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Fluorimetric Determination of Some Sulfur Containing Compounds Through Complex Formation with Terbium (Tb^{+3}) and Uranium (U^{+3})

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Abstract Two simple, sensitive and specific fluorimetric methods have been developed for the determination of some sulphur containing compounds namely, Acetylcysteine (Ac), Carbocisteine (Cc) and Thioctic acid (Th) using terbium Tb^{+3} and uranium U^{+3} ions as fluorescent probes. The proposed methods involve the formation of a ternary complex with Tb^{+3} in presence of Tris-buffer method (I) and a binary complex with aqueous uranyl acetate solution method (II). The fluorescence quenching of Tb^{+3} at 510, 488 and 540 nm (λ_{ex} 250, 241 and 268 nm) and of uranyl acetate at 512 nm (λ_{ex} 240 nm) due to the complex formation was quantitatively measured for Ac, Cc and Th, respectively. The reaction conditions and the fluorescence spectral properties of the complexes have been investigated. Under the described conditions, the proposed methods were applicable over the concentration range (0.2–2.5 μg ml^{-1}), (1–4 μg ml^{-1}) and (0.5–3.5 μg ml^{-1}) with mean percentage recoveries 99.74 ± 0.36, 99.70 ± 0.52 and 99.43 ± 0.23 for method (I) and (0.5–6 μg ml^{-1}), (0.5–5 μg ml^{-1}), and (1–6 μg ml^{-1}) with mean percentage recoveries 99.38 ± 0.20, 99.82 ± 0.28 and 99.93 ± 0.32 for method (II), for the three cited drugs, respectively. The proposed methods were successfully applied for the determination of the studied compounds in bulk powders and in pharmaceutical formulations, as well as in presence of their related substances. The results obtained were found to be in agree statistically with those obtained by official and reported ones. The two methods were validated according to USP guidelines and also assessed by applying the standard addition technique.

Introduction

Acetylcysteine (*N*-acetyl-3-mercaptoalanin), Carbocisteine (*R*)-2-amino-3-{(carboxymethyl) thio} propionic acid, and Thioctic (alpha Lipoic) acid (1, 2-dithiolane-3- pentanoic acid) are three biologically active substances. Ac and Cc are two amino acids playing an important role in reducing viscosity of pulmonary secretions and facilitating their removal, therefore they are used as mucolytic drugs [1]. Ac is also able to promote the detoxication of paracetamol over dosage (poisoning) [2]. Thioctic acid is a biologically active substance, working as an important co-factor in biological oxidation. It is used for treatment of liver dysfunction, diabetes neuropathy, optic neuritis and antidote to poisonous mushrooms (Amanita species) [2]. They have the following structure.

Acetylcysteine (Ac)   Carbocisteine (Cc)

Thioctic acid [Th]
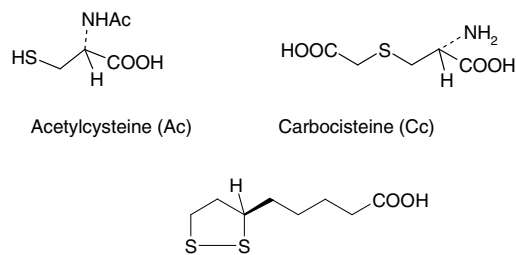
Structure of the three studied drugs.

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B.P describes an iodometric and a non-aqueous titrimetric method for the determination of Ac and Cc [3] while USP describes HPLC method for determination of Ac and Th [4]. Other methods were reported for their determination such as spectrophotometric [5–8], spectrofluorimetric [9–12], electrochemical [13–15] and HPLC [16–18] methods.

Fluorescence spectra of the lanthanide ions especially, where they are chelated with ligands constituted the basis of a technique in microanalysis of many organic compounds. The main advantages of lanthanide chelates in fluorescence spectrometry include large stokes shifts, narrow emission bands and long fluorescence life time [19]. In all applications of lanthanide ions, the intense luminescence originates from an intramolecular energy transfer through the excited state of the ligand to the emitting level of the lanthanide ion. Conversely, energy transfer from an organic compound to a lanthanide ion can be used to improve the fluorimetric analysis of organic analytes.

In some instances, when the organic compound has a triplet—state level below the excited—state level of the lanthanide ion, the organic compound can quench the back ground luminescence of the ion [20]. The sensitizing or quenching effect is more important with chloride than the nitrate salts of the lanthanide, because the probability of collision leading to energy transfer is larger for the chloride salt [21].

