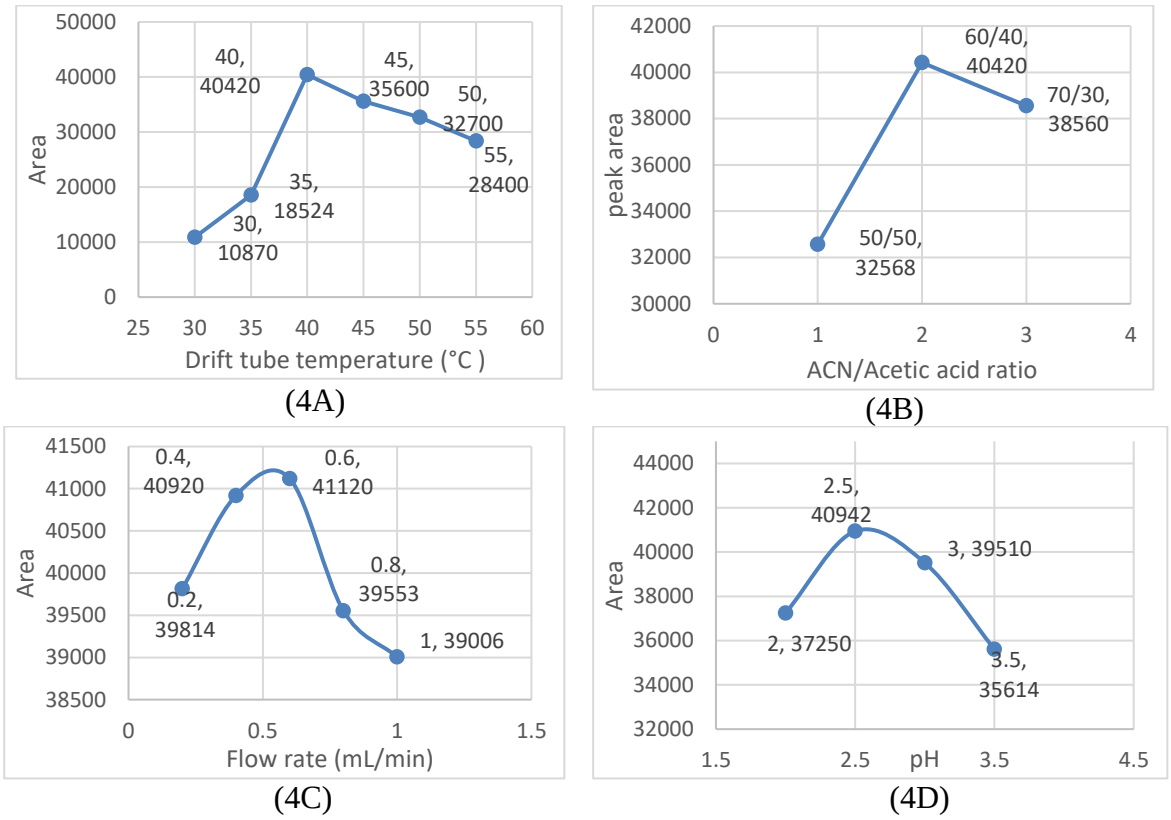

Figure 4: Study of parameters for ALA determination, (4A) Drift tube temperature study, (4B) Mobile phase Ratio study, (4C) Flow rate study and (4D) Mobile phase pH study.

