Spectrophotometric and Kinetic Determination of Some Sulphur Containing Drugs in Bulk and Drug Formulations

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Received August 2, 2003

Two simple and sensitive spectrophotometric methods were developed for the determination of carbocisteine, penicillamine, ethionamide and thioctic acid in bulk and in their pharmaceutical preparations using alkaline potassium permanganate as an oxidizing agent. The first one involves determination of ethionamide and thioctic acid by spectrophotometric investigation of the oxidation reaction of the two drugs. The second method involves determination of carbocisteine and penicillamine by kinetic studies of the oxidation reaction of these two drugs at room temperature for a fixed time of 20 minutes. The absorbance of the colored manganate ions was measured at 610 nm in both methods. 1-10 μg/mL of ethionamide and thioctic acid could be determined by the spectrophotometric method with detection limits of 0.11 and 0.089 μg/mL for the two drugs respectively. 2-10 μg/mL of carbocisteine and penicillamine could be determined by the kinetic method with detection limits of 0.14 and 0.21 μg/mL respectively. The two methods were successfully applied for the determination of these drugs in their dosage forms.

Key Words : Kinetic determination, Carbocisteine, Potassium permanganate, Pharmaceutical analysis, Spectrophotometry

Introduction

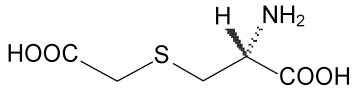
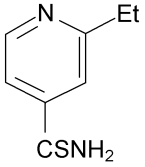
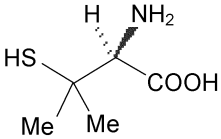
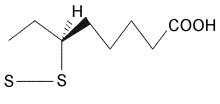
The importance of these sulphur compounds is due to their wide spread and different pharmacological effects. Carbocisteine (I) is a mucolytic drug used for treatment of disorders of the respiratory tract associated with excessive mucus, ethionamide (II) is an antibacterial drug (tuberculostatic agent), Penicillamine (III) is used for treatment of rheumatoid arthritis and in treatment of lead poisoning as a chellating agent, it also used for elimination of copper in treatment of hepatolenticular degeneration (Wilson's disease) and thioctic acid (IV) is used for treatment of liver dysfunction and diabetic neuropathy and antidote to poisonous mushrooms [Amantia species].[1,2]

The published reported methods for the determination of these drugs included titrimetry,[3-5] spectrophotometry,[6-11] fluorometry,[12] electro-analysis[13-16] and chromatography.[17-22]

The catalytic kinetic spectrophotometric method is one of the most attractive approaches for the ultratrace determination of certain chemicals and has many advantages:

1. Selectivity due to the measurement of the evolution of the absorbance with the time of reaction instead of the measure of a concrete absorbance value.
2. Possibility of no interference of the colored and/or turbidity background of the samples.
3. Possibility of no interference of other active compounds present in the commercial products, if they are resisting the chemical reaction conditions established for the proposed kinetic method.[23]

The aim of the present work was to study the reaction between carbocisteine, penicillamine, ethionamide, which contain thiol group and thioctic acid (disulphide group) with alkaline potassium permanganate in alkaline medium spectrophotometrically or kinetically in an attempt to evaluate them in their dosage forms. The proposed methods were simple and did not need sophisticated instruments or special skill, sensitive, rapid and readily adaptable to both the bulk drug and dosage forms.

Compound	Carbocisteine	Ethionamide	Penicillamine	Thioctic acid
Structural formula				
	I	II	III	IV
(Molecular Weight)	(179.2)	(166.25)	(149.2)	(206.3)

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Experimental Section

Apparatus. UV-1601, Shimadzu recording spectrophotometer (P/N 206-67001) equipped with kinetic accessory provided with temperature controlled cell (TCC-240A) thermoelectrical temperature. Recording range, 0-1; wavelength, 610 nm; factor 1; number of cell, 1; reaction time, 20 min; cycle time, 0.1 min.

Materials. The studied drugs were kindly obtained from different companies. Carbocisteine (Amyria Pharmaceutical Industries, Egypt). penicillamine (Biochemie Company, Egypt), ethionamide (Alexandria, Theraplix) and thioctic acid (Eva Pharma for Pharmaceuticals & Medical Appliances, Egypt).

The purities of these drugs were determined by applying the official methods.[3] Pharmaceutical preparations containing the studied compounds were purchased from commercial sources in the local market.

Reagents. All the reagents used were of analytical grade and water was always double distilled. Aqueous solution of 3.79×10^{-2} M potassium permanganate (Merck, Germany), 0.4 M NaOH (BDH, UK) and 5 M HCl (Prolabo) were prepared.