Based on energy level considerations and luminescence quantum yields, Tb^{3+} and Eu^{3+} are the best lanthanide ions to be applied to the determination of organic compounds. Several compounds have been determined by using Tb^{3+} such as cefazolin sodium, cefoperazone sodium and ceftriaxone sodium [20]. The terbium metal ion (Tb^{3+}) has been the lanthanide of choice in most applications of ternary—complex formation.

Uranyl acetate is a yellow free flowing, crystalline, water soluble uranium compound. It has been used as a chromogenic agent for the spectrophotometric determination of different drugs through complex formation [22–24]. It is also used as a fluorescent indicator for TLC chromatographic detection of complexing agents [25]. The first objective of this paper was to study the formation of ternary complex between (Tb^{3+}—Tris buffer) and the three cited compounds whereas the second aim was to determine these mentioned drugs through the formation of binary complexes between them and uranyl acetate.

Experimental

Instrumentation

1) SHIMADZU RF—1501 Spectrofluorimeter using quartz cell ($1 \times 1 \times 4.5$ cm).

2) Digital pH meter, PW 9409 Pye Unicum.

Materials and reagents

All chemicals, solvents and reagents were of analytical grade.

- Acetylcysteine and Carbocicteine were kindly supplied by Sideco. Co. Egypt. The purity of the samples were found to be 99.48 ± 0.13 and $99.60 \pm 0.22\%$ for the two drugs, respectively according to the official titrimetric methods [3]. Parvolex ampoules 2 g Ac/10 ml B.N.13140 were purchased from Celletch Pharmaceuticals Limited. U.K. Mucosol capsules 375 mg Cc B.N.11966 (MUP. Co. Egypt).
- Thioctic acid purity $99.93 \pm 0.17\%$ according to official HPLC method [3, 4], Thiotacid Tablets 300 mg and Thiotacid ampoules 300 mg/10 ml (Eva. Co. Egypt).
- L-cystine. Otsoka Co, Egypt. certified to be 99.50%
- L-arginine. Otsoka Co. Egypt. Certified to be 99.50%
- Oxidized form of thioctic acid was prepared by adding about 3 ml of 30% hydrogen peroxide solution to (10 mg thioctic acid in 15 ml methanol), the solution was left for 15 min and the volume was completed to the mark with methanol. This solution was warmed over heating water bath till nearly dry and the resulting residue was dissolved in 25 ml methanol.
- Reduced form of Thioctic acid (metabolite, dihydrolipoic acid) [26] was prepared by adding about 5 g Tin metal and 5 ml of 20% v/v hydrochloric acid to (10 mg thioctic acid in 15 ml methanol). This mixture was left for 3 h till complete reduction and the resulting solution was heated till nearly dry. The residue left was dissolved in 25 ml methanol.

  The complete oxidation and reduction was confirmed by the disappearance of the characteristic peak of thioctic acid at 334 nm and by TLC using ethyl acetate, methanol, ammonia and water (20:20:5:0.5) as mobile phase. More over the structure of the oxidized form of thioctic acid was confirmed by IR and mass spectroscopy.
- Terbium chloride (5×10^{-3} M in H_2O). (Sigma. Co). Its purity (99.99%)
- Tris buffer (Sigma. Co.) was prepared by dissolving 2 g of Tris buffer and 2.4 g of sodium chloride in 100 ml distilled water, pH was adjusted with 1 M NaOH or 1 M HCl. And then the volume was diluted to 200 ml with distilled water.
- Methanol (Aldrich. Co).
- Sodium hydroxide and hydrochloric acid (El Nasr. Co. Egypt.)
- Uranyl acetate 0.2% w/v aqueous solution (Sigma. Co.)

Preparation of standard solutions

1. Ac (0.1 mg ml^{-1}) in distilled water.

Table 1 Validation report obtained by applying the two suggested methods for the determination of the three cited drugs in bulk powder

Items	Method (I)			Method (II)		
	Ac	Cc	Th	Ac	Cc	Th
Linearity range (μg ml^{-1})	0.2–2.5	1–4	0.5–3.5	0.5–6	0.5–5	1–6
Slope	119.800	30.928	27.00	41.618	56.192	50.657
SE of slope	1.093	0.311	0.111	0.248	0.265	0.135
Intercept	6.424	1.964	0.714	11.460	1.671	6.533
SE of intercept	1.657	0.838	0.247	0.895	0.804	0.526
Correlation coefficient (r)	0.9998	0.9997	0.9999	0.9999	0.9998	1.00
SE of (r)	0.50×10^{-4}	0.56×10^{-4}	0.34×10^{-4}	0.75×10^{-4}	0.98×10^{-4}	0.83×10^{-4}
Accuracy (mean* $\pm$ %RSD)	99.74 $\pm$ 0.36	99.70 $\pm$ 0.52	99.43 $\pm$ 0.23	99.38 $\pm$ 0.20	99.82 $\pm$ 0.28	99.93 $\pm$ 0.32
Interday precision	$\pm$ 0.26	$\pm$ 0.46	$\pm$ 0.28	$\pm$ 0.61	$\pm$ 0.59	$\pm$ 0.69
Intraday precision	$\pm$ 0.46	$\pm$ 0.28	$\pm$ 0.58	$\pm$ 0.52	$\pm$ 0.56	$\pm$ 0.61

*Mean of five different experiments.

