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Determination of ethionamide in pharmaceutical by spectrophotometry through oxidation reaction with potassium iodate

Nagib A. S. Qarah, Kanakapura Basavaiah

Department of Studies in Chemistry, University of Mysore, Manasagangothri, Mysore, Karnataka, India

Corresponding Author:
Kanakapura Basavaiah, Department of Studies in Chemistry, University of Mysore, Manasagangothri, Mysore, Karnataka, India.
Tel: 9448939105.
Mob.: 0821-2419659.
Fax.: 0091-821-2421263.
E-mail: kanakapurabasavaiah@gmail.com

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ABSTRACT

Objective: To described two new spectrophotometric methods for the determination of ethionamide (ETM) in pure and tablet forms. **Methods:** The developed first method is based on the oxidation of ETM by KIO3 in H2SO4 medium, and the in situ generated iodine is extracted into chloroform and measured at 520 nm, While in the second method, the same iodine is complexed with starch and the intense blue colored complex is measured at 580 nm. Different variables affecting the reactions were carefully studied and optimized. **Results:** Beer's law is obeyed in the concentration ranges of 20–400 and 5–125 μg/mL with Methods A and B, respectively, with the respective molar absorptivity (ε) values of 4.04 × 102 and 1.39 × 103 L/mol/cm. The Sandell sensitivity values were calculated to be 0.4113 and 0.1193 μg/mL. The limits detection and quantification were calculated to be 0.85 and 2.56 μg/mL for Method A and 0.27 and 0.81 μg/mL for Method B. The intraday and interday precisions expressed as relative standard deviation (%RSD) were found to be in the range 1.46–2.32%. The accuracy expressed as relative error (%RE) was better than 2.35%. The methods were also found to be robust and rugged as found from low values of RSD. **Conclusion:** The two developed methods were applied to the determination of ETM in tablets and no interference was observed from common tablet excipients.

INTRODUCTION

Ethionamide (ETM), chemically called 2-ethylthioisonicotinamide, is a second line agent with activity against various mycobacteria, including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium avium*-intracellulare [1,2]. Ethionamide is the official in the United States Pharmacopoeia [3] which describes an ultraviolet-spectrophotometric method for its assay. In British Pharmacopoeia [4], the drug is determined by titration with $HClO_4$ in anhydrous acetic acid medium with potentiometric endpoint detection.

A survey of the literature reveals that a few chromatographic methods have been reported for its determination in biological fluids [5-11], various methods for the determination of ETM in pharmaceuticals have been proposed and include fluorimetry [12], visual titrimetry [13] and potentiometric titrimetry [14,15], and spectrophotometry [16-27], The spectrophotometric methods are based on the reaction of ETM with several organic or inorganic reagents such as osmic acid [16], sodium nitroprusside,[17,18] iron (III),[19] iron (III)-p-phenylenediamine,[20] pyridylazoresorcinol-vanadium,[21] alizarin violet 3B and alizarin brilliant violet R [22], dichlone,[23,24] and *N*-bromosuccinimide celestine blue [25]. Using permanganate [26] and iodine [27] as oxidants, two kinetic methods have also been described.

The methods based on redox reactions [25-27] are complicated since the concentrations of the reagents employed are to be known accurately and the kinetic methods require careful and meticulous control of all experimental variables.

The aim of this work was to develop easy, rapid, and reliable methods based on oxidation reaction employing potassium iodate which finds extensive application in spectrophotometric assay of pharmaceuticals [28-35]. The methods are based on the oxidation of ETM by KIO_3 in H_2SO_4

medium, and determination of *in situ* the generated iodine either by extracting into chloroform for measurement at 520 nm (Method A) or by complexing with starch followed by the measurement of the resultant blue species at 580 nm (Method B). In both the methods, the amount of iodine, thus determined is related to the amount of ETM.

METHODS

Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) with matched 1-cm quartz cells was used for absorbance measurements.

