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## Quantitative Determination of Alpha-Lipoic Acid in SLS Micellar Medium Utilizing Inhibitory Kinetic Approach

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A novel, repeatable, and swift kinetic approach for determining alpha-lipoic acid (ALA) in sodium lauryl sulfate (SLS) micellar medium has been presented, and it has been connected to ALA determination in drug formulations. The approach is based on ALA inhibitory property. ALA (containing two sulfur atoms) forms a chelate with  $\text{Hg}^{2+}$ , lowering the effective  $[\text{Hg(II)}]$ , and ultimately, the  $\text{Hg}^{2+}$  catalyzed cyanide substitution rate from  $[\text{Ru(CN)}_6]^{4-}$  by nitroso-R-salt (N-R-salt). Fixed times of 5 and 10 min were chosen under optimal reaction conditions with  $[\text{N-R-salt}] = 0.45 \text{ mM}$ ,  $\text{pH} = 7.0 \pm 0.02$ ,  $\text{Temp} = 45 \pm 0.2 \text{ }^\circ\text{C}$ ,  $I = 0.1 \text{ M (NaClO}_4)$ ,  $[\text{Ru(CN)}_6]^{4-} = 42.5 \text{ } \mu\text{M}$ ,  $[\text{Hg}^{2+}] = 0.25 \text{ mM}$ , and  $[\text{SLS}] = 8.5 \text{ mM}$  to calculate the absorbance at 525 nm associated with the final substitution product  $[\text{Ru(CN)}_5 \text{ N-R-salt}]^{3-}$ . ALA's inhibiting influence on the  $\text{Hg}^{2+}$  catalyzed cyanide substitution with N-R-salt from  $[\text{Ru(CN)}_6]^{4-}$ , has been represented by a modified mechanistic approach. The concentration of ALA in various water specimens can be measured at the micro-level down to  $2.5 \text{ } \mu\text{M}$  using the established kinetic spectrophotometric approach. The suggested method is highly reproducible and has been effectively applied to accurately quantify the ALA in pharmaceutical samples. Even as much as 1000 with  $[\text{ALA}]$ , Excipients used in medications do not significantly hinder the determination of ALA.

**Keywords:** Inhibitory kinetic approach, Alpha-lipoic acid, SLS micellar medium, Pharmaceutical samples, Excipients

### INTRODUCTION

Due to their surface-active characteristics, surfactants are frequently employed in modern industries [1-3]. Surfactants' amphiphilic structure, which includes both a tail (hydrophobic) and head (hydrophilic), is what gives them their surface activity [4]. At modest concentrations, the water-based solution of the surfactant behaves as an electrolyte. Micellization takes place in an aquatic environment because one substrate contains both hydrophilic and hydrophobic components. The critical micelle concentration (CMC) is the typical concentration of

surfactant at which micellization initiates automatically [5]. Due to the repulsive and attractive interactions that exist between surfactant molecules, the molecules will self-associate beyond CMC and constitute micelles of different shapes and sizes. At 298 K, the CMC value of the anionic surfactant sodium lauryl sulfate (SLS) ranges from 8.0 mM to 8.5 mM [6-8]. In comparison to pure solvents, micelle-bound reactants experience a totally distinct reaction environment [7]. The extent of substrate interaction with the micelle aggregates in a micellar medium determines the reaction rate.

The body naturally produces alpha-lipoic acid (ALA), often referred to as thioctic acid (TA), which is necessary for the operation of certain oxidative metabolic enzymes [9,10].

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Its main function is to use oxygen to turn blood sugar (glucose) into energy [11,12]. ALA is a sulfur-containing short-chain fatty acid (Fig. 1). ALA is a powerful antioxidant with numerous metabolic benefits such as antiobesity, glucose reduction, insulin sensitization, and cholesterol reduction. ALA is biologically significant and possesses anti-inflammatory, detoxifying, neuroprotective, anti-aging, and cardiovascular properties [13-17]. ALA is a good contender for metal detoxification because it can efficiently and selectively bind the majority of divalent metal ions both *in vitro* and *vivo via* different mechanistic pathways to form organometallic complexes [16]. Due to the existence of two sulfur with lone pairs of electrons, ALA can form chelates with metal ions.