Stock Solutions. Stock solutions of the studied drugs were prepared by dissolving 100 mg of carbocisteine or thioctic acid in a least amount of 0.4 M NaOH, ethionamide in a least amount of 5 M HCl and penicillamine in distilled water. All solutions were completed to 100 mL with distilled water. Other concentrations were prepared by dilution with distilled water.

General Procedures. *Construction of calibration graphs for the spectrophotometric method:* An aliquot solution of ethionamide or thioctic acid containing 10-100 μg/mL was transferred into a 10 mL volumetric flask; 1 mL or 0.8 mL of 0.4 M NaOH (for thioctic acid or ethionamide respectively) was added followed by 1.6 mL of 3.79×10^{-2} M potassium permanaganate, the mixture was shaken well and completed to volume with distilled water. The absorbance was measured at 610 nm at ambient temperature (25 ^{o}C) against an appropriate blank prepared simultaneously. The values of the absorbance were plotted against the concentration of each drug in μg/mL.

Construction of calibration graphs for the kinetic methods: An aliquot solution of carbocisteine or penicillamine containing 20-100 μg/mL was transferred into a 10 mL volumetric flask; 0.6 mL or 1 mL of 0.4 M NaOH (for Carbocisteine or penicillamine respectively) was added followed by 2 mL of 3.79×10^{-2} M potassium permanganate. The mixture was shaken well and completed to the volume with distilled water. The absorbance was scanned during 20 min. at ambient temperature (25 ^{o}C) at 610 nm against an appropriate blank, prepared simultaneously. The drug concentration was determined by measuring the rate of the reaction as the tangent to kinetic curve during the first 20 min. of the reaction and using the appropriate graphs. Log reaction rate versus log concentration of the drug was plotted to get the order of the reaction after 20 min. and plot the values of the absorbance against the final concentration in μg/mL. Alternatively, the regression equation was derived.

Procedure for Spectrophotometric Determination of the Studied Compounds in Dosage Forms. An accurately weighed quantity of the mixed contents of 10 capsules or powdered tablets, or an accurately measured volume of the syrup or ampoule equivalent to 100 mg of the drug was transferred into a 100 mL volumetric flask. Artamine capsules were extracted into water. Thiotacid ampoules were diluted with water. Rhinathiol syrup or thiotacid tablets were extracted with 3×10 mL of chloroform (95%). 10 mL of 0.05 M sodium lauryl sulphate and 10 mL of 50% acetic acid were added in case of Rhinathiol syrup.

Trecator tablets were extracted with 3×10 mL of hot acetone (95%) of temperature 80 ^{o}C, filtered into a 100 mL volumetric flask . The combined extracts were evaporated to dryness and made up to the mark with distilled water (3 mL of 0.4 M NaOH were firstly added for rhinathiol syrup and thiotacid tablets and 3 mL of 5 M HCl for trecator tablets). Take an aliquot and apply the above procedures. The nominal content was calculated either from a previously plotted calibration graph or using the regression equation.

Results and Discussion

Different oxidants have been used to determine the drug, such as 10% H_2O_2 and potassium persulphate in alkaline medium and potassium periodate in strong acid medium. In case of H_2O_2 and persulphate, complete decomposition of the drug was observed, as revealed by the absence of any chromophoric groups in the absorption spectrum of the reaction product. In case of periodate, oxidation of the drug resulted in hypsochromic shift and hypochromic effect, with maximum absorbance at 236 nm. and this was in agreement with the reported results of oxidation of amino-alcohol compounds.[24]

Potassium permanganate in alkaline medium oxidizes the sulphur drugs and yields the green color of manganate ion, which absorbs at 610 nm (Fig. 1). The color was developed and reached its maximum immediately for ethionamide and thioctic acid, therefore, a direct spectrophotometric method was used for determination of these two drugs while in case of carbocisteine and penicillamine the intensity of the color increases with time, and so, a kinetic method was developed for the determination of these two drugs.

Study of the Experimental Parameters. *Effect of temperature:* At room temperature (25 ^{o}C), the reaction rate of carbocisteine, penicillamine, ethionamide and thioctic acid increased substantially as the color development increased. Higher temperature causes precipitation of MnO_2, therefore, room temperature was selected as the optimum temperature.

Effect of $KMnO_4$: The reaction rate and maximum absorbance increased with increasing $KMnO_4$ concentration. It was found that 1.6 mL of 3.79×10^{-2} M $KMnO_4$ was adequate for the maximum absorbance of ethionamide and thioctic acid while penicillamine and carbocistiene needed 2 mL as shown in Figs. 2 and 3.

