

## Kinetic Spectrofluorometric Determination of Thioctic Acid in Bulk and Pharmaceutical Preparations *via* its Oxidation with Cerium(IV)

F. A. Ibrahim, F. A. Ali, S. M. Ahmed and M. M. Tolba\*

*Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt*

A highly simple and sensitive kinetic spectrofluorometric method was developed for the determination of thioctic acid. The method is based on the oxidation of the studied drug with cerium(IV) ammonium sulfate in acidic medium. The fluorescence of the produced Ce(III) was measured at 365 nm after excitation at 255 nm. The different experimental parameters affecting the development and stability of the reaction product were carefully studied and optimized. The method is applicable over the concentration range of 0.02 to 0.12  $\mu\text{g/mL}$  with a detection limit of  $6.06 \times 10^{-3} \mu\text{g/mL}$  and a quantification limit of 0.02  $\mu\text{g/mL}$ . The method was successfully applied for the assay of the studied drug in pharmaceutical formulations. The results obtained were in good agreement with those obtained with the reference method.

**Keywords:** Spectrofluorometry; Thioctic acid; Ce(IV); Dosage forms.

### INTRODUCTION

Thioctic acid (Fig. 1) is 1,2-dithiolane-3-pentanoic acid. It is used for treatment of liver dysfunction and diabetic neuropathy and as an antidote to poisonous mushrooms [Amantia species].<sup>1,2</sup>

The reported methods for the determination of the drug include spectrophotometry,<sup>3,4</sup> electro-analysis,<sup>5-9</sup> capillary electrophoresis,<sup>10</sup> liquid chromatography-mass spectroscopy,<sup>11</sup> gas chromatography-mass spectroscopy<sup>12</sup> and high performance liquid chromatography.<sup>13-22</sup>

All these methods are either insufficiently sensitive<sup>3-10</sup> or tedious, and require highly sophisticated and dedicated instrumentation.<sup>11-22</sup> To the best of our knowledge, no spectrofluorometric method has been reported for the analysis of thioctic acid up till now. The results obtained were promising.

Since official and reported methods for determination of the studied drug were found to be laborious, expensive and time consuming, the aim of this work was to develop a

new spectrofluorometric method for determination of the drug, that is, more sensitive, simple, rapid and less expensive than the reported and official methods. The suggested spectrofluorometric method depends simply on oxidation of the studied drug using Ce(IV) in acidic medium, and the relative fluorescence intensity of the formed Ce(III) was monitored at 365 nm after excitation at 255 nm.

Cerium(IV) has been utilized as a useful oxidizing agent for the determination of certain pharmaceutical compounds such as antiviral drugs,<sup>23</sup> some psychoactive drugs,<sup>24</sup> aztreonam,<sup>25</sup> isoxsuprine hydrochloride,<sup>26</sup> macrocyclic polyaza polycarboxylate ligands TETA and DOTA,<sup>27</sup> diphenylamine<sup>28</sup> and Arnold's base.<sup>29</sup>

### EXPERIMENTAL

#### Apparatus and Manifold

The fluorescence spectra and measurements were carried out using a Perkin-Elmer UK model LS 45 luminescence spectrometer, equipped with a 150 Walt Xenon arc lamp, grating excitation and emission monochromators for all measurements and a Perkin-Elmer recorder. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used.

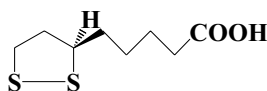


Fig. 1. Structural formulae of thioctic acid.

\* Corresponding author. Fax: ++20502247496; E-mail: manar2kareem@yahoo.com

### Materials and Reagents

All reagents and solvents were of Analytical Reagent Grade.

- Thioctic acid was provided by Eva Pharma for Pharmaceuticals & Medical Appliances, Cairo, Egypt.
- Cerium(IV) ammonium sulfate, (BDH, Pool, UK),  $5 \times 10^{-4}$  M aqueous solution was freshly prepared in 1.0 M sulfuric acid.
- Sulfuric acid (Prolabo, France), 1.0 M aqueous solutions.
- Methanol, Spectroscopic grade (Winlab, UK).
- Acetonitrile, Spectroscopic grade (BDH, Pool, UK).
- Dimethyl sulfoxide and Dimethyl formamide, Spectroscopic grade (Merck, Darmstadt, Germany).