Materials and Reagents

Doubly distilled water and analytical reagent grade chemicals were used. A 0.5% aqueous solution of KIO_3 (Merck, Mumbai, India), 0.1M HCl (Merck, Mumbai, India sp. gr. 1.18) were prepared in the usual manner. Spectroscopic grade organic solvents (Merck, Mumbai, India) were used in the study. Starch indicator was prepared by adding a paste of 1 g starch in water to 100 mL boiling water, boiled for 1 min, and cooled.

Pure ethionamide (ETM) sample certified to be 99.86% pure was provided from Panacea Biotic Ltd. as gift and used as received.

A 500 μg/mL standard solution of ETM was prepared by dissolving 250 mg of pure drug in 0.1M HCl and diluted with the same solvent in a 500 mL calibrated flask. ETM containing tablets: Ethide (Lupin Ltd., Mumbai, India), Ethiokox (Radicura Private Ltd., New Delhi, India), and Myobid (Panacea Biotic, New Delhi, India) each labeled contain 250 mg of ETM were purchased from local commercial sources.

General Procedures

Preparation of calibration graph

- Method A**.** Aliquots of standard ETM solution (0.5–8.0 mL; 500 μg/mL) were placed in a series separating funnels, and the volume in each was brought to 10 mL with 0**.**1 HCl**.** Then, 2 mL of KIO_3 solution was added and the contents mixed well. 10 mL of chloroform was added from a burette to each funnel and shaken for 1 min, and the layers were allowed to separate. The chloroform layer was dried by passing over anhydrous Na_2SO_4 and the absorbance measured at 520 nm against the reagent blank.
- Method B**.** Varying aliquots (0**.**1–2**.**5 mL**)** of standard 500 μg/mL ETM solution were accurately transferred into a series of 10 mL calibrated flasks, and the volume was diluted to 3 mL with 0.1 HCl. To each flask, 2 mL of KIO_3 and 1 mL of starch were added successively, the volume was then made up to the mark with water, mixed well, and absorbance measured at 580 nm versus the reagent blank**.**

A calibration graph was prepared in both methods, and the unknown concentration was computed from the regression equation derived from the absorbance concentration data.

Procedure for Tablets

Twenty tablets were weighed accurately and ground into a fine powder. A portion of the powder equivalent to 50 mg of ETM was weighed accurately and transferred into a 100 mL calibrated flask, 60 mL of 0.1 M HCl was added, and the content was shaken for 20 min. The volume was diluted to the mark with 0.1 M HCl, mixed well, and filtered using Whatman 42 filter paper. The filtrate (500 μg/mL in ETM) was subjected to assay using 4 mL in Method A and 1 mL in Method B, in five replicates.

Procedure for Placebo and Synthetic Mixture Analyses

A placebo blank of the composition: 20 mg talc, 30 mg starch, 20 mg sucrose, 20 mg lactose, 10 mg gelatin, 20 mg sodium alginate, 30 mg magnesium stearate, and 20 mg methylcellulose was prepared by homogeneous mixing in a mortar. 25 mg of placebo was placed in a 50 mL calibration flask and its extract was prepared as described under "Procedure for Tablet." 2 mL of the extract was subject to analysis following the general procedures. To 20 mg of the placebo blank prepared above, 25 mg of pure ETM was added, mixed thoroughly and the mixture was quantitatively transferred into a 100 mL calibrated flask; and then, steps described under "procedure for tablets" were followed.

RESULTS AND DISCUSSION

The proposed methods are direct and essentially based on the determination of iodine released *in situ* upon reacting ETM with KIO_3 in acid medium. The concentration of the iodine thus determined is related to the concentration of the drug responsible for the iodine released. In Method A, the iodine released is extracted into chloroform and measured at 520 nm, whereas Method B entails complexation of iodine with starch and subsequent measurement of absorbance of the colored species at 580 nm [Figure 1]. Possible reaction pathway is shown in [Figure 2].