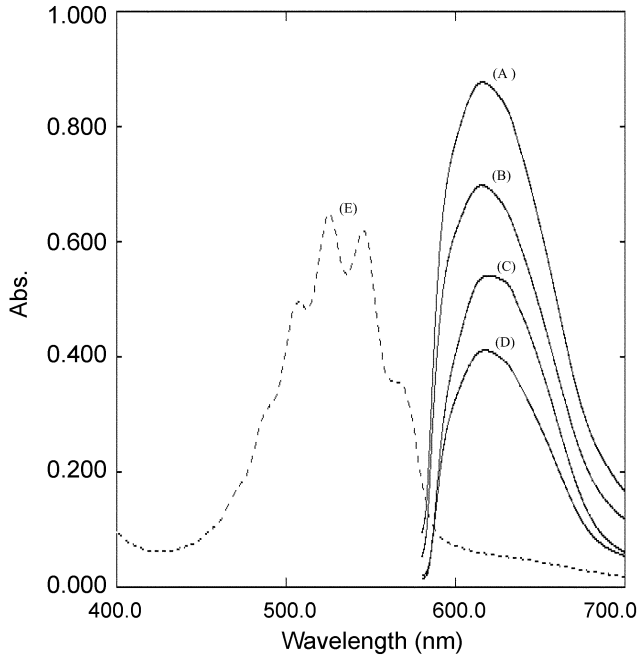

Figure 1. Absorption spectra of the reaction product with $KMnO_4$/NaOH system: (A) Reaction product of ethionamide (10 μg/mL). (B) Reaction product of thioctic acid (10 μg/mL). (C) Reaction product of carbocisteine (10 μg/mL). (D) Reaction product of penicillamine (10 μg/mL). (E) $KMnO_4$ (1.97×10^{-4} M).

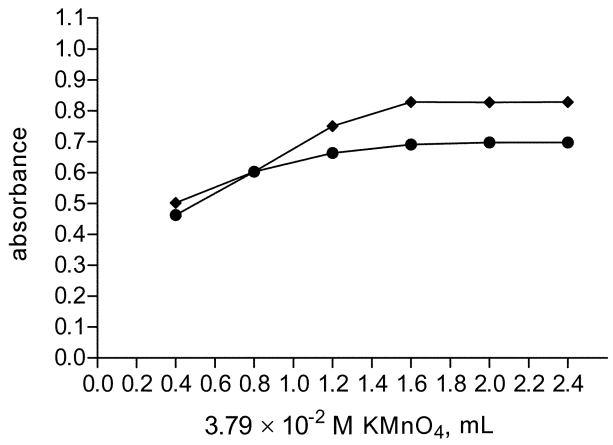

Figure 2. Effect of $KMnO_4$ ● Thioctic acid; ◆ Ethionamide (10 μg/mL of each drug).

Effect of NaOH: Trials were made to determine the drug through oxidation with $KMnO_4$ in neutral, acidic and alkaline media, but no oxidation had been observed in neutral or acidic solution.

It was found that increasing the volume of 0.4 M NaOH would increase absorbance of the reaction product up to 0.8 mL for ethionamide, 0.6 mL for carbocisteine and 1 mL for thioctic acid and penicillamine after that NaOH has no effect on the absorbance as shown in Figs. 4 and 5.

The rate of reaction was found to be dependent on carbocisteine and penicillamine concentrations. The rates were followed at room temperature with various concentrations in the range of 2-10 μg/mL keeping $KMnO_4$ and NaOH concentrations constant.

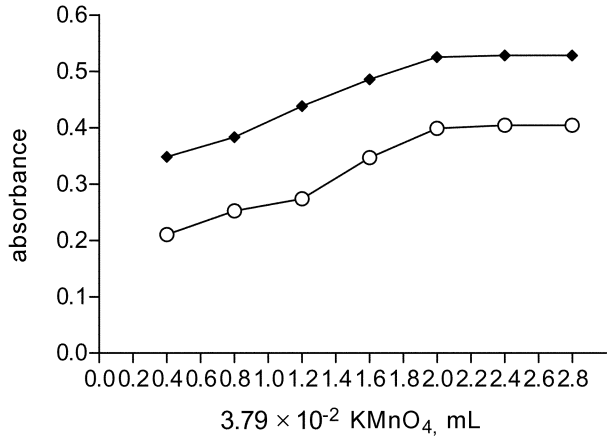

Figure 3. Effect of $KMnO_4$ ◆ Carbocisteine; ○ Penicellamine (10 μg/mL of each drug).

