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Detection of Thioamides: Determination of Ethionamide with 2,3-Dichloro-1,4-naphthoquinone

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Abstract □ 2,3-Dichloro-1,4-naphthoquinone is reacted with thioamides in microquantities in the presence of ammonia in an alcoholic medium to give colored products showing absorbance maxima between 530 and 540 nm. The reaction is specific for thioamides and forms the basis of its spot test. The reaction is also used for the quantitative determination of ethionamide in tablets. The results compare favorably with those obtained by the official BP method. The method is simple, accurate, and precise.

Keyphrases □ Ethionamide—determination using 2,3-dichloro-1,4-naphthoquinone □ Thioamides—determination of ethionamide using 2,3-dichloro-1,4-naphthoquinone □ 2,3-Dichloro-1,4-naphthoquinone—colorimetric determination of ethionamide □ Colorimetry—determination of ethionamide using 2,3-dichloro-1,4-naphthoquinone

Thioamides are used widely as antitubercular agents (1). They are usually detected by fluorescein-1,3,6,8-tetramercuritetraacetate (2), Dragendorff reagent (3), sodium nitroferricyanide (4), sodium pentacyanoamine ferroate (5), and ammoniacal copper sulfate solution (6). Hydrogen sulfide, evolved on reacting thioamides with hydrazine hydrate, is detected with lead acetate paper (7).

Recently, 2,3-dichloro-1,4-naphthoquinone has been used in the detection and determination of sulfur-containing compounds such as thiosemicarbazones, thiosemicarbazides, and thioureas (8, 9). Therefore, it was of interest to use this reagent in the detection of thioamides and the determination of drugs containing the thioamide moiety. In the present work, reaction conditions were sought for a spot test for thioamides with 2,3-dichloro-1,4-naphthoquinone. The absorption maxima of colored products, obtained on reacting various thioamides with 2,3-dichloro-1,4-naphthoquinone, were determined.

Several methods for the estimation of ethionamide include iodometric (10, 11), acidimetric–alkalimetric (12–14), gravimetric (15), complexometric (16, 17), polarographic (18, 19), and spectrophotometric (20–29) procedures. However, most of them are not specific for the thioamide moiety of the molecule. In view of the specificity and sensitivity of this color reaction, 2,3-dichloro-1,4-naphthoquinone reagent is used for the quantitative estimation of ethionamide BP, a well-known antitubercular drug. Optimum conditions for the reaction have been studied.

EXPERIMENTAL

Apparatus—All spectral measurements were made with a spectrophotometer[1] having four matched 10-ml cells of 1-cm light path.

Reagents and Materials—Ethionamide BP, thioacetamide[2], thionicotinamide[3], and absolute alcohol (Ind.P.) were used. Thiobenzamide (30), thioisonicotinamide (31), and 2,3-dichloro-1,4-naphthoquinone (32) were synthesized by known methods. All other reagents were of analytical grade. Various brands of ethionamide tablets were obtained from the market.

Preparation of 2.5% Ethanolic Ammonia—Dry ammonia was passed into absolute alcohol at −5° until the weight had increased by about 20%. The solution was diluted with absolute alcohol to obtain 2.5% (w/v) of ammonia and was stored in a refrigerator.

2,3-Dichloro-1,4-naphthoquinone Reagent Solution—The concentration used was 0.026% (w/v) in absolute alcohol.

Standard Ethionamide Solution—A 0.03% (w/v) solution in absolute alcohol was prepared.

Detection of Thioamides—*Plate Method*—About 3 drops of ethanolic solution of thioamide (0.1 mg/ml) were spotted on a

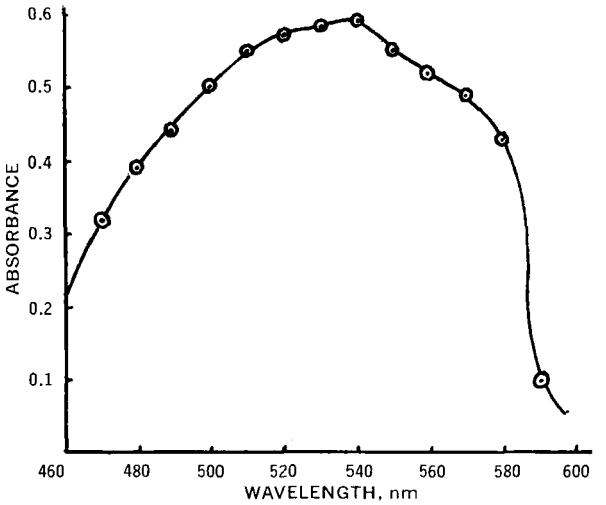


Figure 1—*Visible spectrum of the colored product obtained on reacting ethionamide with 2,3-dichloro-1,4-naphthoquinone reagent.*

[1] Spectronic 20, Bausch & Lomb.
[2] British Drug Houses.
[3] Aldrich.