Method Development

Preliminary experiments were performed to determine the optimum concentration of KIO_3 and starch to give the maximum response. 2 mL of 5% KIO_3 was found optimum in both the methods, and 1 mL of 1% starch was found sufficient to complex the iodine released in Method B. Hydrochloric acid *(*0.1 M*)* was found ideally suited to prepare the drug solution and no additional acid was required to cause the redox reaction that facilitated the assay. The redox reaction between ETM and KIO_3, subsequent release of iodine, and also the complexation reaction between I_2 and starch were instantaneous. The extracted colored species measured was stable for 24 h and 60 min in Methods A and B, respectively. Of the several organic solvents tried to extract the iodine in Method A, chloroform was most suitable because of high sensitivity and stability of the colored species. A shaking time for 1 min was necessary to quantitatively extract the iodine to the chloroform layer and the two layers got clearly separated within 1 min (Method A).

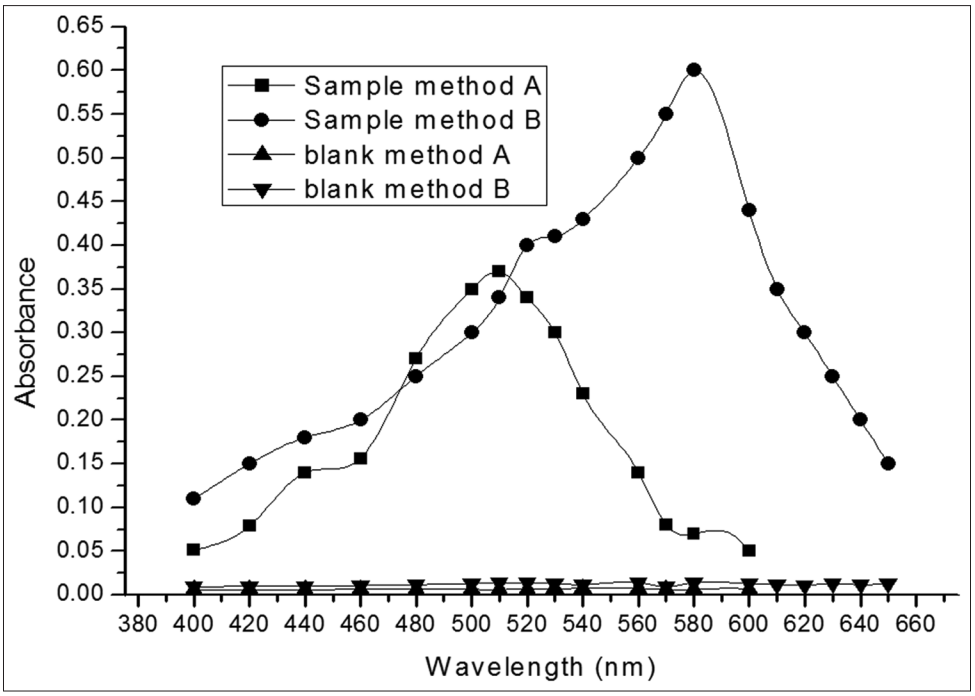

Figure 1: Absorption spectra colored species (150 μg/mL ETM for Method A, and 70 μg/mL ETM for Method B)

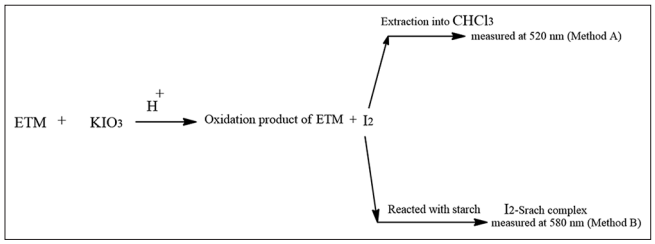

Figure 2: Possible reaction pathway

Method Validated

Linearity and sensitivity

The calibration graphs obtained follow Beer's law up to 400 and 125 μg/mL ETM for Methods A and B, respectively as shown in Figure 3. Regression parameters along with the respective dynamic linear ranges, molar absorptivity, and Sandell sensitivity values, and the detection and quantification limits are given in Table 1.