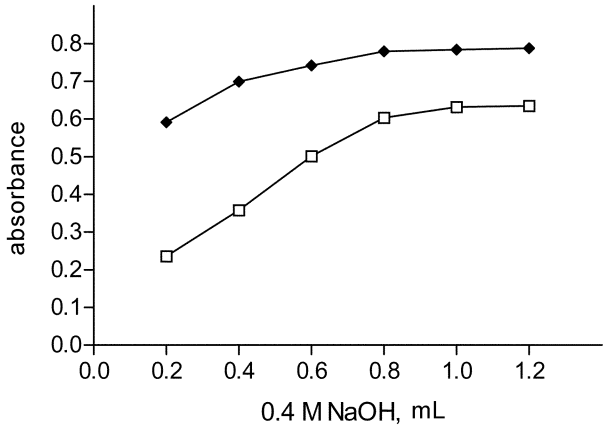

Figure 4. Effect of NaOH ◆ Ethionamide; □ Thioctic acid (10 μg/mL of each drug).

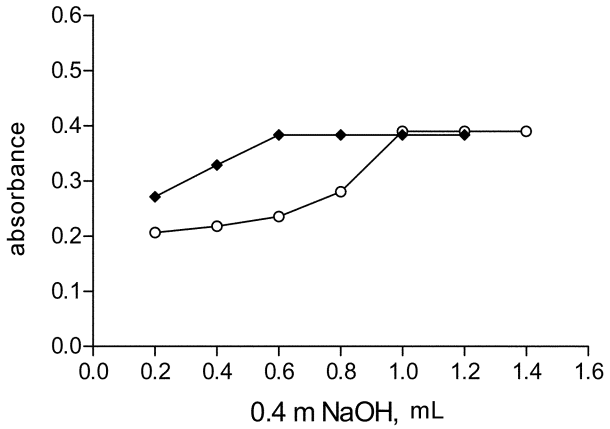

Figure 5. Effect of NaOH ◆ Carbocisteine; ○ Penicillamine (10 μg/mL of each drug).

The reaction rate was found to obey the following equation:

$$\text{Rate} = K'[\text{drug}]^n \quad (1)$$

Table 1. Lograthims of rates for different concentrations of carbocisteine and penicillamine at room temperatures and 610 nm

Log ΔA/Δt	Log. [Carbocisteine] (M)	Log ΔA/Δt	Log [Penicillamine] (M)
-4.10	-4.95	-4.18	-4.87
-3.76	-4.65	-3.88	-4.57
-3.59	-4.47	-3.71	-4.40
-3.45	-4.35	-3.58	-4.27
-3.36	-4.25	-3.48	-4.17

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

The rate of the reaction may be estimated by the variable time method measurement[25] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations (Table 1), Eq. (1) is transformed into:

$$\log(\text{rate}) = \log \frac{\Delta A}{\Delta t} = \log K' + n \log[\text{drug}] \qquad (2)$$

log(rate) versus log (drug) gave the regression equation (for carbocistiene):

log rate = 1.127 + 1.05 log C (r = 0.9997)

Hence K' = 13.4 S^{-1} and the reaction is first order (n = 1.05) and for penicillamine.

Log rate = 9.48×10^{-3} + 0.845 log C (r = 0.9999)

Hence K' = 1.022 S^{-1} and the react is first order (n = 0.845).

Evaluation of the Kinetic Methods. The quantitation of drug under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations where $KMnO_4$ concentration was at least 138 times of the initial concentration of carbocisteine or 116 times of the initial concentration of penicillamine. However, the rate will be directly proportional to drug concentration in a pseudo-first rate equation as follows:

$$\text{Rate} = K'[\text{drug}] \qquad (3)$$

Where K' is the pseudo-order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to Eq. (3). Initial rate, rate constant, fixed- concentration and fixed time methods[27,28] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

Table 2. Values of K' calculated from slopes of log A Vs time graphs at 610 nm

K' (S^{-1})	[Carbocisteine] (M)	K' (S^{-1})	[Penicillamine] (M)
-9.58×10^{-4}	1.11×10^{-5}	-2.24×10^{-4}	1.34×10^{-5}
-8.40×10^{-4}	2.23×10^{-5}	-1.96×10^{-4}	2.68×10^{-5}
-7.35×10^{-4}	3.35×10^{-5}	-1.68×10^{-4}	4.02×10^{-5}
-6.26×10^{-4}	4.46×10^{-5}	-1.42×10^{-4}	5.36×10^{-5}
-5.50×10^{-4}	5.58×10^{-5}	-1.10×10^{-4}	6.7×10^{-5}

Table 3. Values of reciprocal of time taken at fixed absorbance for different rates of variable concentrations of carbocisteine and penicillamine at constant concentrations of NaOH and $KMnO_4$ at room temperature

1/t (S^{-1})	[Carbocisteine] (M)	1/t (S^{-1})	[Penicillamine] (M)
8.40×10^{-4}	3.35×10^{-5}	1.25×10^{-3}	5.36×10^{-5}
2.75×10^{-3}	4.46×10^{-5}	1.67×10^{-3}	6.03×10^{-5}
4.80×10^{-3}	5.58×10^{-5}	2.10×10^{-3}	6.70×10^{-5}