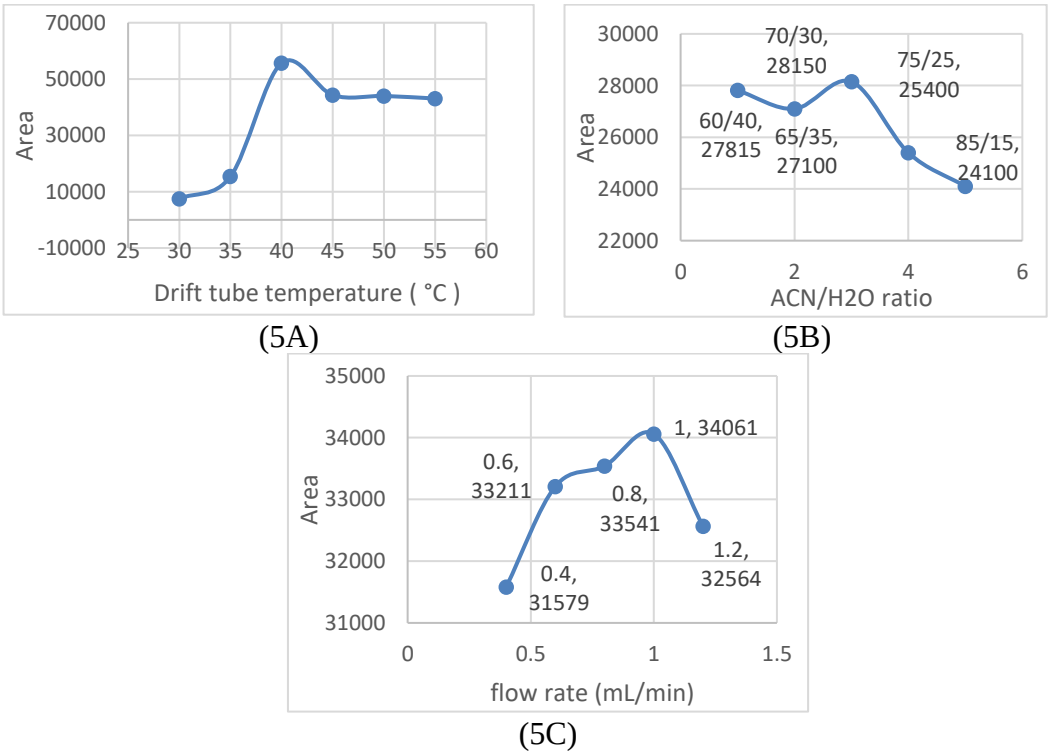

Figure 5: Study of parameters for SUC determination, (5A) Drift tube temperature study, (5B) Mobile phase Ratio study and (5C) Flow rate study

SUC. According to ICH guidelines,[42] at least five concentrations must be used.

The obtained exponential responses are being linearized by logarithmic transformation[38] using the log values of peak areas versus the corresponding log values of concentrations (n= 5 for ALA and n= 6 for SUC, respectively). Regression analysis for the calibration curves showed good linear relationships over the concentration ranges of 100-750 µg/mL and 16-500 µg/mL for ALA and SUC, respectively. Good linearity was indicated from the correlation coefficient values (r= 0.996 and 0.995) for method ALA and SUC, respectively.

Table (2) represents the performance data and statistical parameters, including linear regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept (S_a), slope (S_b).

3.3.2. Accuracy and precision

The accuracy and within-day (intra-day) precision for the proposed methods were examined at three concentration levels within the studied linearity ranges (100, 250, and 500 µg/mL) for both ALA and SUC using three replicate determinations for each concentration within one day. Similarly, the accuracy and between-day (inter-day) precision were tested by analyzing the same three concentrations for each compound using three replicate determinations repeated on three consecutive days. Recoveries were calculated using the corresponding regression equations, and they were satisfactory.

The percentage relative standard deviation (RSD %) and percentage relative error (RE) % did not exceed 2.0 % proving the high precision and accuracy of the proposed methods for the estimation of the ALA and SUC in their bulk form Table (3).

3.3.3. Robustness

The robustness of the analytical procedure measures its capability to remain unaffected by slight but deliberate variations in method parameters and indicates its reliability during normal usage.[42] In these experiments, one parameter was changed, the others were kept constant, and the recovery was calculated each time.