Precision and Accuracy

Precision and accuracy of the methods were evaluated by replicate analyses of the pure drug solution at three concentration levels within the linear range. The analyses were performed on the same day (intraday) and on five consecutive days (interday) using the solutions prepared afresh each day. The precision expressed as relative standard deviation (%RSD) was satisfactory with the RSD values <2.5%. The accuracy of the methods expressed as relative error (%RE) was better than 2.5% revealing fair precision and accuracy of the methods [Table 2].

Table 1: Sensitivity and regression parameters

Parameter	Method A	Method B
$\lambda_{max,}$ nm	520	580
Color stability	24 h	60 min
Linear range, µg/mL	20–400	5.0–125
Molar absorptivity (ε), L/mol/cm	4.04×10^{2}	1.39×10^{3}
Sandell sensitivity*, µg/cm	0.4113	0.1193
Limit of detection (LOD), µg/mL	0.85	0.27
Limit of quantification (LOQ), µg/mL	2.56	0.81
Regression equation, y**		
Intercept (a)	−0.003	0.0063
Slope (b)	0.0025	0.0078
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	2.8×10^{-4}	8.9×10^{-4}
Regression coefficient (r)	0.9964	0.9994

*Limit of determination as the weight in µg/mL of solution, which corresponds to an absorbance of A=0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l=1 cm. **y=a+bx, where y is the absorbance, x concentration in µg/mL, a intercept, and b slope

Robustness and Ruggedness

Robustness was examined by evaluating the influence of a small variation of the method variables on the performance of the methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the %RSD was calculated each time. It was revealed from the values of %RSD that small alterations in the experimental variables did not affect the methods significantly. Ruggedness was tested by applying the proposed methods to the determination of ETM using the same optimum conditions

but using three different instruments in three different laboratories, and also using the single instrument but by three different analysts. Results obtained from instrument-to-instrument as well as analyst-to-analyst variations did not affect the performance of the methods. The results of this study are presented in Table 3.

Selectivity

The placebo blank when subjected to analysis by the proposed methods yielded absorbance values close to those of the reagent blanks. On analyzing the synthesis mixture using the two methods, the percent recoveries recorded were 98.46 ± 0.85 (n = 5) (Method A), 101.3 ± 1.42 (n = 5) (Method B) ensuring that the methods are selective for the determination of ETM in tablets.

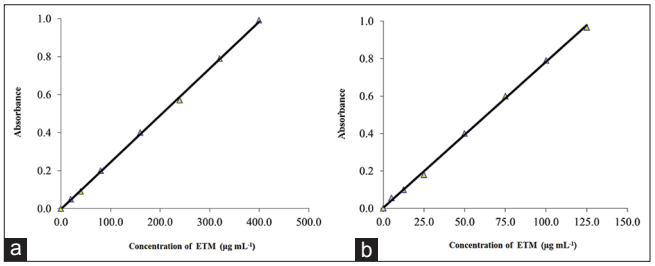

Figure 3: (a) Standard graph (Method A), (b) standard graph (Method B)

Table 2: Evaluation of intraday and interday accuracy and precision

Method	ETM taken (μg/mL)	Intraday accuracy and precision (n=7)			Interday accuracy and precision (n=5)		
		ETM found[a] (μg/mL)	RSD[b] %	RE[c] %	ETM found (μg/mL)	RSD[b] %	RE[c] %
A	100	100.77	1.88	0.77	100.85	1.84	0.85
	200	203.81	1.66	1.91	204.11	2.32	2.01
	300	305.43	1.97	1.81	304.44	2.11	1.48
B	40	40.44	1.59	1.10	40.67	1.89	1.68
	80	80.95	1.46	1.19	81.88	1.77	2.35
	120	122.04	1.65	1.70	121.89	2.19	1.58

[a]Mean value of seven determinations, [b]relative standard deviation (%), [c]relative error *(%), RSD*: Relative standard deviation

Table 3: Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	ETM taken (μg/mL)	Robustness (%RSD)	Ruggedness (%RSD)	
		Parameters altered	Interanalysts (n=3)	Interlaboratories (n=3)
		Volume of KIO_3*		
A	100	1.86	1.44	1.57
	200	1.88	1.63	1.78
	300	2.34	1.06	2.43
B	40	1.98	1.85	1.79
	80	2.23	1.73	2.31
	120	2.44	1.68	1.97