Rate-constant Method. Graphs of log absorbance versus time for carbocisteine and penicillamine concentration in the range of 1.11×10^{-5} - 5.58×10^{-5} M and 1.34×10^{-5} - 6.7×10^{-5} M respectively were plotted and all 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 Table 2. Regression of (C) versus K' gave equations:

K' = -1.050×10^{-3} + 9.2212 C
(r = 0.9975) for carbocisteine
K'= -2.526×10^{-4} + 2.104 C
(r = 0.9995) for penicillamine

Fixed-concentration Method. Reaction rates were recorded for different concentrations of carbocisteine and penicillamine in the range of 3.35×10^{-5} - 5.58×10^{-5} M and 5.36×10^{-5} - 6.7×10^{-5} M respectively. A preselected value of the absorbance (0.3) was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of drug. (Table 3) was plotted and the following equations of the calibration graphs were obtained:

1/t = -5.1296×10^{-3} + 177.5866 C
(r = 0.99984) for carbocisteine

Table 4. Regression equation for carbocisteine and penicillamine at different fixed time over the range of 1.11×10^{-5} - 5.58×10^{-5} M and 1.34×10^{-5} - 6.7×10^{-5} M

Time (min.)	Carbocisteine		Penicillamine	
	Regression Equation	Correlation coefficient (r)	Regression Equation	Correlation C + oefficient (r)
5	A = -0.05658 + 0.04196 C	0.9998	A = -0.04044 + 0.03906 C	0.9976
10	A = -0.04706 + 0.04408 C	0.9831	A = -0.01856 + 0.0391 C	0.9999
15	A = -0.02149 + 0.05032 C	0.9998	A = -3.49×10^{-3} + 0.03922 C	0.9999
20	A = -9.8×10^{-3} + 0.05368 C	0.9999	A = 2.14×10^{-3} + 0.03946 C	0.9998

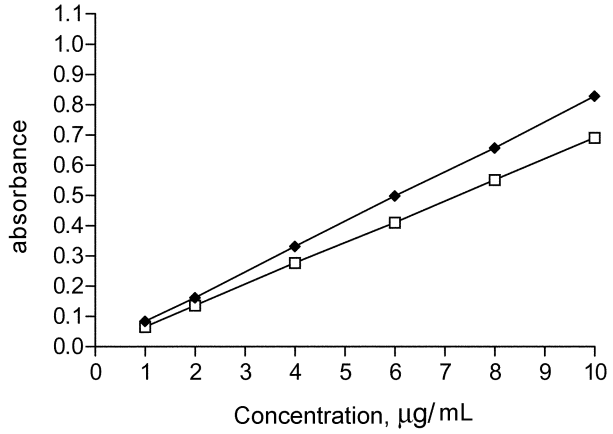

Figure 6. Spectrophotometric calibration curves of ethionamide ◆ and Thioctic acid □ .

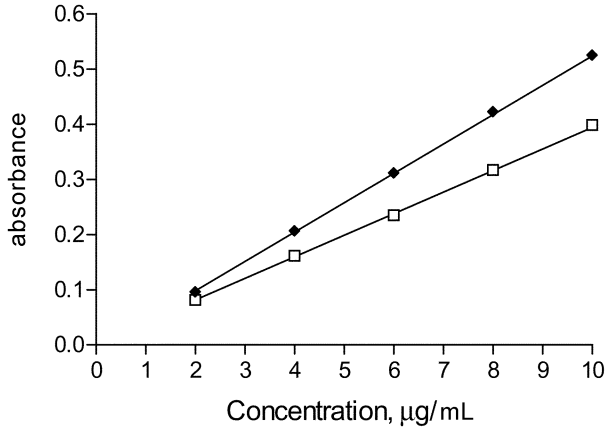

Figure 7. Kinetic spectrophotometric calibraion f penicillamine □ and carbocisteine ◆ .