### Standard Solutions

Stock solution of thioctic acid was prepared by dissolving 10.0 mg of the studied compound in 0.4 mL of 0.5 M NaOH, then it was completed to 100 mL with distilled water. The working standard solution was prepared by further dilution with distilled water. The standard solution was stable for 10 days when kept in the refrigerator.

### General Procedure

Aliquot volumes of thioctic acid standard solutions covering the working concentration range were transferred into a series of 10 mL volumetric flasks followed by 0.5 mL of  $5 \times 10^{-4}$  M Ce(IV) solution. The flasks were heated in a thermostatically controlled water-bath at 100 °C for 25 min, cooled and diluted to the mark with distilled water. A blank experiment was performed simultaneously. The relative fluorescence intensity (FI) of the solutions was measured at 365 nm after excitation at 255 nm. The observed fluorescence was corrected by subtracting the fluorescence intensity measured using a reagent blank. The corrected FI was plotted vs final concentration of the drug ( $\mu\text{g/mL}$ ) to get the calibration graph. Alternatively, the corresponding regression equation was derived.

### Procedure for the Dosage Form

An accurately weighed quantity of the mixed contents of 10 powdered tablets equivalent to 10.0 mg was transferred into a small conical flask. 0.4 mL of 0.5 M NaOH was firstly added. The contents of the flask were sonicated for 5 min. The extract was filtered into a 100 mL volumetric flask. The conical flask was washed with a few mls of distilled water. The washings were passed into the

same volumetric flask and completed to the mark with distilled water. Aliquots covering the working concentration range were transferred into 10 mL volumetric flasks. The general procedure was then performed and the nominal content of tablets was determined either from a previously plotted calibration graph or from the corresponding regression equation.

## RESULTS AND DISCUSSIONS

As the fluorescence intensity of the liberated Ce(III) increases with time, this fact was used as a basis for a useful kinetic method for the determination of thioctic acid in bulk and pharmaceuticals. In the present study, oxidation of the studied drug with Ce(IV) in an acid medium yields a highly fluorescent Ce(III) which exhibits maximum fluorescence at 365 nm after excitation at 255 nm (Fig. 2). The oxidation product was found not to be fluorescent. This confirmed the fluorescence induced in the oxidation of the investigated drug with Ce(IV) was not attributed to its oxidation product; however, it was mainly due to the formation of Ce(III).

### Optimization of experimental conditions

The spectrofluorometric properties of the reaction product as well as the different experimental parameters affecting the fluorophore development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors included the Ce(IV) concentration, type of acid and its concentration, heating time, temperature and diluting solvents.

### Effect of Ce(IV) concentration

The influence of Ce(IV) concentration on the fluorescence intensity of the reaction product was studied using increasing volumes of  $5 \times 10^{-4}$  M Ce(IV) solution. It was found that maximum and constant fluorescence intensity was attained using 0.5 mL. A larger volume of Ce(IV) caused a dramatic decrease in the fluorescence intensity.

### Effect of acid type and its concentration

The oxidation reaction of Ce(IV) has to be performed in acid medium to prevent precipitation of  $\text{Ce}(\text{OH})_3$ . Different acids such as sulfuric acid, hydrochloric acid, nitric acid and perchloric acid were tested to determine the most

suitable acid for the reaction. Nitric acid is not preferred to be used owing to the inhibitory effect of nitrate ions on the fluorescence of Ce(III).<sup>30</sup> In the presence of hydrochloric acid, perchloric acid and sulfuric acid the reaction rate and the fluorescence of Ce(III) were found to be high. However, hydrochloric acid and perchloric acid gave high blank readings, so sulfuric acid was selected for the study. The effect of sulfuric acid concentration on the fluorescence intensity was studied using concentrations ranging from 0.25 to 2 M of sulfuric acid. It was found that the relative fluorescence intensity increased by increasing sulfuric acid concentration up to 1.0 M. So, this was used as the optimum concentration of sulfuric acid throughout the study.