Sulfur has remained the dominant heteroatom for centuries in the wide range of fungicides, insecticides, and bioactive compounds. Organosulfur compounds function specifically as structural proteins and enzymes in various metabolic processes [18-20]. Analytical chemists are constantly sought after by the pharmaceutical sector for the most effective technology for detecting and quantifying sulfur-containing bioactive compounds and medicines in various samples. The basic importance and applicability of the oxidation-reduction/ligand exchange processes of complexes containing transition metals in analytical, organometallic, and synthetic chemistry encouraged a large number of scientists to explore their kinetics [21-24]. Kinetic analyses of the Fe(II) and Co(II) complexes oxidation and metal-catalyzed cyanide substitution from  $[\text{Ru}/\text{Fe}(\text{CN})_6]^{3-}$  by nitrogen heterocyclic ligands have been reported by several authors [25-27]. The aforementioned processes have also successfully been employed for the trace-level evaluation of catalysts and medications/moieties that have a profound affinity for catalysts [28-30].

Different methods are frequently employed to quantify thio compounds in analytical, biological, and pharmacological materials. The quantification methodology includes potentiometry [31], spectrophotometry [32,33], chromatography [34-36], fluorimetry [37], NMR-spectrometry [38], flow injection analysis [39], colorimetry [29], and voltammetry [40]. The aforementioned techniques may have some limitations, including a significant initial investment, a lengthy process, heavy equipment, and an elevated cost for the analysis of samples. A relatively small



**Fig. 1.** Structure of alpha lipoic acid.

number of investigations have been conducted using the kinetic approach, which only requires a UV-Visible spectrophotometer to quantify thio compounds [28-30,41]. The proposed kinetic technique will provide a solution to issues with complicated methodologies, high initial costs, lengthy processes, and heavy instrumentation for ALA quantification. The procedure is inexpensive because it only needs a visible spectrophotometer and cheap reagents. The developed method for determining ALA is straightforward, quick, and highly repeatable.

Several publications have demonstrated metal-catalyzed Ag(I)/Hg(II), cyanide exchange by heterocyclic ligands containing nitrogen from Fe(II)/Ru(II) cyano complexes [26,27]. The micro-level evaluation of utilized catalysts and medications or compounds with a potent affinity for catalysts has also been effectively accomplished using these reactions [28-30]. Through the formation of a stable complex with  $\text{Hg}^{2+}$ , organosulfur compounds significantly reduce the catalytic effectiveness of Hg(II), which reduces the exchange of cyanide to a significant extent. This inhibiting property of thio moieties (containing sulfur as S-, -S-, and -SH) can be employed for their quantification at trace levels utilizing the kinetic spectrophotometric approach. ALA (containing two "S") similarly reduces Hg(II) catalytic efficacy, lowering the cyanide exchange rate from  $[\text{Ru}(\text{CN})_6]^{4-}$  by N-R-salt. Since the uncatalyzed reaction between  $[\text{Ru}(\text{CN})_6]^{4-}$  and N-R-salt was not seen at the examined reaction conditions, a more accurate result will be found having the reaction at disposal [42]. Due to the ALAs' potent inhibitory effect on Hg(II)'s catalytic effectiveness, we developed a straightforward, repeatable, and quick kinetic spectrophotometric technique for the micro-level detection of ALA in various water specimens down to 2.5  $\mu\text{M}$ . The developed procedure has also been used to quickly and accurately quantify ALA in various commercial samples with excellent consistency. The

technique can be used to determine numerous medicines and biological substances at trace levels that have the potential to severely reduce Hg(II)'s catalytic efficacy.

## MATERIALS AND METHODS

### Reagent Used

All chemicals that were utilized were pure and of analytical grade. The stock solution of each reagent was directly prepared by weighing its accurate amount and further dissolving it in double-distilled water. To avoid the potential photo-decomposition of  $K_4[Ru(CN)_6] \cdot 3H_2O$  (Sigma-Aldrich, USA), an amber-colored bottle was used to preserve its stock solution ( $10^{-2}$  M). Alpha-lipoic acid (ALA), N-R-salt, and sodium lauryl sulfate were purchased from Himedia India and utilized directly. The solution of  $HgCl_2$  (Fisher Scientific, India) was made every day by dissolving its computed amount.  $NaClO_4$  (Fisher Scientific, India) was utilized to control the reaction mixture's ionic strength, while Molechem's (India)  $NaOH/HCl$  and potassium hydrogen phthalate was employed for adjusting the reaction medium's pH.

