Scientific Paper

FLUORIMETRIC DETERMINATION OF CARBOCISTEINE AND ETHIONAMIDE IN DRUG FORMULATION

Mohamed I. Walash,* Amina M. El-Brashy, Mohamed E.-S. Metwally, and Amina A. Abdelal

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

Received 05-08-2003

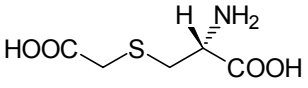
Abstract

A highly sensitive and specific fluorimetric method was developed for the determination of carbocisteine and ethionamide in their dosage forms. The proposed method based on the reaction of carbocisteine and ethionamide with roth's reagent (*o*-phthaldehyde) to get a highly fluorescent isoindole product emits strong fluorescence at 431 nm and 424 nm after excitation at 329 nm and 339 nm for carbocisteine and ethionamide, respectively. The thiol group present in these compounds is responsible for the formation of highly fluorescent complexes of improved stability and enhanced fluorescence. The different experimental parameters affecting the intensity of the fluorescence were carefully studied and incorporated into the procedure. Under the described conditions, the method was applicable over the concentration range of 0.05-0.9 μg/mL and 0.25-2.5 μg/mL with detection limits of 5 ng/mL and 26 ng/mL for carbocisteine and ethionamide, respectively. The proposed method was successfully applied for determination of carbocisteine and ethionamide in their dosage forms.

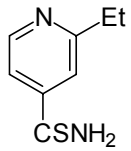
Key words: flourimetry, *o*-phthaldehyde, carbocisteine, ethionamide, dosage forms

Introduction

The importance of carbocisteine [I] and ethionamide [II] is due to their widespread use and different pharmacological effects. Carbocisteine (S-carboxymethyl-L-cysteine) is a mucolytic drug used for treatment of disorders of the respiratory tract associated with excessive mucus. Ethionamide (2-ethyl-thioisonicotinamide) is a tuberculostatic agent used in treatment of isoniazid-resistant tuberculosis.[1-2]



[I]



[II]

The published methods for the determination of these drugs include titrimetry,[3-4] spectrophotometry,[5-9] electro-analysis,[10] and chromatography.[11-16] The above mentioned

methods were either not sufficiently sensitive or were tedious and require highly sophisticated instrumentation. Therefore, there was still a need for a much more sensitive and simple method for the determination of carbocisteine and ethionamide. Reviewing the literature revealed that no fluorimetric methods were reported for these drugs. As for *o*-phthaldehyde, it has been recently used for determination of different thiols,[17] prazosin hydrochloride,[18] acidic polysaccharides,[19] and amino acids.[20-21] The aim of the present work is to study the reaction of *o*-phthaldehyde and the primary amino group in presence of thiol group in these thiocompounds in an attempt to develop a highly sensitive fluorimetric method to be used for their determination in their dosage forms.

Experimental

Equipment

ARF-1501 Shimadzu spectrofluorophotometer with xenon lamp. A 1 cm quartz cell was used for all measurements.

Reagents and Materials

All reagents used were of analytical reagent grade and the water was always double distilled water. Carbocisteine was offered by Amyria Pharmaceutical Industries, Egypt. Ethionamide was offered by Alexandria Theraplix Company, Egypt. The purities of these drugs were determined by applying the official methods.[3]

Stock solutions of the studied drugs were prepared by dissolving 5 mg of carbocisteine and ethionamide in 3 mL of 0.2 M NaOH and 3 mL of 5 N HCl respectively then completed to 100 mL with distilled water. Other concentrations were prepared by dilution with distilled water.

0.05% *o*-phthaldehyde (Sigma, UK) was freshly daily prepared in methanol. Aqueous solutions of 0.2 M NaOH (BDH, UK) and 5N HCl (Prolabo) were prepared.