#### Effect of temperature and heating time

Oxidation of the studied drug with Ce(IV) was carried out at different temperature sets ranging from 25-100 °C for various periods of time ranging from 5 to 60 min. At 25 °C, the reaction proceeds slowly. However, heating the reaction solution was found to increase both reaction rate and the fluorescence intensity. The results revealed that the

optimum temperature was 100 °C. Complete reaction was attained upon boiling for 25 min, and a longer heating time decreased the relative fluorescence intensity.

#### Effect of diluting solvents

Dilution with different solvents such as water, methanol, acetonitrile, dimethyl sulfoxide and dimethyl formamide was attempted. It was found that water was the best solvent for dilution as it gave the highest fluorescence intensities and the lowest blank reading. A distinct and sharp decrease in the fluorescence intensities was attained upon using acetonitrile and methanol, while dimethyl sulfoxide and dimethyl formamide quench the fluorescence completely.

#### Study of the kinetic parameters

The rate of the reaction was found to be dependent on the concentration of the studied drug. The rate was followed with various concentrations in the range of 0.02-0.12 µg/mL keeping Cerium(IV) and H<sub>2</sub>SO<sub>4</sub> concentrations constant at the recommended levels mentioned before.

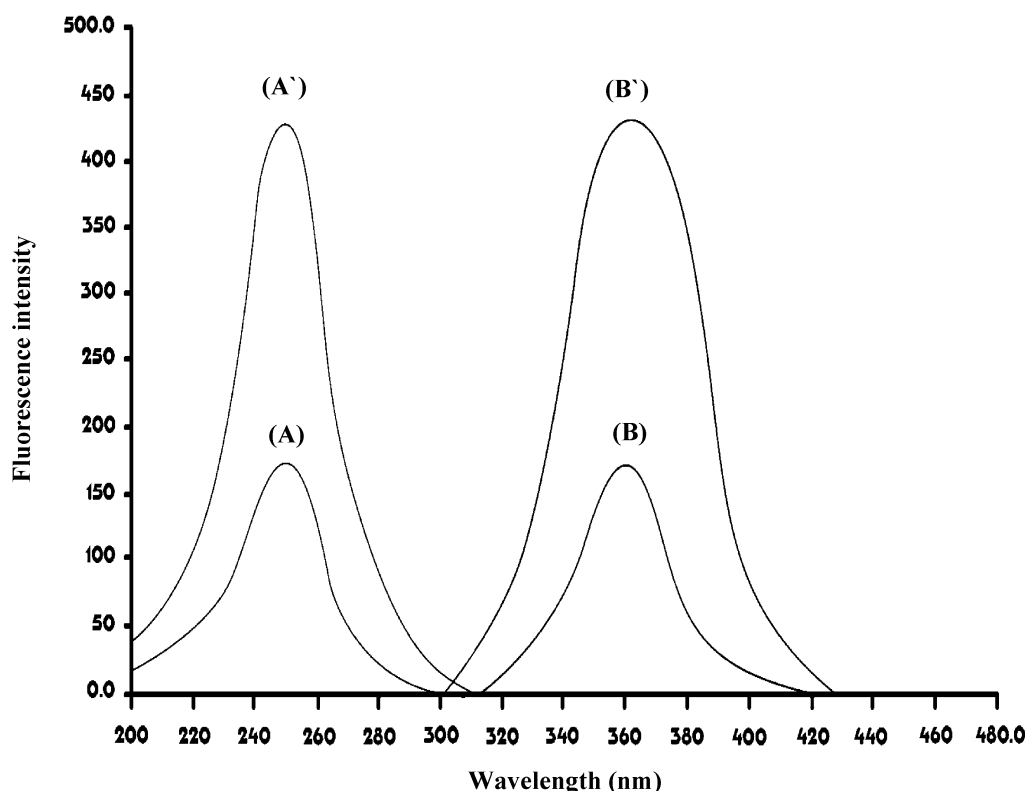


Fig. 2. Excitation and emission spectra of the reaction product induced by oxidation of the studied drug (0.12 µg/mL) with Ce(IV).

The rate of the reaction was found to obey the following equation:

$$\text{Rate of the reaction} = \Delta F/\Delta t = K'[\text{drug}]^n \quad (1)$$

where  $K'$  is the rate constant and  $n$  is the order of the reaction.