### Instrumentation

Using a DD LAB (model LAB.PHM.66800620) auto digital pH meter, verified with a predefined buffer solution, the pH of the reacting solution was monitored. A double-beam UV-Visible spectrophotometer made by Electronics India, model 2375, was deployed for measuring absorbance at 525 nm.

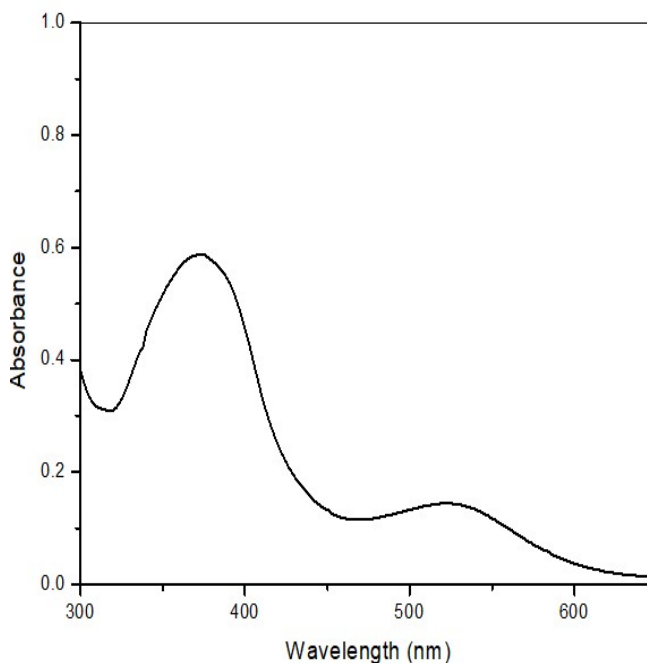
### Kinetic Procedure

The absorption values were not modified since, with the exception of the final product, none of the reacting solutions exhibit significant absorption at the pertinent wavelength. An optimum reaction setting that showed a considerable change in absorbance at 525 nm was carefully chosen from the reaction's extensive kinetic investigation. Due to the limited solubility of ALA in water, its kinetic quantification was performed in SLS micellar medium. Following 30 minutes of thermal equilibration at 45 °C, all of the reactive solutions were quickly mixed in the sequence:  $HgCl_2$ , buffer, SLS, N-R-salt,  $NaClO_4$ ,  $[Ru(CN)_6]^{4-}$  and ALA. Immediately following a thorough shaking, the reacting mixture was

poured into the spectrophotometric cell. An ingeniously constructed system of circulating water arrangement kept the cell compartment at a constant temperature. The absorbance increase related to the final product was documented. The quantification of ALA was done using a graph (calibration curve) of absorbance versus altering [ALA].

## RESULTS AND DISCUSSION

The reaction involving N-R-salt and  $[Ru(CN)_6]^{4-}$ , which is accelerated by Hg(II), resulting in the final substitution product  $[Ru(CN)_5 \text{ N-R-salt}]^{3-}$  [42]. By analyzing the end reaction product's mole ratio and slope ratio, it was determined that the reaction between N-R-salt and  $[Ru(CN)_6]^{4-}$  occurs in a 1:1 mole ratio [42]. No modification to the absorption values was made because, except for the ultimate reaction product, none of the reacting solutions displayed any discernible absorption at the examined wavelength. The prominent absorption band at 525 nm corresponds to the finished product  $[Ru(CN)_5 \text{ N-R-salt}]^{3-}$  (Fig. 2).