For this method, robustness was examined by making small changes in drift tube temperature (40±0.2 ºC), flow rate (0.6±0.05 mL/min), mobile phase ratio (ACN: 0.1 M acetic acid 60/40 ± 2%), and pH of acetic acid solution (2.5±0.2) for ALA and drift tube temperature (40±0.2ºC), flow rate (1±0.05 mL/min), mobile phase ratio (ACN: H2O 70/30±2%) for SUC and recording the chromatograms was done after each deliberate change.

These variations did not significantly affect the measured responses (peak areas) of ALA or SUC. Also, recovery and %RSD remain unaffected, which indicates methods robustness **(Table 4)**.

Table 2: Regression and statistical parameters for the proposed HPLC/ELSD method.

Parameters	ALA	SUC
Linearity Range (µg/mL)	100-750	16-500
Regression Equation	Y=1.2896 X + 0.8554	Y=1.029 X + 1.6414
Intercept (a)	0.855	1.641
S_a	0.157	0.099
Slope (b)	1.289	1.029
S_b	0.065	0.049
(S_b %)	5.01	4.83
Correlation Coefficient (r)	0.996	0.995
$S_{y/x}$	0.049	0.063
F value	398.26	428.486
Significant F	$2.7x10^4$	$3.21x10^{-5}$
LOD (ppm)	0.403	0.320
LOQ (ppm)	1.22	0.97

ALA: Alpha-lipoic acid, SUC: Sucralose, (Sb%) is RSD% of the slope, (Sy/x) is the residual standard deviation, and (F) is the Variance ratio.

Table 3: Evaluation of accuracy and precision of the proposed HPLC/ELSD method

		Nominal Values (µg/mL)	% Recovery ± SD [a]	RSD(%) [b]	RE (%) [C]
ALA	**Within-day**	500	101.59±0.19	0.193	1.59
		250	100.29±0.09	0.094	0.29
		100	98.59±0.79	0.800	-1.41
	Between-days	500	101.53±0.26	0.260	1.53
		250	100.32±0.68	0.679	0.32
		100	98.48±1.51	1.530	-1.18
SUC	**Within-day**	500	99.08±0.13	0.126	-0.92
		250	98.64±0.43	0.434	-1.36
		100	102.14±1.72	1.690	2.14
	Between-days	500	99.16±0.14	0.140	-0.84
		250	98.75±0.34	0.340	-1.25
		100	102.54±1.36	1.320	2.54

ALA: Alpha-lipoic acid, SUC: Sucralose, [a] % mean Recovery ± standard deviation of three determinations, [b] % Relative standard deviation, C % Relative Error

Table 4: Evaluation of robustness of the proposed HPLC/ELSD method

Drug	Parameter	Mean recovery ± SD	RSD%
ALA	**Flow rate** (0.6±0.05mL/min)	99.86± $4.9x10^{-3}$	0.11
	Drift Tube temperature (40±0.2 ºC)	99.96± $9.7x10^{-3}$	0.21
	Mobile phase Ratio ACN: 0.1 M acetic acid (60:40 ±2%)	99.96± $2.2x10^{-3}$	0.05
	pH of Mobile phase (2.5 ±0.2)	99.93± $3.7x10^{-3}$	0.08
SUC	**Flow rate** (1±0.5mL/min)	99.99± $9.6x10^{-4}$	0.21
	Drift Tube temperature (40 ± 0.2 ºC)	99.97± 0.01	0.23
	Mobile phase Ratio ACN: H2O (70:30 ±2%)	99.93± 0.02	0.43

ALA: Alpha-lipoic acid, SUC: Sucralose

3.3.4. Limit of detection and limit of quantitation (lod and loq)

LOD is the lowest analyte concentration detected but not necessarily quantitated under the applied experimental conditions. At the same time, the limit of quantitation is the lowest concentration that can be determined with acceptable precision and accuracy. LOD and LOQ were calculated according to the ICH guidelines. LOD is defined as the concentration of the analyte, which has a signal-to-noise ratio of 3:1. For LOQ, the ratio considered is 10:1,[42] given in Table (2).