The rate of the reaction may be estimated by the variable time method measurement,<sup>31</sup> where  $F$  is the fluorescence intensity and  $t$  is the time in seconds. Taking logarithms of rates and drug concentrations, the previous equation is transformed into:

$$\text{Log (rate)} = \text{Log } \Delta F/\Delta t = \text{Log } K' + n \text{Log [drug]} \quad (2)$$

A plot of log reaction rate versus log concentration of the drug (Fig. 3) gave the regression equation (for thioctic acid):

$$\text{Log rate} = 5.590 + 1.018 \text{ log } C \quad (r = 0.9999)$$

Hence  $K' = 3.90 \times 10^5 \text{ S}^{-1}$  and the reaction is first order ( $n = 1.018$ ).

These results indicate that the reaction is pseudo first order, depending on the drug concentration.

#### Selection of the best kinetic method

Several kinetic techniques were adopted for the selection of the best method. Rate constant, fixed fluorescence and fixed time methods<sup>32,33</sup> were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, i.e. the slope of the calibration graph and the correlation coefficient ( $r$ ).

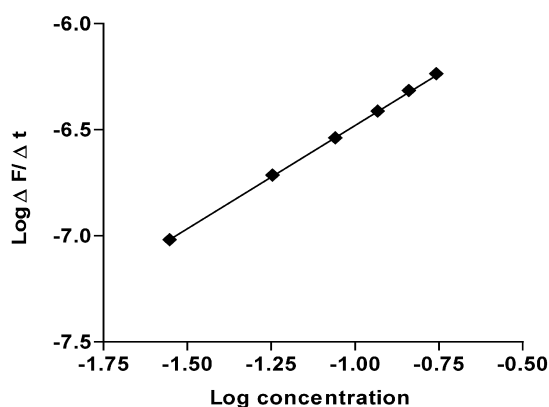


Fig. 3. Plot of Log reaction rate ( $\text{Log } \Delta F/\Delta t$ ) versus Log concentration of thioctic acid.

#### Rate constant method

A graph of log absorbance versus time for thioctic acid concentration in the range of  $1.93 \times 10^{-7}$ – $5.81 \times 10^{-7}$  M was plotted and appeared to be rectilinear. Pseudo-first order rate constants ( $K'$ ) corresponding to different drug concentrations ( $C$ ) were calculated from the slopes multiplied by  $-2.303$  and are presented in Fig. 4.

Regression of ( $C$ ) versus  $K'$  gave equations:

$$K' = -8.863 \times 10^{-4} + 181.134 C \quad (r = 0.996)$$

where  $C$  is the molar concentration of the drug.

#### Fixed fluorescence method

Reaction times required to reach specific fluorescence of redox reaction for different concentrations of thioctic acid in the range of  $1.93 \times 10^{-7}$ – $5.37 \times 10^{-7}$  M were recorded (Fig. 5). A preselected value of the fluorescence 85 was fixed and the time was measured in seconds. The reciprocal of time ( $1/t$ ) versus the initial concentration of drug was plotted. The following equation of the calibration graph was obtained:

$$1/t = -6.900 \times 10^{-4} + 7570.109 C \quad (r = 0.995)$$

where  $C$  is the molar concentration of the drug and  $t$  = time in second.

#### Fixed time method

At a preselected fixed time, which was accurately determined, the fluorescence was measured. Calibration graphs of fluorescence versus initial concentrations of thioctic acid at fixed times (5, 10, 15, 20 and 25 min.) were established (Fig. 6) with the following regression equations and

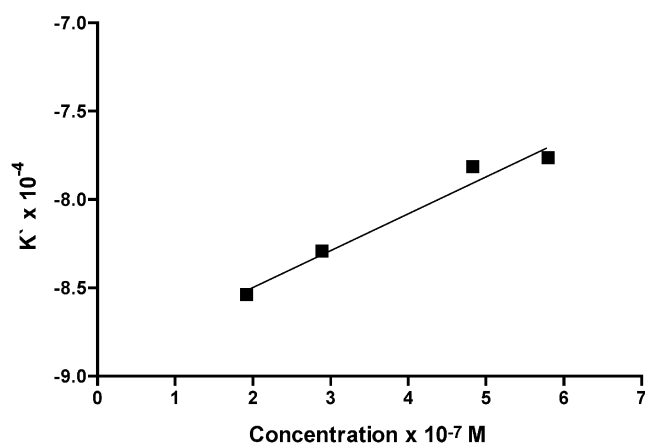


Fig. 4. Plot of rate constant ( $K'/\text{S}^{-1}$ ) versus the molar concentration of thioctic acid.