Procedures

Construction of calibration graphs

Transfer aliquots of carbocisteine or ethionamide equivalent to 0.05-0.9 μg/mL or 0.25-2.5 μg/mL respectively into a series of 10 mL volumetric flasks. 0.8 mL or 1.5 mL

of 0.05% *o*-phthaldehyde was added to the 2 drugs respectively followed by 1 mL of 0.2M NaOH. The volume was adjusted to the mark with distilled water. The fluorescence was measured at λ Em. 431 or 424 nm after excitation at 329 nm or 339 nm for carbocisteine or ethionamide after 45 min, at ambient temperature, respectively. A blank reagent was prepared simultaneously.

Procedure for the dosage forms

An accurately weighed quantity of the mixed contents of 10 pulverized tablets or measured volume of the syrup equivalent to 5 mg of the drug was transferred into a 100 mL volumetric flask and made up to the mark with distilled water. 3 mL of 0.2 M NaOH or 3 mL of 5N HCl were firstly added in case of Rhinathiol® syrup or Trecator® tablets, respectively. The contents of the flask were sonicated for 5 min, filtered, and the above procedure was completed as before. The amount of carbocisteine or ethionamide was calculated either from a previously prepared calibration graph or using the regression equation.

Results and Discussion

Carbocisteine and ethionamide are sulfur compounds containing both sulfur and primary amino groups that react with *o*-phthaldehyde giving a highly fluorescent isoindole products[22] at 431/329 nm and 424/339 nm ($\lambda_{em.}/\lambda_{ex.}$) for these two drugs (figures 1 and 2).

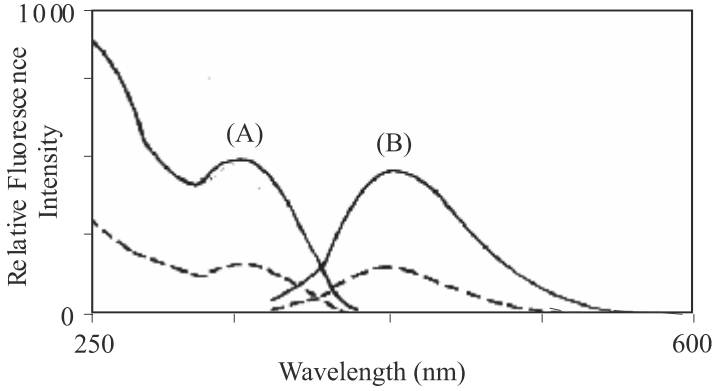


Figure 1. Fluorescence spectra of Carbocisteine (0.9 μg/mL) after reaction with *o*-phthaldehyde *o*-phthaldehyde at $\lambda_{ex.}$ = 329 nm (A), $\lambda_{em.}$ = 431 nm (B).

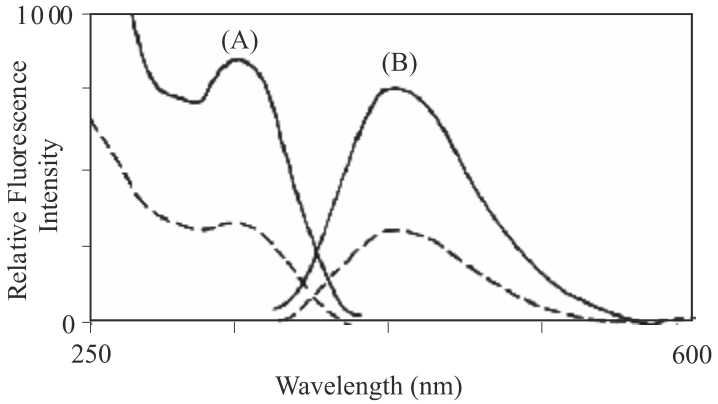


Figure 2. Fluorescence spectra of Ethionamide (2.5 μg/mL) after reaction with *o*-phthaldehyde *o*-phthaldehyde at $\lambda_{ex.}$ = 339 nm (A), $\lambda_{em.}$ = 424 nm (B).