The LOD values are 0.403 and 0.320 µg/mL, and the LOQ values are 1.22 and 0.970 µg/mL for ALA and SUC respectively, Both LOD and LOQ values confirm the sensitivity of the proposed HPLC/ELSD procedures.

3.3.5. Specificity

The successful resolution of the peaks can partially demonstrate method specificity. Figures (2) and (3) show typical chromatograms for ALA and SUC, respectively, considering their respective spectra at peak start, apex, and end positions of the peak.

3.3.6. Stability

The stability of ALA and SUC standard solutions, as well as the sample solutions in acetonitrile, was examined through several days' analysis to ensure that retention time is the same and the peak areas remained unchanged each day, once the retention time or area had changed, so the deterioration or degradation of the drug solution began. Retention times and peak areas of both drugs remained unchanged, and no detectable degradation was observed during two days at room temperature (± 25℃). For five days, when stored refrigerated at 4℃, taking into consideration, the ALA should be protected from light.

3.4.Application to dosage forms

The developed HPLC-ELSD method was applied for the assay of these dietary supplements in its commercial pharmaceutical preparations (Thiotacid® capsules) labeled to contain 300 mg ALA per capsule and Nocal® sachets for SUC.

Recovery values were calculated. Assay results revealed satisfactory accuracy and precision as indicated from %recovery, SD, and RSD% values **(Table 5)**. Good recoveries indicated the absence of any interference from commonly encountered inactive ingredients that may be present.

Table 5: Statistical comparison between the proposed HPLC/ELSD methods and the reported methods for determination of ALA and SUC.

	ALA		SUC	
Parameter	**HPLC/ ELSD Proposed method**	**HPLC/UV Reported method*[5]**	**HPLC/ ELSD Proposed method**	**HPTLC Reported method*[17]**
Mean	99.30	99.08	97.75	99.23
SD	0.81	1.93	0.77	1.42
Variance	0.59	0.08	0.59	6.23
t-test (2.31)**	2.29		1.67	
F-test (6.39)**	6.21		0.095	

ALA: Alpha-lipoic acid, SUC: Sucralose, ** Reference values for t- and F-tests

Furthermore, a simple reported HPLC/UV method was adopted to estimate ALA and reported the HPTLC method for SUC. Including selective pre-column derivatization of the thiol group of ALA with 1-benzyl-2-chloropyridinium bromide, the UV-detector was set at 332 nm, and 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 monophosphate at pH 2.5 (45:55 v/v) at a flow rate of 0.8 mL/min,[5] and SUC was determined on HPTLC amino plates whose amino groups reacted with sucralose to fluorescent zones by just heating the plate after chromatography and fluorescence measurement at 366-400 nm.[17]

The recoveries obtained from the proposed methods were statistically compared with those reported using F- (two samples for variances) and t- (two samples for means) tests. Both calculated values did not exceed the critical value, indicating insignificant differences between the proposed methods versus the reported ones **(Table 5)**. Consequently, the proposed HPLC/ELSD methods apply to the assay of ALA and SUC in commercial products with a satisfactory level of accuracy and precision.

4. CONCLUSION

This study provides simple, selective and reliable chromatographic methods for the assay of ALA and SUC in bulk and in their pharmaceutical dosage forms using isocratic elution HPLC-ELSD. It achieves estimations of two non-chromphoric dietary supplements without the need of derivatization or any additional complicated practical steps. Each parameter is optimized regarding sensitivity and peak sharpness. Reliability has been guaranteed by testing various validation parameters of the method and the successful application to commercial dosage forms. Proposed methods are statistically compared with reported methods for ALA and SUC. Obtained results show agreeable precision, accuracy and applicability of the proposed methods. The ELSD discloses efficient chromatographic separation and estimation in the direct screening of compounds of desperate absorptivities**.**

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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