correlation coefficients:

5 min	$A = 1.667 + 757.143 C$	$r = 0.9950$
10 min	$A = -5.333 + 1492.857 C$	$r = 0.9990$
15 min	$A = -1.133 + 1561.429 C$	$r = 0.9993$
20 min	$A = -0.733 + 2012.857 C$	$r = 0.9994$
25 min	$A = -2.400 + 2198.571 C$	$r = 0.9999$

It is clear that the slope increases with the time and the most acceptable values of the correlation coefficient ( $r$ ) were chosen as the most suitable time interval for measurement. As a conclusion, the fixed time method was chosen

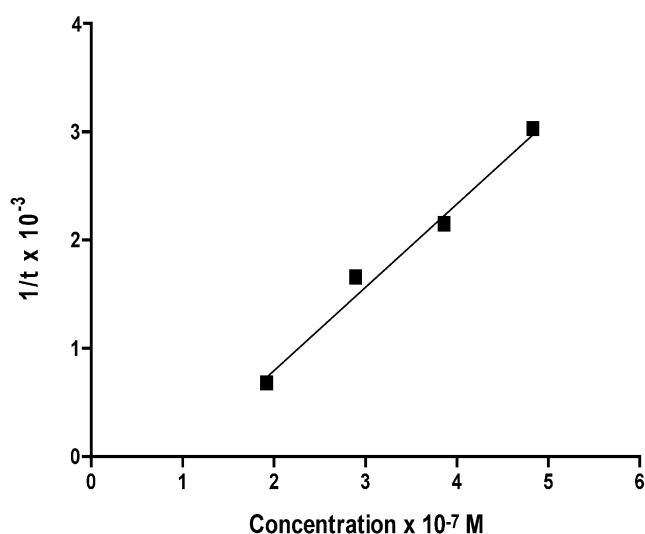


Fig. 5. Plot of reciprocal of time ( $1/t$ ) versus the molar concentration of the drug.

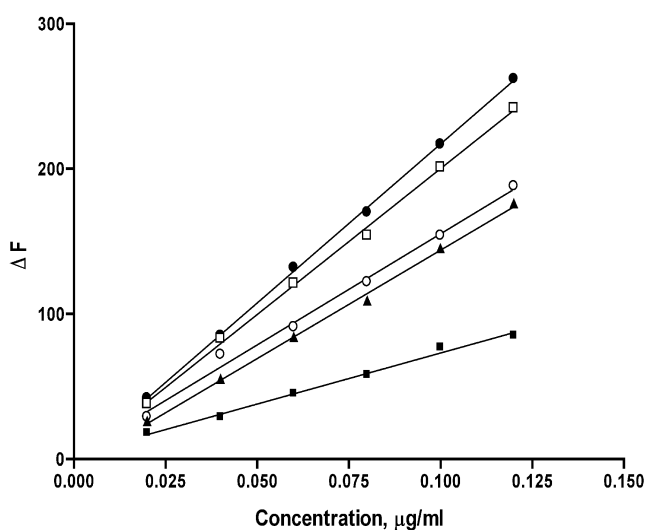


Fig. 6. Calibration curves of thioctic acid at different times.

for quantification because it gives the best correlation coefficient in a reasonable time.

#### Analytical Performance

The fluorescence-concentration plot for the studied drug was linear over the range of 0.02-0.12  $\mu\text{g/mL}$ .

Linear regression analysis of the data gave the following equation:

$$F = -2.400 + 2198.571 C \quad (r = 0.9999)$$

where  $F$  is fluorescence intensity,  $C$  is the concentration of the drug ( $\mu\text{g/mL}$ ) and  $r$  is correlation coefficient.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured, below which the calibration graph is non linear and was found to be 0.02  $\mu\text{g/mL}$ . The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected and was found to be  $6.06 \times 10^{-3}$   $\mu\text{g/mL}$ .

The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation which were found to be 0.220 and 0.539, respectively.