**Fig. 2.** UV-Visible spectra of final product  $[Ru(CN)_5 \text{ N-R-salt}]^{3-}$ .

### Kinetic Quantification of Alpha-lipoic Acid (ALA)

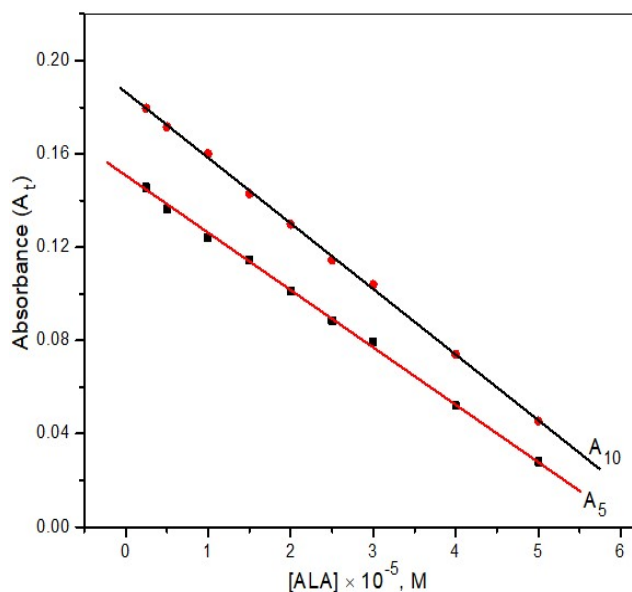
The prior research on acetylcysteine, thioglycolic acid, D-penicillamine, and other compounds that include sulfur acknowledges that the cyanide substitution rate from  $[\text{Ru}(\text{CN})_6]^{4-}$  by nitrogen heterocyclic ligands, accelerated by  $\text{Hg}(\text{II})$  is reduced by the sulfur compounds [28-30]. The effective concentration of  $\text{Hg}(\text{II})$  decreases due to the stable complex formation between the  $\text{Hg}(\text{II})$  and added sulfur compounds, which ultimately leads to a slower reaction rate. By the same token, ALAs (containing two sulfur atoms) also forms a chelate with  $\text{Hg}^{2+}$ , lowering the effective  $[\text{Hg}(\text{II})]$ , and ultimately, the substitution rate. At a set period (5 and 10 min after reactant mixing), the absorbance ( $A_t$ ), associated with the finished substitution product  $[\text{Ru}(\text{CN})_5 \text{ N-R-salt}]^{3-}$ , with altering  $[\text{ALA}]$ , was documented. ALA was quantified using absorbance against  $[\text{ALA}]$  plot (calibration curve) that was found linear in the 2.5 to 5.0  $\mu\text{M}$  ALA concentration band (Fig. 3). So with the help of the proposed method, ALA can be determined or quantified down to 2.5  $\mu\text{M}$ . Eqs. (1) and (2) express the regression line linking  $A_t$  and  $[\text{ALA}]$ .

$$A_5 = 0.1491 - 2.4 \times 10^3 [\text{ALA}] \quad (1)$$

$$A_{10} = 0.1852 - 2.83 \times 10^3 [\text{ALA}] \quad (2)$$

For the  $A_5$  and  $A_{10}$  plots ( $A_t$  against  $[\text{ALA}]$ ), the computed linear regression coefficient and standard deviation were 0.9948, 0.9954, and 0.0030, 0.0033, respectively. After 5 min

of injecting the estimated quantity of ALA into the reaction system, the absorbance was recorded. The amount of recovered ALA, estimated using the calibration curve is documented in Table 1. The recovered ALA validates the method's repeatability and accuracy.

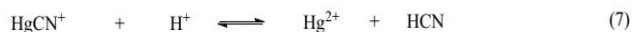
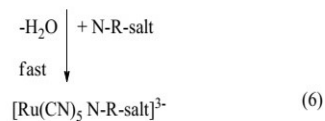
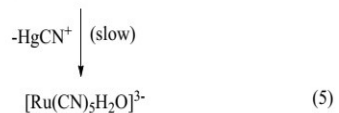
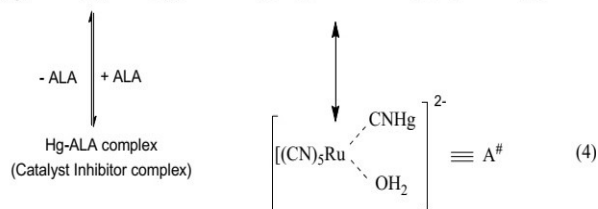


**Fig. 3.** Calibration curve for the determination of alpha-lipoic acid at  $[\text{N-R-salt}] = 0.45 \text{ mM}$ ,  $\text{pH} = 7.0 \pm 0.02$ ,  $\text{Temp} = 45 \pm 0.2 \text{ }^\circ\text{C}$ ,  $[\text{Ru}(\text{CN})_6^{4-}] = 42.5 \text{ } \mu\text{M}$ ,  $I = 0.10 \text{ M}$  ( $\text{NaClO}_4$ ),  $[\text{Hg}(\text{II})] = 0.25 \text{ mM}$ , and  $[\text{SLS}] = 8.5 \text{ mM}$ .