2. Cc (0.1 mg ml^{-1}), was prepared as follows, (10 mg Cc in 100 ml conical flask, 0.5 ml of 0.1 N NaOH and 50 ml distilled water were added, the resulting mixture was sonicated till dissolved, transferred quantitatively to 100 ml measuring flask and the volume was completed to the mark with distilled water.
3. Thioctic acid (0.1 mg ml^{-1}) in methanol.

General procedures

Method I: Using terbium chloride

Into a series of 10 volumetric flasks, 0.5 ml of aqueous terbium chloride solution (5×10^{-3} M) was transferred. Aliquots of the three cited drugs, each according to the working range 2–25, 10–40 and 5–35 μg ml^{-1} for Ac, Cc and Th, respectively were added, followed by, 1 ml of Tris-buffer pH 8.5 for Ac, pH 9.5 for Cc and pH 10.5 for Th. The volume was completed to the mark with distilled water. The fluorescence quenching at 510, 488 and 540 nm was measured using 250, 241 and 268 nm as excitation wavelengths for the three drugs, respectively. To obtain the standard calibration graphs concentrations were plotted versus decrease in fluorescence intensities, and the linear regression equations were computed.

Method II: Using uranyl acetate

Into a series of 10 ml volumetric flasks 1 ml for Ac and Cc or 1.5 ml for Th of 0.2% w/v aqueous uranyl acetate was transferred. Aliquots of the three cited drugs, each according to the working range 5–60, 5–50 and 10–60 μg ml^{-1} for Ac, Cc and Th, respectively were added, and the volume was completed to the mark with distilled

water. The fluorescence quenching at 512 nm was measured using 240 nm, as excitation wave length for the three cited compounds. The standard calibration graphs were obtained by plotting concentrations versus decrease in fluorescence intensities. The linear regression equations were derived.

Procedures for dosage forms

For ampoules: (Parvolex ampoules 2 g Ac/10 ml and Thiotacid ampoules 300 mg Th/10 ml) The contents of three ampoules were mixed well in 50 ml dried beaker. Aliquots equivalent to 10 mg Ac and Th were transferred into 100 ml measuring flask and diluted with distilled water to volume .An accurately measured volumes of the obtained solutions in the linearity range shown in Table 1 were assayed as described under general procedure.

For capsules: (Mucosol capsules 375 mg Cc) The contents of 10 capsules were mixed thoroughly, an accurate weight equivalent to 10 mg Cc was transferred into 100 ml conical flask, and 0.5 ml of 0.1 M NaOH and 50 ml water were added. This mixture was sonicated for 25 min, filtered, transferred quantitatively to 100 ml measuring flask and the volume was completed to the mark with distilled water. Aliquots of this solution in the linearity range shown in Table 1 were assayed as described under general procedure.

For tablets: (Thiotacid tablets 300 mg) Five tablets were pulverized well, an accurate weight of the powdered tablets equivalent to 10 mg Th was transferred into 100 ml conical flask. 50 ml methanol were added, shaked well, filtered and transferred quantitatively to 100 ml measuring flask. The volume was completed to the mark with methanol, and proceeded as described under general procedure.