The sulfur group present in these compounds is responsible for formation of highly fluorescent complexes of improved stability and enhanced fluorescence. The effect of temperature on the fluorescence intensity was studied in the range 30-80 °C. It was observed that the reaction not quantitized at higher temperature.

Optimization of the reaction conditions

Effect of o-pthaldehyde

From Figure 3, it was found that increasing the volume of 0.05% *o*-phthaldehyde up to 0.8 mL and 1.5 mL for carbocisteine and ethionamide respectively, would increase the fluorescence intensity of the reaction and after that the fluorescence intensity decreases.

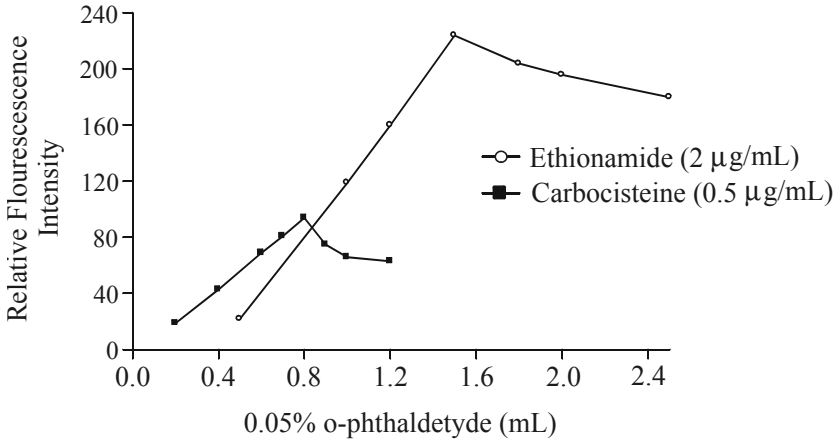


Figure 3. Effect of *o*-pthaldehyde on the Flourescence intensity.

Effect of NaOH

From Figure 4, increasing the volume of 0.2M NaOH up to 1 mL for both compounds would increase the fluorescence intensity of the product and after that the fluorescence intensity decreases.

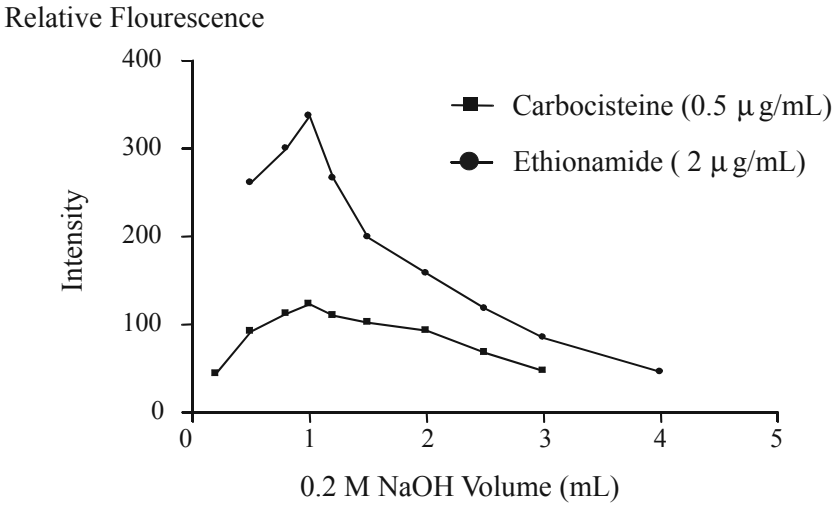


Figure 4. Efect of NaOH volume on the fluorescence intensity.

Effect of time

The fluorescence intensity of the products of the two studied drugs increases with time up to 45 min (at room temperature), after that it remains stable for more than one hour Figure 5.