Table 5. Validity of the proposed method for the determination of the studied compounds

Compound	Proposed method			Official method [3]	
	Taken (μg/mL)	Found (μg/mL)	Rec.* (%)	Taken (mg)	Rec. (%)
1-Carbocisteine	10	9.96	99.60		
	8	8.05	100.62	100	100.36
	6	5.99	99.83	150	99.16
	4	4.03	100.75	200	99.46
	2	1.97	98.50		
Mean ± S. D			99.87 ± 0.846	99.66 ± 0.624	
Student's *t*-test			0.341	(2.447)**	
F-test			1.84	(19.25)**	
2-Penicillamine	10	10.05	100.50		
	8	7.98	99.75	100 mg	101.49
	6	5.91	98.50	150	99.50
	4	4.03	100.75	200	100.75
	2	2.02	101.00		
Mean ± S. D			100.10 ± 1.001	100.58 ± 1.058	
Student's *t*-test			0.625	2.447	
F-test			1.01	6.94	
3-Ethionamide	10	10.01	100.10		
	8	7.95	99.38	100 mg	99.67
	6	6.02	100.33	150	98.56
	4	4.00	100.00	200	100.58
	2	1.96	98.00		
	1	1.00	100.00		
Mean ± S. D			99.63 ± 0.862	99.60 ± 1.012	
Student's *t*-test			0.047	2.365	
F-test			1.39	5.79	
4-Thioctic acid	10	100.56	100.56		
	8	08.03	100.34		
	6	05.98	99.67	50	99.91
	4	04.06	101.40	100	101.63
	2	02.01	100.65	150	99.68
	1	0.99	99.82		
Mean ± S. D			100.407 ± 0.626	100.410 ± 1.066	
Student's *t*-test			0.006	2.365	
F-test			2.90	5.79	

*Each Result is the average of three separate experiments.
**The values between brackets are the tabulated students t-test and variance ratio test (at P=0.05)[26].

Table 6. Application of the proposed method to the determination of the studied sulphur compounds in dosage forms

Compound	Proposed method			Reference method	
	Taken (μg/mL)	Found (μg/mL)	Rec.* (%)	Taken, (μg/mL)	Rec. (%)
1-Rhinathiol Syrup	10	10.23	102.34	10	101.20
(2 gm Carbocisteine/100 mL)	6	6.16	102.73	20	100.00
	4	4.11	102.85	30	98.57
				40	98.93
				50	101.02
Mean ± S. D			102.64 ± 0.3	99.95 ± 1.19	
2-Trecator Tablet	10	9.99	99.88	50	98.92
(250 gm/tablet)	8	8.06	100.69	100	99.81
	6	6.09	101.41	150	98.14
Mean ± S. D			100.66 ± 0.76	98.96 ± 0.84	
3-Artamine Capsule	1	0.99	98.55	3	99 .00
(250 mg/capsule)	0.8	0.79	98.43	8	100.25
	0.6	0.59	98.16	12	100.33
Mean ± S. D			98.38 ± 0.20	100.35 ± 0.66	
4-Thiotacid ampoule	8	8.01	100.04	400	99.47
(300 mg/10 mL)	6	6.01	100.10	300	99.95
	4	4.01	100.22	200	100.02
Mean ± S. D			100.12 ± 0.10	99.58 ± 0.63	
4-Thiotacid Tablet	8	7.95	99.31	400	99 .00
(300 mg/tablet)	6	5.95	99.13	300	99.50
	4	3.98	99.49	200	100.25
Mean ± S. D			99.31 ± 0.18	99.58 ± 0.63	

N.B.: *The results are the average of 6 separate determinations. 1. Amyria Pharmaceutical Company, Egypt. 2. Alexandria Theraplix Company, Egypt. 3. Biochemie Company, Austria. 4. Eva Pharma for Pharmaceuticals & Medical appliances, Egypt. 5. **Concentration at mg.

$1/t = -2.137 \times 10^{-3} + 63.2\ C$
(r = 0.9999) for penicillamine

Fixed-time Method. Reaction rates were determined for different concentrations of drug. At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentrations of carbocisteine and penicillamine were established at fixed times of 5, 10, 15, 20 min. with the regression equations assembled in Table 4.

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept chosen as the most suitable time interval for measurement.

Calibration Graphs. After optimizing the reaction conditions, the fixed time was applied to the determination of carbocisteine and penicillamine in pure form over the concentration range 2-10 μg/mL. Carbocisteine, penicillamine, ethionamide and thioctic acid with molar absorbtivities of 9.41×10^4, 5.95×10^4, 1.38×10^5 and 1.43×10^5 respectively.

Analysis of the data gave the following regression equations:

$A = -9.48 \times 10^{-3} + 0.05368\ C$
(r = 0.9999) → for carbocisteine
$A = 2.14 \times 10^{-3} + 0.03946\ C$