*1.8, 2.0 and 2.2 mL in both methods. RSD: Relative standard deviation

Table 4: Results of analysis of tablets by the proposed methods and statistical comparison of the results with the official method

Tablet brand name	Nominal amount	Found* (% of nominal amount±SD)		
		Official method	Proposed methods	
			Method A	Method B
Ethide	250	99.66±1.09	99.92±0.79	97.88±1.23
			t=2.10	t=1.08
			F=1.90	F=1.25
Ethiokox	250	100.10±0.75	101.36±1.36	101.45±1.64
			t=1.81	t=1.67
			F=3.29	F=4.78
Myobid	250	99.59±1.64	98.97±1.51	100.3±1.21
			t=0.59	t=0.81
			F=1.18	F=1.84

*Mean value of five determinations. Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77. Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39

Application to tablets

Results of determination of ETM in three brands of commercial tablets by the proposed methods are presented in Table 4 and are in agreement with the label claim as well as those obtained by the reference method [4], in which the drug is determined by titration with $HClO_4$ in anhydrous acetic acid medium with potentiometric endpoint detection.

Statistical comparison of the results by applying the Student's *t*-test for accuracy and variance ratio F-test for precision indicate no significant difference between the proposed methods and the reference method with respect to accuracy and precision when the calculated t-and F-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom.

Table 5: Results of recovery experiment through standard-addition method

Method	Tablet studied	ETM in tablet, µg/mL	Pure ETM added, µg/mL	Total found, µg/mL	Pure ETM recovered Percent±SD*
A	Ethide 250	149.58	75	225.23	102.40±1.87
		149.58	150	302.15	101.01±1.74
		149.58	225	384.55	103.80±1.77
B	Ethide 250	44.02	22.5	67.33	101.90±1.44
		44.02	67.5	113.22	101.91±1.55
		44.02	112.5	157.98	101.90±1.76

*Mean value of three determinations

Table 6: Comparison of the proposed and the existing methods

Reagents	Methodology	Linear rang µg/mL	LOD/LOQ (µg/mL)	Remark	Reference number
Sodium nitroprusside	Orange colored product in basic medium measured at 510 nm	-	-	-	20
Sodium nitroprusside	Orange-red complex measured at 510 nm	5–32	-	-	21
DCNQ	Orange colored product in ethanol measured at 440 nm	-	-	-	16
DCNQ	Red colored product formed in the presence of ammonia in alcohol medium measured at 530 nm	-	-	-	17
PAR-V (V)	Ternary complex (1:1:1) extracted into chloroform and measured at 560 nm	0.2–20	-	30 min contact time, extraction step	22
Iron (III)	Purple-violet colored complex in acid medium measured at 510 nm	0–36	-	Longer contact time	18
Iron (III)-PPD	Thionine compound measured at 600 nm	-	-	Multiple step reaction involved	19
Osmic acid	Light yellow colored formed at pH 4 measured at 375 nm	0.25–40	-	60 min contact time, pH adjustment required	23
NBS-CB	Unbleached dye color measured in acid medium at 540 nm	0.2–5.0	-	Critical acid concentration; unstable reagent used; concentrations of both reagents to be known	25
$KMnO_4$	Blue colored manganite in alkaline medium measured at 610 nm (direct method) Absorbance at a fixed time of 20 min measured (kinetic method)	1–10 1–10	-	Critical NaOH concentration reaction rate precariously dependent on experimental variables	26
Sodium azide-iodine	Decreases in absorbance at the 5th min measured at 348 nm (kinetic method	10–100	0.7/-	Reaction rate precariously dependent on experimental condition	27
KIO_3 KIO_3-starch	I_2 in chloroform measured at 520 nm I_2-starch complex measured at 580 nm	20–400 5–125	0.85/2.56 0.27/0.81	No drastic experimental conditions, instantaneous reaction, more sensitive	Present methods

DCNQ: Dichloronaphthoquinone, PAR: Pyridylazoresorcinol, PPD: P-phenylenediamine, NBS: N-bromosuccinimide, CB: Celestine blue

Accuracy by Recovery Experiment

To further establish the accuracy and reliability of the proposed methods, recovery experiment through standard-addition procedure was performed. To the preanalyzed tablet powder, pure ETM was added at three levels and the total found by the proposed methods. Each determination was performed in triplicate. From the percent recovery values of pure drug, added to the tablet powder, summarized in Table 5, it is clear that coformulated substances in the tablets did not interfere in the determination, and thereby complementing the results of the selectivity study.