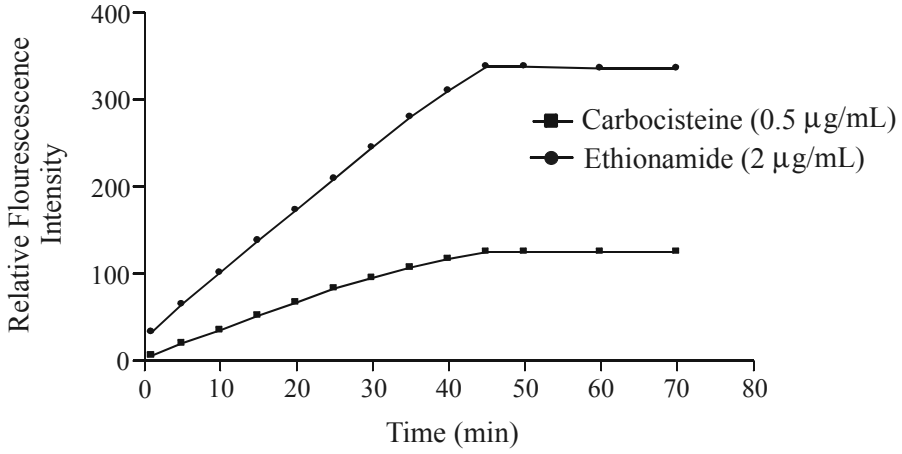


Figure 5. Effect of reaction time on the reaction between the studied compounds and *o*-phthaldehyde.

Calibration graphs

After optimizing the conditions, it was found that the relation between relative intensity (RI) and final concentration of carbocisteine and ethionamide was rectilinear over the range 0.05-0.9 μg/mL and 0.25-2.5 μg/mL with detection limit of 5 ng/mL and 26 ng/mL for the two drugs respectively Figure 6.

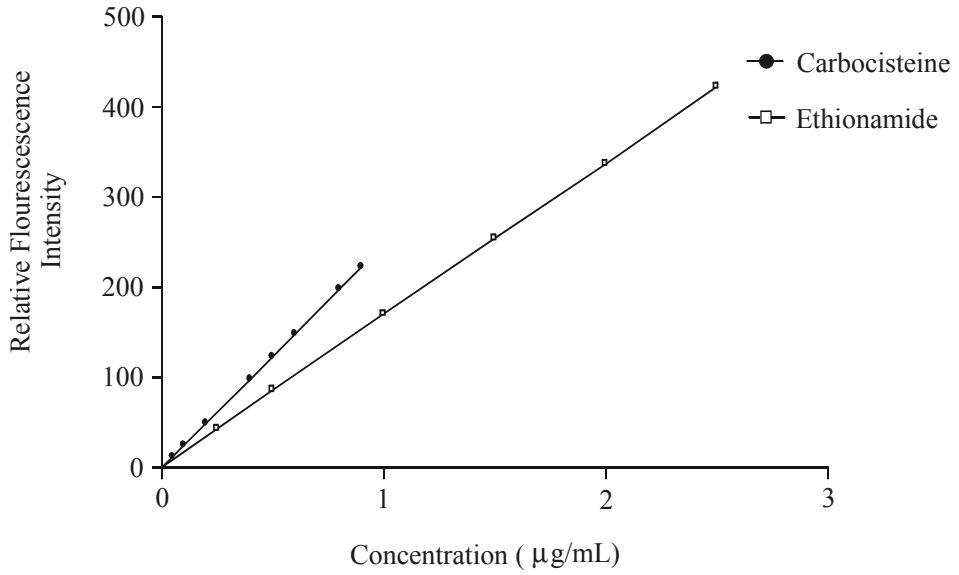


Figure 6. Calibration Curve of Carbocisteine and Ethionamide after reaction with 0.05% *o*-phthaldehyde. The linear regression analysis of the results are: RI = -0.983+247.877 C (r = 0.9999) for carbocisteine and RI = 1.518+168.134 C (r = 0.9999) for ethionamide.

Where: C=final concentration in μg/mL, RI = relative fluorescence intensity (fluorescence intensity of the product – fluorescence intensity of the *o*-phthaldehyde) and r = correlation coefficient.

The % recoveries of these studied drugs compared with that obtained by the official methods[3] were given in table 1. These official methods recommends non-aqueous titration for the two drugs.