**Table 1.** ALA Present *versus* the Recovered ALA Experimental Condition:  $[\text{NR-salt}] = 0.45 \text{ mM}$ ,  $\text{pH} = 7.0 \pm 0.02$ ,  $\text{Temp} = 45 \pm 0.2 \text{ }^\circ\text{C}$ ,  $[\text{Ru}(\text{CN})_6^{4-}] = 42.5 \text{ } \mu\text{M}$ ,  $I = 0.10 \text{ M}$  ( $\text{NaClO}_4$ ),  $[\text{Hg}(\text{II})] = 0.25 \text{ mM}$ , and  $[\text{SLS}] = 8.5 \text{ mM}$

$[\text{ALA}] \times 10^5 \text{ M}$ (Present)	ALA recovered using $A_5$		ALA recovered using $A_{10}$	
	$[\text{ALA}] \times 10^5 \text{ M}$	Error	$[\text{ALA}] \times 10^5 \text{ M}$	Error
0.40	$0.43 \pm 0.051$	+0.075	$0.42 \pm 0.056$	+0.050
0.60	$0.58 \pm 0.062$	-0.033	$0.60 \pm 0.038$	0.000
1.35	$1.35 \pm 0.021$	0.000	$1.39 \pm 0.037$	+0.030
1.75	$1.73 \pm 0.039$	-0.011	$1.79 \pm 0.052$	+0.023
2.25	$2.29 \pm 0.074$	+0.018	$2.27 \pm 0.038$	+0.009
3.00	$3.00 \pm 0.049$	0.000	$2.95 \pm 0.041$	-0.017
3.75	$3.79 \pm 0.067$	+0.011	$3.72 \pm 0.065$	-0.008
4.50	$4.45 \pm 0.092$	-0.011	$4.50 \pm 0.061$	0.000

A modified mechanistic approach (Eqs. (3)-(7)) established ALA's inhibitory influence on  $\text{Hg}^{2+}$  accelerated cyanide substitution from  $[\text{Ru}(\text{CN})_6]^{4-}$  by N-R-salt. The hypothesized mechanism is similar to the enzyme-catalyzed process in the presence of an inhibitor [28-30].



Taking into account hexacyanoruthenate(II) as a sole substrate having initial concentration  $S_o$ . The catalyzed reaction rate can be developed in parallel with the enzyme-catalyzed process when an inhibitor (ALA) is present. The catalyzed reaction's rate ( $V_o$ ) during ALA absence is represented by Eq. (8) [43-44].

$$V_o = \frac{V_{\max}}{1 + \frac{K_m}{[S_o]}} \quad (8)$$

Here,  $V_{\max}$  and  $K_m$  represent the optimum rate at higher reactant concentrations and, correspondingly, the Michaelis-Menten constant. The above equation's straight-line version ( $1/V_o$  vs.  $1/[S_o]$ ), given by Eq. (9), is consistent with the Lineweaver-Burk expression [45]. It has a slope ( $K_m/V_{\max}$ ), intercept ( $1/V_{\max}$ ), and linear regression coefficient of 0.9992.

$$\frac{1}{V_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \frac{1}{[S_o]} \quad (9)$$

Figure 4's slope and intercept were used to compute the  $K_m$  value, which came out at  $0.2448 \pm 0.017$  mM.

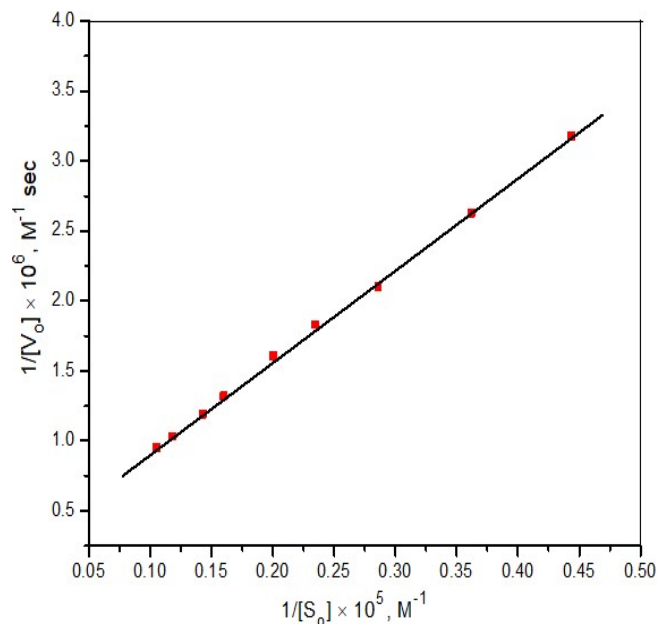
When the inhibitor is present and the catalyst concentration is constant, the apparent M-M constant " $K'_m$ " can be expressed as [43-44]:

$$K'_m = K_m \left( 1 + \frac{[I_o]}{K'_{CI}} \right)$$

Where initial [ALA] is denoted by  $I_o$ , and  $K'_{CI}$  is the dissociation constant of the catalyst-inhibitor complex (C-I). Equation (10) can be used to express the initial rate ( $V_i$ ) in the presence of the inhibitor at a fixed  $\text{Hg}(\text{II})$  concentration [46].

$$V_i = \frac{V_{\max}}{1 + \frac{K'_m}{[S_o]}} \quad (10)$$

$$V_i = \frac{V_{\max}}{1 + \frac{K_m}{[S_o]} \left( 1 + \frac{[I_o]}{K'_{CI}} \right)} \quad (11)$$

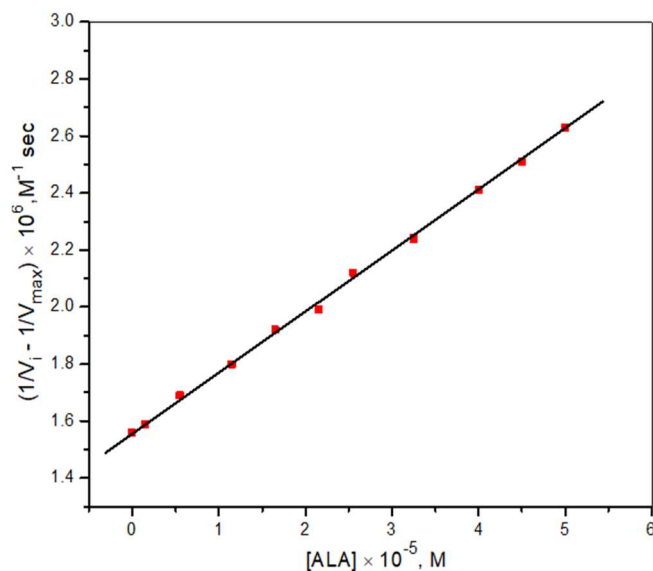


**Fig. 4.** The Lineweaver-Burk plot at constant  $[\text{Hg}^{2+}]$  in the absence of ALA at  $[\text{N-R-salt}] = 0.45$  mM,  $\text{pH} = 7.0 \pm 0.02$ ,  $\text{Temp} = 45 \pm 0.2$  °C,  $I = 0.10$  M ( $\text{NaClO}_4$ ),  $[\text{Hg}(\text{II})] = 0.25$  mM, and  $[\text{SLS}] = 8.5$  mM.

Equation (12) represents the straight-line variant of Equation (11) according to the Lineweaver-Burk equation.

$$\frac{1}{V_i} - \frac{1}{V_{\max}} = \frac{K_m}{[S_o]V_{\max}} + \frac{K_m}{[S_o]V_{\max}} \frac{[I_o]}{K'_{CI}} \quad (12)$$

The  $K'_{CI}$  and  $K_m$  (in the presence of ALA) values were calculated using the slope and intercept of the linear plot between the initial [ALA] and  $(\frac{1}{V_i} - \frac{1}{V_{\max}})$  (Fig. 5) and were discovered to be  $7.36 \times 10^{-5} \pm 0.24$  and  $0.2481 \pm 0.009$  mM, respectively. The computed  $K_m$  value is nearly the same whether ALA is present or absent. The catalyst inhibitor complex appears to be quite stable, as indicated by the reduced dissociation constant value ( $7.36 \times 10^{-5} \pm 0.16$ ).



**Fig. 5.** The plot of  $(1/V_i - 1/V_{\max})$  vs. initial [ALA] at [N-R-salt] = 0.45 mM, pH =  $7.0 \pm 0.02$ , Temp =  $45 \pm 0.2$  °C,  $[Ru(CN)_6^{4-}] = 42.5 \mu M$ , I = 0.10 M (NaClO<sub>4</sub>),  $[Hg(II)] = 0.25$  mM, and [SLS] = 8.5 mM.