Results and discussion

Method (I) using terbium chloride

The solution of Tb^{3+} in Tris buffer has an intense fluorescence, if compared with its solution in hydrochloric acid [20]. On adding the studied compound solutions immediate fluorescence quenching was observed. The relative emission spectra are shown in Fig. 1a–c. The decrease in fluorescence intensity was proportional to the concentration of the added compounds. (Ac, Cc and Th)—Tb^{3+}—Tris buffer) ternary complexes were found to exhibit fluorescence quenching at $\lambda_{ex}/\lambda_{em}$, 248/510, 250/528 and 268/540 for Ac, Cc and Th, respectively.
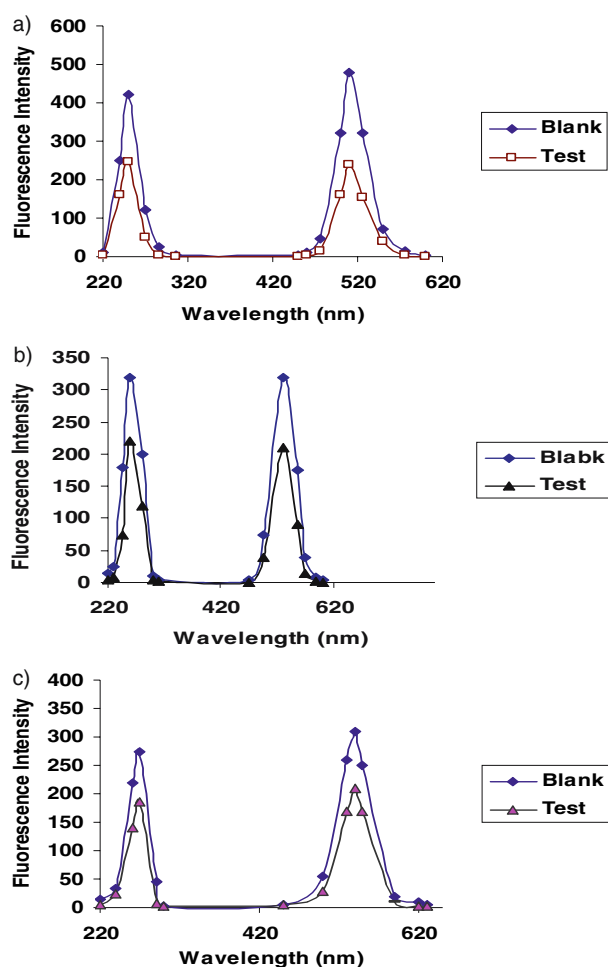
Fig. 1 **(a)** Excitation and Emission Spectra of the reaction product of Ac (2 μg ml^{-1}) and terbium chloride 0.5 ml of (5×10^{-3} M) solution. **(b)** Excitation and Emission Spectra of the reaction product of Cc (3.5 μg ml^{-1}) and terbium chloride 0.5 ml of (5×10^{-3} M) solution. **(c)** Excitation and Emission Spectra of the reaction product of Th (3.5 μg ml^{-1}) and terbium chloride 0.5 ml of (5×10^{-3} M) solution

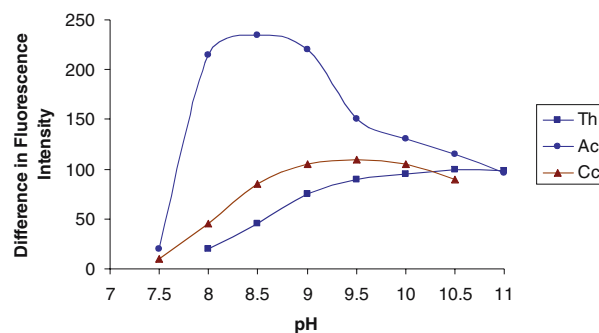**Fig. 2** Effect of different pH on the reaction between Terbium chloride and Ac (2 μg ml^{-1}), Th (3.5 μg ml^{-1}) and Cc (3.5 μg ml^{-1})

The optimum conditions for complex formation were carefully studied. The maximum fluorescence quenching of the drug solutions was measured over a pH range 7.5–11 by using Tris buffer (Fig. 2). The fluorescence quenching was observed at pH 8.5 for Ac, pH 9.5 for Cc and at pH 10.5 for Th. Also the effect of volume of Tris buffer was studied, and it was found that 1 ml was adequate for complete reaction (Fig. 3). The effect of Tb^{3+} concentration on fluorescence intensity was studied by using different volumes of terbium chloride (5×10^{-3} M). It was found that 0.5 ml was appropriate for fluorescence intensity for the three studied compounds (Fig. 4). The fluorescence signal was linearly related to the concentration in the range (0.5–2.5 μg ml^{-1}) for Ac, (1–4 μg ml^{-1}) for Cc and (0.5–3.5 μg ml^{-1}) for Th, with mean percentage recoveries 99.74 ± 0.36, 99.70 ± 0.52 and 99.43 ± 0.23 for the three cited drugs, respectively. The regression equations were computed as shown in Table 1. The ternary complex is formed immediately and the fluorescence signal remains stable for at least 1 h.