Statistical analysis of the results obtained by the proposed and official methods[3] revealed that no significant difference between the two methods regarding the accuracy and precision as indicated by the F-test and student's t-test.[23]

The proposed method was successfully applied for the determination of the studied sulfur drugs in their dosage forms, as shown in Table 2.

Table 1. Fluorimetric determination of carbocisteine and ethionamide by *o*-phthaldehyde in pure form.

Compound	Proposed method			Official method[3]	
	Taken (μg/mL)	Found (μg/mL)	Rec.* %	Taken (mg)	Rec.* %
Carbocisteine	0.05	0.049	98.00		
	0.1	0.100	100.00	100	100.36
	0.2	0.198	99.00		
				150	99.16
	0.4	0.399	99.75		
	0.5	0.496	99.20	200	99.46
	0.6	0.601	100.16		
	0.8	0.802	100.25		
	0.9	0.899	99.89		
Mean ± S. D.			99.53± 0.76	99.66 ± 0.624	
t-test			0.635	2.262**	
F-test			1.483	19.36	
Ethionamide	0.25	0.247	98.80		
	0.5	0.502	100.40		
				100	99.67
	1	1.002	100.2		
	1.5	1.501	100.06	150	98.56
	2	1.995	99.75		
				200	100.58
	2.5	2.501	100.04		
Mean ± S. D.			99.88± 0.568	99.60± 1.012	
t-test			0.813	2.365**	
F-test			3.17	5.79	

* Each result is the average of three separate experiments. ** The values are the tabulated student's t-test and Variance ration test (at P = 0.05).[23]

The results obtained by the proposed method are compared with that of the reference methods.[5,6] The reference methods recommends spectrophotometric methods for the two drugs, where the absorbance methanolic solution of ethionamide was measured at 290 nm[5] and complexation of carbocisteine with nickel and measuring the absorbance of the formed complex at 259 nm.[6]

Conclusions

The proposed method was simple, accurate, precise, reproducible, highly sensitive, and relatively selective compared to the official method.

Table 2. Fluorimetric determination of carbocisteine and ethionamide by *o*-phthaldehyde in their dosage forms.

Compound	Proposed method			Reference method	
	Taken (μg/mL)	Found (μg/mL)	Rec.* %	Taken	Rec.* %
1) Carbocisteine®syrup					6
(Carbocisteine, 2g/100mL)	0.4	0.406	101.63	10 μg/mL	101.20
	0.6	0.605	100.95	20 μg/mL	100.36
	0.8	0.808	101.12	30 μg/mL	98.57
				40 μg/mL	98.93
				50 μg/mL	101.02
Mean ± S. D.		101.23 ± 0.354		99.95 ± 1.189	
2) Trecator®tablets					5
(Ethionamide, 250 mg/tablet)	0.5	0.494	98.84	50 mg	98.92
	1	1,01	100.59	100 mg	99.81
	2.5	2.488	99.53	150 mg	98.14
Mean ± S. D.		99.65 ± 0.881		98.96 ± 0.836	

The results are the average of 6 separate determinations. 1) Amyria Pharmaceutical Company, Egypt. 2) Alexandria Theraplix Company, Egypt.

Furthermore, the proposed method does not require elaboration of procedures which are usually associated with chromatographic methods. The proposed method could be applied successfully for determination of carbocisteine and ethionamide in pure form as well as in different dosage forms.

Our method allows the determination of lower amounts of the studied compounds than those detectable by the official methods that require non-aqueous titration for carbocisteine and ethionamide.[3] The proposed method was found to be with detection limits of 5 ng/mL and 26 ng/mL and quantification limits of 0.05 μg/mL and 0.25 μg/mL for carbocisteine and ethionamide, respectively.