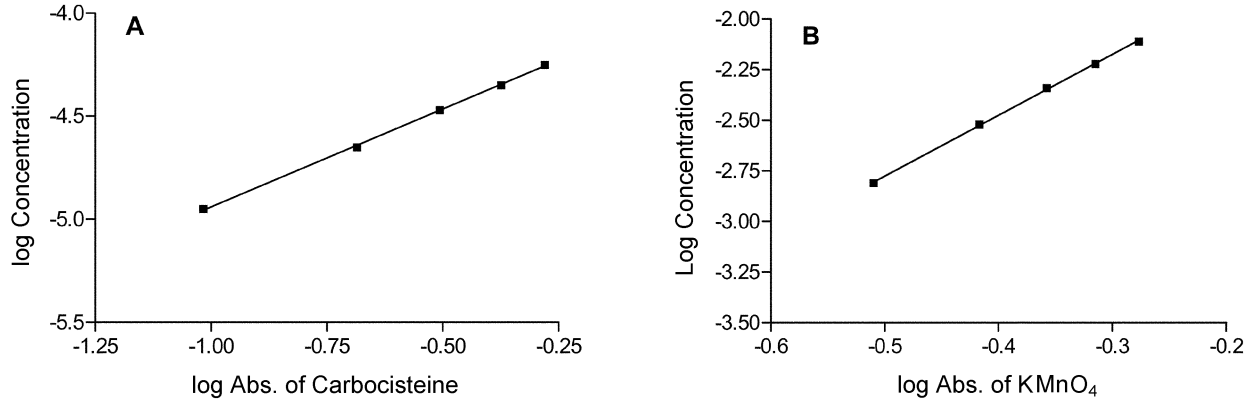

Figure 8. Stoichiometry of the reaction between carbocistiene and $KMnO_4$ adopting limiting logarithmic method A: carbocistiene B: $KMnO_4$.

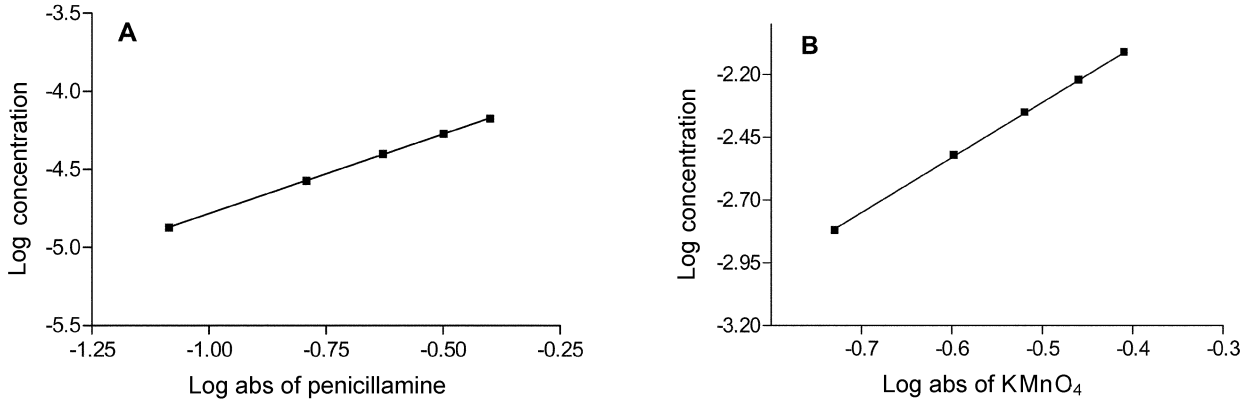

Figure 9. Stoichiometry of the reaction between penicillamine and $KMnO_4$ adopting limiting logarithmic method A: Penicillamine B: $KMnO_4$.

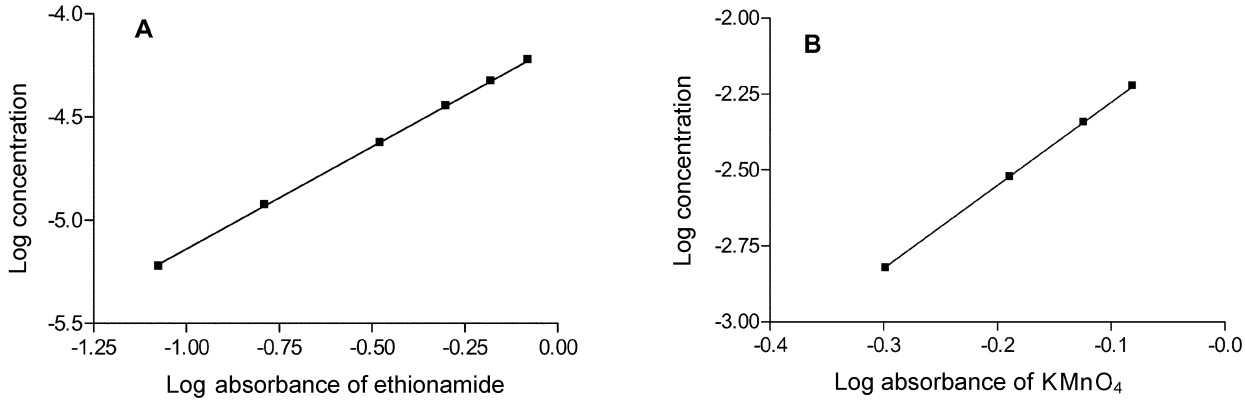

Figure 10. Stoichiometry of the reaction between Ethionamide and $KMnO_4$ adopting limiting logarithmic method A: Ethionamide B: $KMnO_4$.