The Stoichiometry of the reaction was studied by the molar ratio and it was found to be (1 Tb: 3 drug) for the three studied drugs.
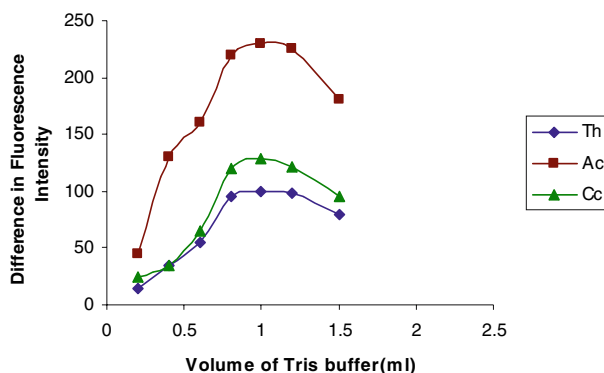

Fig. 3 Effect of volume of Tris buffer on the reaction between Terbium chloride and Ac (2 μg ml^{-1}), Th (3.5 μg ml^{-1}) and Cc (3.5 μg ml^{-1})
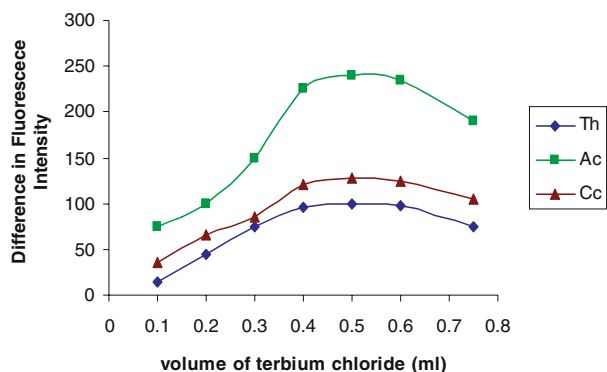
Fig. 4 Effect of ml of Terbium chloride (5×10^{-3} M) on the reaction between terbium chloride and Thioctic acid (3.5 μg ml^{-1}), Acetylcysteine (2 μg ml^{-1}) and Carbocisteine (4 μg ml^{-1})

Method (II) using uranyl acetate

Aqueous solution of uranyl acetate has high fluorescence intensity. When the solutions of the studied compounds were added immediate fluorescence quenching was observed at 512 nm (λ_{ex} 240 nm) [27]. The decrease in fluorescence intensity and the relative emission spectra of test and blank solutions are shown in Fig. 5.

The decrease in fluorescence intensity was proportional to the concentration of the added compounds. Ac, Cc and Th form binary complexes with aqueous uranyl acetate resulting in fluorescence quenching at 510 nm, using 240 nm as excitation wavelength.

Optimum conditions for binary complex formation were studied. Different concentrations of aqueous uranyl acetate ranging from (0.05–0.5% w/v) were tried Fig. 6 and different volumes of 0.2% w/v solution were also used Fig. 7. It was found that 1 ml for Ac and Cc or 1.5 ml for Th of 0.2% w/v aqueous solution was adequate for best results. The reaction was also done in different media e.g. alkaline medium using dilute Na OH, acidic medium using dilute HCl and also in presence of acetate buffer of different pH (4–6). Differ-
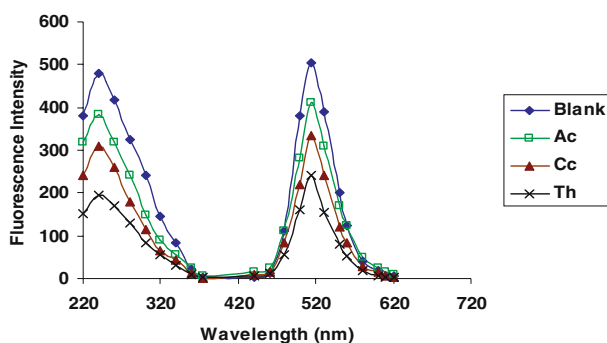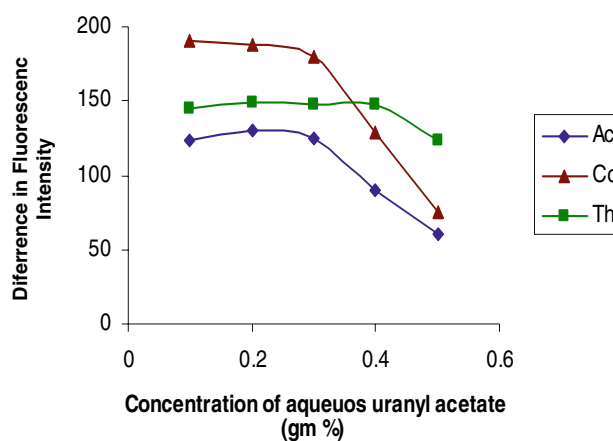