#### Validation of the Method

##### Linearity

The proposed method was tested for linearity, specificity, precision, and reproducibility. A linear regression equation was obtained. The regression plot showed a linear dependence of FI value on drug concentrations over the calibration range. The lower detection limit as well as the slope and intercept were also clarified. Validation of the method was evaluated by statistical analysis of the regression line regarding standard deviation of the residuals ( $S_{y/x} = 0.515$ ), the intercept ( $S_a = 4.441$ ), and the slope ( $S_b = 6.155$ ). The small values given point to the low scattering of the points around the calibration curve.

The % recoveries of the studied drug compared with those obtained by the comparison method<sup>4</sup> are given in Table 1. The comparison method involved the spectrophotometric determination of the drug by alkaline  $\text{KMnO}_4$ .

Statistical analysis<sup>34</sup> of the results, obtained by the proposed and the comparison method<sup>4</sup> using the Student's  $t$ -test and variance ratio  $F$ -test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

Table 1. Application of the proposed and comparison method to the determination of thioctic acid in pure form

Parameters	Recovery, %			
	Proposed method		Comparison method <sup>4</sup>	
	Concentration taken ( $\mu\text{g/mL}$ )	Recovery, (%)	Concentration taken ( $\mu\text{g/mL}$ )	Recovery, (%)
	0.02	101.00	4	99.20
	0.04	99.50	5	100.96
	0.06	100.33	7	99.77
	0.08	99.75		
	0.10	99.80		
	0.12	100.25		
Mean found, $\bar{x}$		100.11		99.99
$\pm$ SD		0.540		0.876
RSD, %		0.539		0.876
Variance		0.292		0.767
Student's <i>t</i> -value		0.259 (2.365)		
Variance ratio <i>F</i> -test		2.63 (19.30)		

N.B. Figures between parenthesis are the tabulated *t* and *F* values, respectively at  $p = 0.05$ <sup>34</sup>

### Accuracy and precision

The intra-day precision was evaluated through replicate analysis of a sample containing 0.1  $\mu\text{g/mL}$  thioctic acid. The concentration was analyzed three times. The mean percentage recovery was  $98.73 \pm 0.945$ . The repeatability and reproducibility of the proposed method are fairly good as indicated by small values of standard deviation (SD).

The inter-day precision was evaluated through replicate analysis of a sample containing the studied compound on three successive days. The mean percentage recovery based on the average of three separate determinations was  $99.40 \pm 0.872$ .

The accuracy of the proposed method was evaluated by analyzing standard solutions of the studied drug. The results obtained by the proposed method were compared with those given by the comparison method.<sup>4</sup>

### Robustness of the method

The robustness of the method adopted in the proposed method is demonstrated by the constancy of the fluorescence intensity with minor changes in the experimental parameters such as  $5 \times 10^{-4}$  M Ce(IV) volume,  $0.5 \pm 0.1$  mL and change in the concentration of sulfuric acid,  $1 \pm 0.1$  M. These minor changes that may take place during the experimental operation didn't affect the fluorescence intensity.

### Pharmaceutical Applications

The proposed method was applied to the determination of the studied drug in its dosage forms. The specificity of the method was investigated by observing any interference encountered from the common excipients, such as talc, lactose, starch, avisil, gelatine, or magnesium stearate. These excipients did not interfere with the proposed method (Table 2). The results of the proposed method were compared with those obtained using the comparison method.<sup>4</sup>

Statistical analysis<sup>34</sup> of the results obtained using the Student's *t*-test and variance ratio *F*-test revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 2).

### Mechanism of the reaction

The stoichiometry of the reaction between the studied drug and cerium(IV) was studied adopting the limiting logarithmic method<sup>35</sup> (Fig. 7). The fluorescence intensity of the reaction product was alternatively measured in the presence of excess Ce(IV) and the studied drug. Plots of  $\log [\text{drug}]$  versus  $\log \Delta F$  and  $\log [\text{Ce(IV)}]$  versus  $\log \Delta F$  gave straight lines; the values of the slopes were 3.553:0.982 (Ce(IV): drug). Hence, it is concluded that the molar reactivity of the reaction is 4:1, i.e. the reaction proceeds in a ratio of 4:1.