(r = 0.9998) → for penicillamine

The spectrophotometric analysis of the data gave the following regression equations:

$A = - 4.8219 \times 10^{-4} + 0.0826\ C$

(r = 0.9999) → for ethionamide

$A = - 2.72 \times 10^{-3} + 0.0693\ C$

(r = 0.9999) → for thioctic acid

The calibration graphs were shown in (Figs. 6 and 7)

The % recoveries of the four studied drugs compared with that obtained by the official methods[3] were given in Table 5.

Statistical analysis[29] of the results obtained by the proposed and reference methods[4,6,10,25] using student's t-test and variance ratio revealed no significant difference between the performance of the two methods regarding the accuracy and precision.

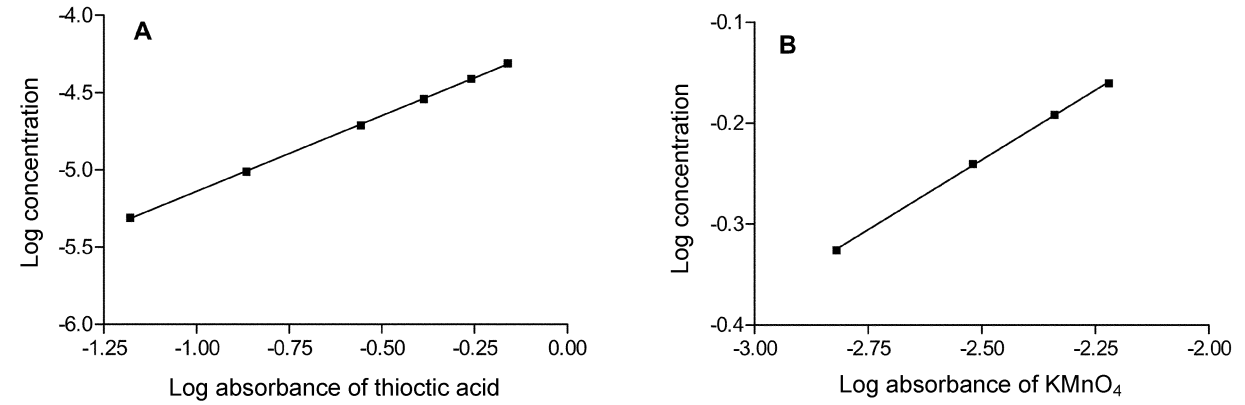

Figure 11. Stoichiometry of the reaction between thioctic acid and $KMnO_4$ adopting limiting logarithmic method A: Thioctic acid B: $KMnO_4$.

The proposed method was successfully applied for determination of the studied sulphur drugs in their different dosage forms, as shown in Table 6, compared with the results obtained by the reference methods.[4,6,10,25]

Mechanism of the Reaction. The stoichiometry of the reaction was studied adopting the limiting logarithmic method [30]. The ratio of the reaction between (carbocisteine, penicillamine and ethionamide (with thiol group)) and $KMnO_4$ in alkaline medium was calculated by dividing the slope of $KMnO_4$ curve over the slope of the drug curve (Figs. 8-11).

It was found that, the ratio was 3 : 1 ($KMnO_4$ to drug). While for thioctic acid (disulphide), the ratio was 4 : 1. The proposal pathway of the reaction is given as follow:

$$R\text{-}SH + 3MnO_4^- + 6\ OH^- \rightarrow R\text{-}SO_3H + 3MnO_4^{2-} + 3H_2O$$

And for thioctic acid

$$R\text{-}S\text{-}S\text{-} + 4MnO_4^- \xrightarrow{OH^-} R-\overset{\overset{O}{\|}}{S}-\overset{\overset{O}{\|}}{S} + 4MnO_4^{2-}$$

Conclusion

The proposed method was simple, accurate, precise, sensitive, rapid, low cost and relating selective compared to the official methods.[3]

Furthermore, the proposed method doesn't require elaboration of procedures, which are usually associated with chromatographic methods. The proposed method could be applied successfully for determination of the studied compounds in pure form as well as in different dosage forms. Moreover, small amounts of the studied compounds could be determined compared with the official methods which required at least 50 mg to be determined by Non-aqueous titration for carbocisteine, penicillamine and ethionamide while thioctic acid isn't official in USP or B.P. and determined by iodometry as a similar compound, cystine.[3]

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