Fig. 6 Effect of concentration of aqueous uranyl acetate on the fluorescence quenching of uranyl acetate after its reaction with Ac (4 ug ml^{-1}), Cc (3 ug ml^{-1}) and Thioctic (3 ug ml^{-1})

ent diluting solvents for example methanol, ethanol, acetone and acetonitrile were also tried. It was found that the reaction takes place only in aqueous medium. Addition of acids, alkali or organic solvents resulted in the disappearance of the fluorescence intensity of the blank solution. The fluorescence signal was linearly related to the concentration in the range (0.5–6 μg ml^{-1}), (0.5–5 μg ml^{-1}), and (1–6 μg ml^{-1}) with mean percentage recoveries 99.38 ± 0.20, 99.82 ± 0.27 and 99.93 ± 0.32, for the three cited drugs, respectively. The binary complex is formed immediately and remains stable for 24 h.

The Stoichiometry of the reaction was studied by the molar ratio and it was found to be (1 U: 2 drug) for the three cited drugs. The two suggested methods were successfully applied for the determination of the three investigated drugs in bulk powders, in pharmaceutical formulations a well as in presence of their related substance as mentioned under specificity.

The suggested scheme of the structure of the formed complexes is represented in Scheme 1 as follows [20, 23].
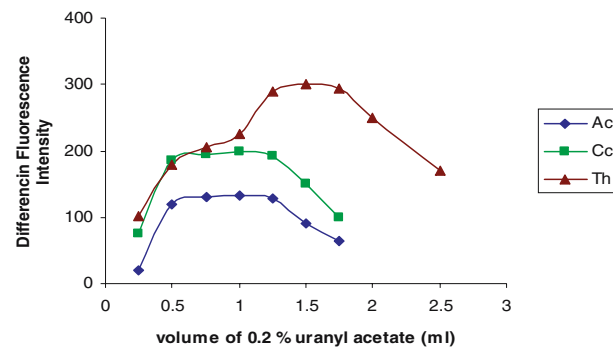

Fig. 5 Excitation and Emission spectra of the reaction between Uranyl aceta (0.2% w/v) and {Thioctic acid (6 μg ml^{-1}), Carbocisteine (3 μg ml^{-1}) and Acetylcysteine (2 μg ml^{-1})}

Fig. 7 Effect of volume of 0.2% w/v aqueous uranyl acetate on fluorescence quenching of uranyl acetate after its reaction with Ac (3 ug ml^{-1}), Cc (3.5 ug ml^{-1}) and Thioctic acid (6 ug ml^{-1})

Scheme 1 The suggested structure of the formed complexes between Tb^{3+} and U^{+3} with the three cited drugs

<table>
<tr><td colspan="2"></td><td>Method (I)</td><td>Method (II)</td></tr>
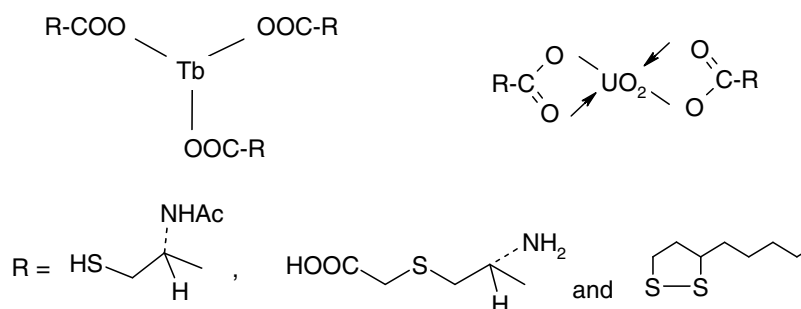</table>

Table 2 Determination of Acetylcysteine (Ac) in presence of its related substance L-cystine using the two suggested methods

Drug %	L-cystine %	Method (I) recovery %	Method (II) recovery %
90	10	97.45	99.15
70	30	96.99	98.55
50	50	98.77	97.75
40	60	98.25	98.84
20	80	97.35	97.99
Mean ± SD		97.76 ± 0.65	98.46 ± 0.52

Table 4 Determination of Thioctic acid in presence of its oxidation product using the two suggested methods

Drug %	Oxidized form %	Method (I) recovery %	Method (II) recovery %
90	10	99.43	99.35
70	30	98.22	99.68
50	50	97.35	97.55
40	60	95.97	96.98
15	85	96.75	98.25
Mean* ± SD		97.54 ± 1.20	98.362 ± 1.03

*Mean of five different experiments.