### Interference Caused by Competing Constituents

Excipients are inert substances used in drug formulations as fillers, coloring agents, and preservatives in addition to the active medicinal component. Recovery tests were carried out under optimized reaction conditions utilizing the A<sub>5</sub> calibration line, comprising  $4.0 \mu g ml^{-1}$  ALA and a variety of

**Table 2.** Results of ALA Recovery in the Presence of Neutral Additives. Experimental Condition: [NR-salt] = 0.45 mM, pH =  $7.0 \pm 0.02$ , Temp =  $45 \pm 0.2$  °C,  $[Ru(CN)_6^{4-}] = 42.5 \mu M$ , [ALA] = 20.0  $\mu M$ , I = 0.10 M (NaClO<sub>4</sub>),  $[Hg(II)] = 0.25$  mM, and [SLS] = 8.5 mM

Excipients	[Excipients]/[ALA]	Recovery ± sd (%)
Maltitol	750	99.2 ± 0.7
Sorbitol	1000	100.9 ± 0.6
Citrate	750	101.3 ± 0.5
Sucrose	1000	99.5 ± 0.7
Magnesium stearate	750	101.7 ± 0.9
Lactose	1000	99.4 ± 0.8
Gelatin	750	100.5 ± 0.8

different excipients to examine the impact of excipients. The recovery findings demonstrate that regular additives included in medications do not significantly hinder the quantification of ALA, even when multiplied by 1,000 (Table 2).

### Application in Medicinal Formulations

By using the recommended kinetic spectrophotometric approach, the powdered amount of 8 capsules or tablets were dissolved in 100 ml of water to determine the amount of ALA within different medication samples. The resulting solution was then sonicated for 20 min, filtered through Whatman filter paper, and diluted to attain the drug concentration into the A<sub>5</sub> calibrating range. The recommended kinetic approach for the measurement of ALA was used for five distinct medications obtained from local pharmacies that only contained ALA and excipients. The outcome has been compared with the approved method (Table 3). The statistical analysis and average recovery (99-101) findings show that the suggested methodology for the ALA determination in pharmaceuticals and water samples is reproducible and accurate [47].

### CONCLUSION

For the quantitative determination of ALA, a novel, swift, and consistently repeatable kinetic approach that relies on the

**Table 3.** Comparison of ALA Determination in Drug Samples with the Approved Method Experimental Condition: [NR-Salt] = 0.45 mM, pH = 7.0 ± 0.02, Temp = 45 ± 0.2 °C, [Ru(CN)<sub>6</sub><sup>4-</sup>] = 42.5 µM, I = 0.10 M (NaClO<sub>4</sub>), [Hg(II)] = 0.25 mM, and [SLS] = 8.5 mM

Samples of Medicine	Recommended methodology [Recovery ± SD (%)]	Approved methodology [Recovery ± SD (%)]
Alafin 600 mg Tablet (Fourrts Labs Pvt. Ltd, Chennai, India)	99.51 ± 0.65	99.87 ± 0.35
Alpha Lipoic Acid 300 mg Tablet (Bliss Lifescience LLP, Indore, India)	99.13 ± 0.71	101.17 ± 0.18
Liplac 600 mg Tablet (Apco Pharm Ltd, New Delhi, India)	100.59 ± 0.92	100.35 ± 0.80
Alpha Lipoic Acid 300 mg Capsule (Medizen Labs Pvt. Ltd, Bengaluru, India)	99.82 ± 0.48	100.09 ± 0.15
Alpha Lipoic Acid 350 mg Capsule (Pharmagenica Healthcare Pvt. Ltd, Bengaluru, India)	100.39 ± 0.30	99.28 ± 0.28

inhibitory behavior of the sulfur-containing molecule ALA towards Hg<sup>2+</sup> has been presented. Even up to 1000 times with [ALA], the general additives used in medications do not significantly hinder the detection of ALA. The suggested kinetic spectrophotometric approach allows for the micro-level measurement of ALA in various water specimens down to 2.5 µM. The optimized methodology was also used to quickly quantify ALA in pharmaceutical samples. The suggested methodology for the determination of ALA in water samples and different drugs is reproducible and accurate, as shown by average recovery (99-101%) and statistical analysis results.

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