Table 2. Application of the proposed method to the determination of the studied drug in dosage form

Compound	Proposed method			Comparison method <sup>4</sup>	
	Taken (µg/mL)	Found (µg/mL)	Recovery, (%)	Taken (µg/mL)	Recovery, (%)
Thiotacid tablet* (300 mg/tablet)	0.02	0.0198	98.75	4	99.25
Batch # 508647	0.04	0.0392	97.88	5	101.02
	0.06	0.0600	100.00	7	99.80
Mean ± S.D			98.88 ± 1.066		100.02 ± 0.906
Student's <i>t</i> -test			1.411 (2.776)**		
F-test			1.380 (19.00)**		

N.B. Each result is the average of three separate determinations.

Figures between parenthesis are the tabulated *t* and *F* values, respectively at  $p = 0.05$ <sup>34</sup>

\* Product of Eva Pharma for Pharmaceuticals & Medical Appliances, Egypt.

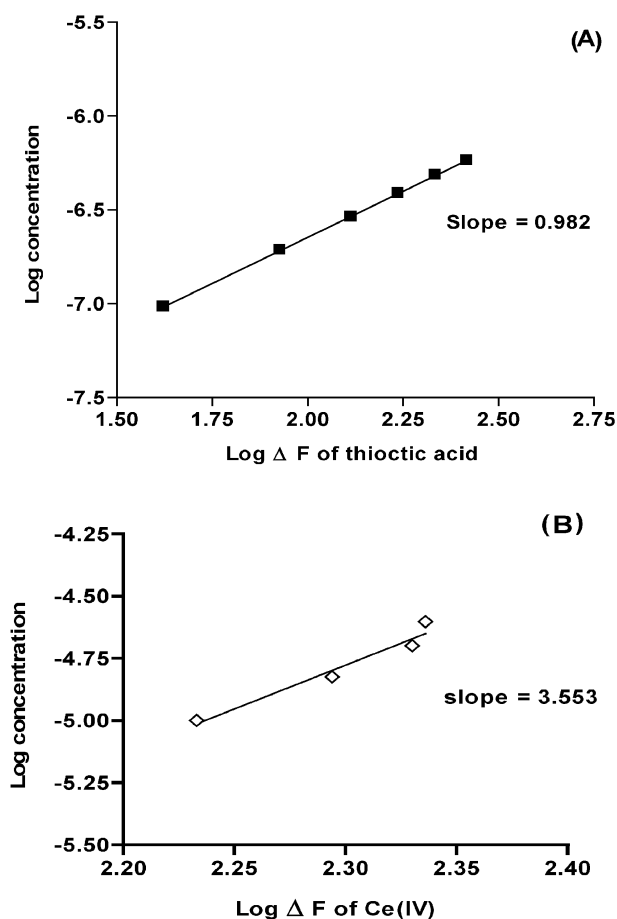
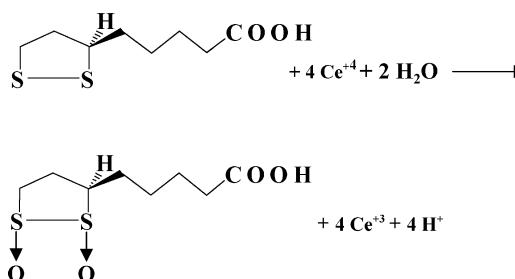


Fig. 7. Stoichiometry of the reaction between thioctic acid and Ce(IV) adopting limiting logarithmic method.<sup>35</sup> (A) Log [thioctic acid] vs log  $\Delta F$ . (B) Log [Ce(IV)] vs log  $\Delta F$ .

Based on the above fact and by analogy to a previous report,<sup>23</sup> a proposal for the reaction between the studied drug and Ce(IV) is shown in the following scheme:

Scheme I Proposal for the reaction between thioctic acid and Ce(IV)



## CONCLUSION

The present work describes a validated spectrofluorometric method for the determination of the studied drug without interference from common excipients. Hence, it could be applied for the routine quality control of the studied drug either in bulk or in its corresponding dosage forms. The methodology appears to be straightforward and results are relevant. Another advantage is that compared to the existing reported methods for determination of this drug, it is several times more sensitive. From an economic point of view, the proposed method is simple, rapid and inexpensive and uses water as diluting solvent. So, it is a good alterna-

tive method to the reported methods and to high cost HPLC methods.

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