Method validation

Linearity range

Under the experimental conditions, Beer's plot for the three cited drugs using the two suggested methods show linear relationship with regression equation shown in Table 1.

Accuracy

The accuracy of the proposed methods was determined by investigating the percentage recovery at five levels each three times in the concentration range 0.5–3 μg ml^{-1} as shown in Table 1. The percentage relative standard deviation (%RSD) revealed high accuracy.

Precision

The intraday precision was evaluated by assaying freshly prepared solutions in triplicate in the concentration range

0.5–3 μg ml^{-1} as shown in Table 1. The percentage relative standard deviations (%RSD) were found to be ±0.255, ±0.456 and ±0.275 for Ac, Cc and Th, respectively using terbium chloride or ±0.610, ±0.588 and ±0.691 for the three drugs, respectively using uranyl acetate. While the interday precision was calculated by assaying freshly prepared solutions in triplicate for three days. The percentage relative standard deviations (% RSD) were found to be ±0.459, ±0.275 and ±0.580 for the three studied drugs, respectively using terbium chloride and ±0.518, ±0.562 and ±0.605 for Ac, Cc and Th, respectively using uranyl acetate.

Specificity

For the specificity determination of synthetic mixtures of different percentages of each drug and its related substance were

Table 3 Determination of Cabocisteine in presence of its related substance L-arginine using the two suggested methods

Drug %	L-arginine %	Method (I) recovery %	Method (II) recovery %
90	10	99.35	98.43
70	30	96.99	99.15
50	50	97.33	96.95
40	60	99.25	98.64
30	70	98.75	98.35
Mean ± SD		98.33 ± 0.99	98.27 ± 0.72

Table 5 Determination of Thioctic acid in presence of its reduction product using the two suggested methods

Drug %	Reduced form %	Method (I) recovery %	Method (II) recovery %
90	10	99.50	99.09
70	30	99.12	99.60
50	50	97.45	97.35
40	60	98.89	97.55
15	85	97.65	97.10
Mean* ± SD		98.52 ± 0.82	98.14 ± 1.09

*Mean of five different experiments.

Table 6 Statistical comparison of the proposed methods and the official ones

Items	Method (I)			Method (II)			Official methods[bc]		
	Ac	Cc	Th	Ac	Cc	Th	Ac[b]	Cc[b]	Th[c]
Mean[c]	99.74	99.70	99.43	99.38	99.82	99.93	99.48	99.60	99.93
% RSD	0.36	0.52	0.23	0.20	0.28	0.32	0.13	0.22	0.17
Variance (V)	0.05	0.04	0.02	0.01	0.02	0.01	0.02	0.05	0.03
SE	0.10	0.09	0.07	0.05	0.07	0.05	0.06	0.10	0.08
t-Test	1.752 (1.86)[d]	0.74	0.14	1.49	0.76	0.60			
F-test	2.630 (6.4)[d]	1.24	0.81	1.52	2.14	2.12			

[a] Mean of five experiments.
[b] Official titrimetric method.
[c] Official HPLC method.
[d] Theoretical values.

Table 7 Quantitative determination of Ac and Cc in pharmaceutical formulations by the proposed methods and compared with official and reported ones

Items	Parvolex ampoules			Mucosol capsules		
	Method (I)	Method (II)	Official method [3]	Method (I)	Method (II)	Reported method [28]
Mean[a]	99.81	99.69	99.99	100.10	100.25	100.02
% RSD	0.25	0.16	0.28	0.28	0.31	0.31
Variance (V)	0.02	0.06	0.02	0.08	0.08	0.10
SE	0.07	0.11	0.07	0.12	0.13	0.14
t-Test	0.54 (1.86)[b]	1.84		1.08	0.26	
F-test	1.09 (6.4)[b]	2.60		1.27	1.22	

[a] Mean of five experiments.
[b] Theoretical values.

prepared, it was found that Ac can be determined in presence of its related substance L-Cystine up to 80% Table 2. While Cc can be determined in presence of L-arginine up to 70% Table 3. Also thioctic acid can be determined in presence of its oxidation product which may result from its exposure to light (photo-oxidation) as well as in presence of its metabolite (dihydrolipoic cid) [26] up to 85% for both. As the oxidized and the reduced forms do not undergo complexation reactions as shown in Tables 4 and 5, so these methods considered to be stability indicating method for the determination of thioctic acid.

Robustness

Two sets of experiments were carried out using two different calibrated Spectrofluorimeters SHIMADZU RF—540 and SHIMADZU RF 1501. Also two sets were carried out on SHIMADZU RF-1501 by two different analysts, no significant difference was obtained between the results in this study.