References

1. Martindale (The Extra Pharmacopoeia)-32 Ed., The Pharmaceutical Press; London, 1999, pp 1056–1057, p 208.
2. Merck Index, 12 Ed., by Merck & Co. Inc.; White House Station, N.J., 1995, p 294, p 638.
3. British Pharmacopoeia, London, 1998, pp 249-250, pp 553–554.
4. H. Sikorska-Tomicka, H. Sobolewska, *Chem. Anal.* (Warsaw) **1986**, *31*, 453–457.
5. The United States Pharmacopoeia XXII. National Formulary XVII; Rockville, MD, USP convention, Inc., 2000.
6. G. G. Pargaonkar, S. G. Kashedikar, *Indian Drugs* **1994**, *31*, 590–592.

7. J. M. Nedeljkovic, V. M. Vasic, V. V. Vukovic, T. S. Jovanovic, *J. Pharma. Biomed. Anal.* **1995**, *13*, 471–475.
8. C. S. P. Sastry, K. R. Srinivas, M. M. Krishna Prasad, *Mikrochim. Acta* **1996**, *122*, 77–86.
9. H. Sikorska-Tomicka, *Chem. Anal.* (Warsaw) **1993**, *38*, 745–751.
10. D. Dion, E. Laviron, *Electrochimica Acta* **1998**, *43*, 2061–2069.
11. M. C. Gennaro, R. Calvino, C. Abigo, *J. Chromatogr. B.* **2001**, *754*, 477–486.
12. J. E. Conte, G. F. Wang, E. T. Lin, E. Zurlinden, *J. Chromatogr. B.* **2001**, *753*, 343–353.
13. L. Suntornsuk, *Pharm. Biomed. Anal.* **2001**, *25*, 165–170.
14. G. B. Steventon, *Chromatographia* **1998**, *48*, 561–568.
15. G. Indrayanto, S. Widjaja, L. Aditama, L. Darmawan, *J. Planar. Chromatogr.* **1998**, *11*, 263–266.
16. R. Ancardio, M. G. Cantalini, F. De-Angelis, M. Gentile, *J. Mass. Spectrom.* **1997**, *32*, 388–394.
17. F. Dai, V. P. Burkert, H. N. Singh, W. L. Hinze, *Micochem. J.* **1997**, *57*, 166–198.
18. P. Parimoo, P. V. Ballimane, *Indian Drugs* **1997**, *34*, 21–23.
19. M. Kobayashi, Y. Chiba, *Anal. Biochem*. **1994**, *219*, 189–194.
20. T. Miura, *Yakugaku-Kenkya-Noshinpo* **1991**, *7*, 87–97.
21. K. Yokotsuka, I. Kato, J. C. Ogbonna, Y. Amano, *J. Ferment. Bioeng.* **1989**, *68*, 222–224.
22. S. S. Simons, D. F. Johnson, *J. Am. Chem. Soc.* **1976**, *98*, 7098.
23. R. Caulcut, R. Boddy, "*Statistics for Analytical Chemists*"; Chapman and Hall, 1983.

Povzetek

Razvili smo občutljiv in specifičen fluorimetrični postopek za določanje karbocisteina in etionamida v farmacevtskih pripravkih. Predlagani postopek temelji na reakcijah karbocisteina in etionamida z *o*-ftalaldehidom, pri čemer nastane fluorescirajoči produkt izoindol, ki pri vzbujevalni valovni dolžini 329 nm fluorescira pri 421 nm za karbocistein oziroma pri 424 nm za etionamid. V le-teh je za tvorbo sorazmerno stabilnih fluorescirajočih spojin pomembna tiolna skupina. Preučili smo različne eksperimentalne parametre, ki lahko vplivajo na intenzivnost fluorescence produkta. Z optimizacijo smo razvili metodo, ki daje uporabne rezultate v koncentracijskem območju 0,05-0,9 μg/mL za karbocistein (meja zaznavnosti 5 ng/mL) oziroma 0,25-2,5 μg/mL za etionamid (meja zaznavnosti 26 ng/mL). Predlagano metodo smo uporabili za določanje karbocisteina in etionamida v farmacevtskih pripravkih.