CONCLUSION

The results demonstrated the usefulness of potassium iodate as a reagent for the spectrophotometric assay of ethionamide in pharmaceuticals. The proposed methods offer the advantages of simplicity selectivity and applicability over wide linear dynamic ranges compared to the previously reported methods [Table 6]. The methods were demonstrated to accurate and precise besides being robust and rugged, since small deliberate alterations in the operating conditions, instruments, and personal were not found to significantly affect their performance.

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REFERENCES

1. Kucers A, Bennett NM, editors. The Use of Antibiotics. 4th ed. Philadelphia, PA: Lippencott; 1988. p. 1426-31.
2. Heifets L. MIC as a quantitative measurement of the susceptibility of *Mycobacterium avium* strains to seven antituberculosis drugs. Antimicrob Agents Chemother 1988;32:1131-6.
3. United State pharmacopoeia. USP 32-NF 27 P 2324. Pharm Forum 2009;33:648.
4. British Pharmacopoeia Commission. Monographs: Medicinal and pharmaceutical substances, ethionamide. British Pharmacopoeia. Vol. 1-2. London, U.K.: British Pharmacopoeia Commission; 2009.
5. Deshpande AY, Gurav S, Punde R, Zambre V, Kulkarni R, Pandey S, *et al*. Development and validation of a highly sensitine LC-MS/MS method for simultaneous quantitation of ethionamide and ethionamide sulfoxide in human plasma: Application to a human pharmacokinetic study. Biomed Chromatogr 2011;25:985-94.
6. Madni AU, Ahmad M, Akhtar N, Ashraf M, Zeba A. An improved HPLC method for the determination of ethionamide in serum. J Chem Soc Pak 2008;30:449-52.
7. Peloquin CA, James GT, McCarthy E. Improved high-performance liquid chromatographic assay for the determination of ethionamide in serum. J Chromatogr Biomed Appl 1991;563:472-5.
8. RuseMJ, Waring RH. HPLC analysis of thioamides and their sulfoxides. Med Sci Res 1990;18:53-4.
9. Harnanansingh AM, Eidus L. Micro method for the determination of ethionamide in serum. Int Z Klin Pharmakol Ther Toxikol 1970;3:128-31.
10. Mohamed IW, Amina MB, Mohamed ES, Amina AA. Fluorimetric determination of ethionamide in pharmaceutical preparation and biological fluids. Chin Chem Soc 2004;51:1059-64.
11. Bhanushali CJ, Zidan AS, Rahman ZM. Ion-pair chromatography for simultaneous analysis of ethionamide and pyrazinamide from their porous microparticles. AAPS Pharm Sci Tech 2013;14:1313-20.
12. Mohamed IW, Amina MB, Mohamed ES, Amina AA. Fluorimetric determination of carbocisteine and ethionamide in drug formulation. Acta Chim Slov 2004;51:283-91.
13. Reddy BS, Krishna RR, Sastry CS. Titrimetric determination of some antitubercular drugs by sodium nitrite and N-bromosuccinimide (NBS) using internal indicators. Indian Drugs 1982;20:28-9.
14. Ciesielski W, Krenc A, Zlobinsha U. Potentiometric titration of thioamides and mercaptoacids with iodine in alkaline medium. Chem Analityczna (Warsaw) 2005;50:397-405.
15. Obtemperanskaya SI, Buzlanova M, Karandki IV, Shakhid R, Kashin AN. Potentiometric determination of some drugs and other physiologically active substances using a silver sufide ion-selective electrode. J Anal Chem 1996;51:419-23.
16. Sikorska TH. Spectrophotometric determination of ethionamide and thionicotinamide with osmic acid. Mikrochemica Acta 1986;3:151-7.