Method validation for dosage forms

The validity of the suggested methods was assessed by applying the standard addition technique by adding Ac, Cc and Th to the previously analyzed pharmaceutical preparations. Statistical comparison of the results obtained by the proposed methods with those obtained by official [3, 4] or reported methods [28, 29] showed that the recommended procedures are more simple and sensitive without any loss of accuracy or precision (Tables 6–8).

Conclusion

The two suggested spectrofluorimeric methods are sensitive, accurate and selective compared with the official titrimetric methods. The data given above reveals that the proposed methods are selective as they could determine Ac and Cc in presence of their related substances and thioctic acid in presence of its oxidation or reduction product with good precision and accuracy.

Table 8 Quantitative determination of Th in pharmaceutical formulations by the proposed methods and compared with official and reported ones

Items	Thiotacid tablets			Thiotacid ampoules		
	Method (I)	Method (II)	Official method [4]	Method (I)	Method (II)	Reported method [29]
Mean[a]	100.02	99.88	99.95	100.04	99.93	99.79
% RSD	0.31	0.32	0.20	0.36	0.32	0.19
Variance (V)	0.10	0.04	0.13	0.15	0.10	0.03
SE	0.14	0.09	0.16	0.17	0.14	0.08
t-Test	0.34(1.86)[b]	0.65		0.79	0.47	
F-test	1.27 (6.4)[b]	3.09		4.33	2.97	

[a] Mean of five experiments.
[b] Theoretical values.

References

1. Gilman AG, Limbird LE, Hardman JG (2006) Goodman and Gilman. The pharmacological basis of Therapeutics, 11th edn. McGraw-Hill, New York, USA, p 694

2. Paefitt KM (2005) The complete drug reference, vol 1116, 34th edn. The Pharmaceutical Press, London, pp 1112–1113

3. British Pharmacopoeia, vol. I Her, Majestys Stationer Office, London, pp 48–49, 354 (2005)

4. The United States pharmacopoeia. The National Formulary USP 29, United States Pharmacopoeial Convention Inc., pp 51–52, 2351 (2006)

5. Pilar Vinas P, Cordoba MH, Sanchez Pedrenot C (1990) Analyst 115:757

6. Pargaonkar GG, Kashedikar SG (1994) Indian Drugs 31:590–592

7. Nedeljkovic JM, Vasic VM, Vukovic VV, Jovanovic TS (1995) J Pharm Biomed Anal 13:471–475

8. Taha EA, Hasan NY, Abdel Aal F, Abdel Fattah LS (2005) Bull Fac Pharm Univ 43(3)

9. Takeyoshi O, Takeo O (2002) J Electroanal Chem 534:181

10. Belal F, Metwally ME-S, Moustfa MA (1991) J Pharm Belg 64:320–324

11. Pentari JG, Efstathiou CE, Koupparis MA (1991) Int J Pharm 77:41–46

12. Kang TF, Shi YY (1994) Analyst 119:1067–1070

13. Kagedal B, Kallberg M (1982) J Chromatogr Biomed 229:409–415

14. Bron J (1986) Pharm Weekbl Sci Ed 8:134

15. Gennaro MC (1991) J Chromatogr Sci 29:410–415

16. Kullman JP, Yu T, Chen XH, Neal R, Ercal N, Armstrong DW (2000) J Liq Chromatogr Relat Technol 23:1941–1952

17. Yang ZP, Wang GF, Zeng WS, Liao HM, Xu KS (2003) Yaown Fenxi Zahi 23:189–191

18. Bailley MP, Rocks BF, Riley C (1987) Anal Chem Acta 201:335

19. Diamandis EP, Christopoulos TK (1990) Anal Chem 62:1149A

20. Bebaway LI, El Kelani K, Abdel Fattah L (2003) J Pharm Biomed Anal 00:1–7

21. Georges J (1993) Analyst 118:1481

22. Rizk M, Belal F, Aly FA, El-Enaany NM (1997) Anal Lett 30(10):1897

23. El-Reis MA (1998) Anal Lett 31(5):793

24. Taha EA (2004) Yaowu Fenxi Zahi 24(4):390–394

25. Ghazi AA, Qamar S, Atta MA (1994) Spectrochim Acta Part B 49(5):527–531

26. Wronski M (1987) J Chem Anal 32(3):315–318

27. Sarbu C, Horm M, Hantz A (1985) J Chromatogr 322(1):272

28. Manufacturer method supplied by MUP. Co. Egypt, through personal communication

29. Manufacturer method supplied by Eva. Co. Egypt, through personal communication