17. Ibrahim FA. Colorimetric estimation of certain thione compounds of pharmaceutical importance. Mansoura J Pharm Sci 1994;10:334-44.
18. Devani MB, Shishoo CJ, Doshi K. A spectrophotometric determination of ethionamide in tablets. Indian J Pharm Sci 1981;43:149-50.
19. Shah AK, Agrawal YK, Banerjee SK. Spectrophotometric method for the rapid determination of microgram amount of ethionamide. Anal Lett 1981;14:1449-64.
20. El-Din MS, Belal F, Hassan S. Spectrophotometric determination of some pharmaceutically important thione-containing compounds. Zentra Pharm Lab 1988;127:133-5.
21. Sikorska TH. Spectrophotometric determination of ethionamide and thionicotinamide with 4-(2-pyridylazo) resorcinol and vanadium. Chem Anal (Warsaw) 1993;38:745-51.
22. Reddy BS, Sastry CS. Ion-pair extraction method for ethambutol, ethionamide and rifampicin determination. J Inst Chem (India) 1983;55:69-70.
23. Bedair MM. Use of 2,3-dichloro-1,4-naphthoquinone for the spectrophotometric assay of five thio compounds of pharmaceutical importance. Alex J Pharm Sci 1991;5:64-7.
24. Devani MB, Shishoo CJ, Mody HJ, Raja PK. Detection of thioamides determination of ethionamide with 2,3-dichloro-1,4-naphthoquinone. J Pharm Sci 1974;63:1471-3.
25. Sastry CS, Srinivas KR, Prasad KM. Spectrophotometric determination of drugs in pharmaceutical formulations with N-bromosuccinimide and Celestine blue. Mikrochimica Acta 1996;122:77-86.
26. Mohamed IW, Amina MB, Mohamed ES, Amina AA. Spectrophotometric and kinetic determination of some sulphur containing drugs in bulk and drug formulations. Bull Korean Chem Soc 2004;25:517-24.
27. Walash MI, Metwally ME, El-Brashy AM, Abdelal AA. Kinetic spectrophotometric determination of some sulfur containing compounds in pharmaceutical preparations and human serum. Farmaco 2003;58:1325-32.
28. Basavaiah K, Nagegowda P. Determination of ranitidine using potassium iodate and dichlorofluorescein. Indian J Chem Technol 2004;11:11-6.
29. Swamy N, Prashanth KN, Basavaiah K. Titrimetric and spectrophotometric assay of diethylcarbamazine citrate in formulations using iodate and iodide mixture as reagents. Braz J Pharm Sci 2015;51:43-52.
30. Gurupadayya BM, Bharatheesha BM. Validated

spectrophotometric method for the assay of diacerein in bulk and pharmaceutical formulations. J Pharm Sci Res 2010;2:638-43.
31. El-Shaboury SR, Mohamed FA, Saleh GA. Kinetic spectrophotometric determination of certain cephalosporines using iodate/iodide mixture. Nat Sci 2010;2:432-43.
32. Ahmed NR. Indirect spectrophotometric determination of captopril in pharmaceutical tablets and spiked environmental samples. Iraqi Natl J Chem 2013;49:1-11.
33. Basavaiah K, Zenita O, Tharpa K, Rajendraprasad N, Anilkumar UR, Hiriyana SG, *et al*. Iodimetric assay of olanzapine in pharmaceuticals using iodate and nile blue as reagents. Chem Ind Chem Eng Q 2009;15:95-102.
34. Zenita O, Basavaiah K, Vinay KB. Iodimetric assay of pantoprazole sodium sesquihydrate in pharmaceuticals using iodate and iodide as reagents. Proc Indian Natl Sci Acad 2010;76:17-25.
35. Raghu MS, Basavaiah K, Prashanth KN, Vinay KB. Iodometric determination of milligram and microgram amounts of levocetirizine in pharmaceuticals. Mosc Univ Chem Bull 2012;53:417-24.