Table I—Absorbance of Colored Products Obtained on Reacting Various Thioamides with 2,3-Dichloro-1,4-naphthoquinone Reagent

Thioamides	λ_{max}, nm	Absorbance at λ_{max}[a]
Thioacetamide[b]	530	0.085
Thiobenzamide[b]	540	0.255
Ethionamide[b]	540	0.590
Thionicotinamide[b]	530	0.450
Thioisonicotinamide[b]	530	0.615
Acetamide[c]	—	—
Benzamide[c]	—	—
Nicotinamide[c]	—	—

[a] Average value of three experiments. [b] Concentration of 5 μmoles/25 ml reaction mixture. [c] Concentration of 50 μmoles/25 ml reaction mixture.

white porcelain spot plate. Ethanolic ammonia (0.3 ml) and the 2,3-dichloro-1,4-naphthoquinone solution (0.5 ml) were added successively to it. After 5 min, the colored spot was compared with the yellowish reference spot of the reagents alone.

Spot Test on Paper—About 3 drops of the ethanolic test solution (0.1 mg/ml thioamide) were transferred onto a 2.54-cm (1-in.) wide filter paper[4] and dried. The 2,3-dichloro-1,4-naphthoquinone reagent (0.3 ml) was also spotted on it and dried. The yellowish spot thus obtained became purple when exposed to ammonia vapor for about 2 min. The colored spot was then compared with a yellowish reference spot of the reagents alone after 5 min.

Determination of Wavelength of Maximum Absorbance—An ethanolic solution of various thioamides (1 ml of a 5 μmole/ml solution) was mixed with ethanolic ammonia (4.5 ml) and the 2,3-dichloro-1,4-naphthoquinone solution (16.0 ml) in a 25-ml volumetric flask, diluted to volume with absolute alcohol, and allowed to stand for 20 min at room temperature. The absorbance was measured at 10-nm intervals from 400 to 600 nm against absolute alcohol. The blank contained the solution of 2,3-dichloro-1,4-naphthoquinone (16.0 ml) and ethanolic ammonia (4.5 ml) diluted to 25 ml with absolute alcohol. The blank readings were used to correct the absorbance of the sample (Table I).

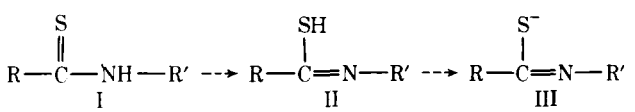
Analysis of Ethionamide Tablets—Twenty tablets were weighed and powdered. A portion of the powder, equivalent to about 30 mg of ethionamide, was accurately weighed. Four equal portions of 20 ml of absolute alcohol were used to extract ethionamide from the tablet powder, and each extract was filtered through filter paper[5]. The residue on the filter paper was then washed with 10 ml of alcohol. The filtrate and the washings were combined in a 100-ml volmetric flask and diluted to volume with ethanol. The solution was assayed as described.

Factors Affecting Reaction—*Concentration of 2,3-Dichloro-1,4-naphthoquinone*—The absorbance at 540 nm of the colored product formed by the reaction of the ethionamide solution (3.0 ml) with 2,3-dichloro-1,4-naphthoquinone increased with the increase in concentration of the reagent. The maximum absorbance was obtained in the presence of 16.0 ml of reagent in 25.0 ml of reaction mixture.

Concentration of Ammonia—A purple color was obtained on the addition of ammonia to the mixture of ethionamide solution (3.0 ml) and 2,3-dichloro-1,4-naphthoquinone reagent (16.0 ml). Maximum color intensity was obtained in the presence of 4.5 ml ammonia solution in 25.0 ml of reaction mixture.

Time of Reaction—The color intensity was found to be at a maximum when the reaction mixture (25.0 ml) containing ethionamide solution (3.0 ml), ethanolic ammonia (4.5 ml), and 2,3-dichloro-1,4-naphthoquinone reagent (16.0 ml) was kept at room temperature for 20 min. The color decreased slowly on standing.

Effect of Concentration of Ethionamide—The absorption at



Scheme I

[4] Whatman No. 1.
[5] Whatman No. 40.

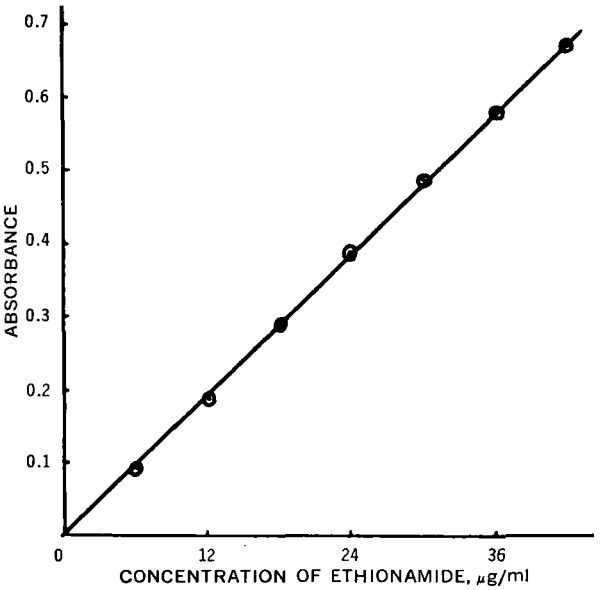


Figure 2—*Standard curve for ethionamide with 2,3-dichloro-1,4-naphthoquinone reagent.*

540 nm was proportional to the amount of ethionamide in the range of 6.0–42.0 μg/ml reaction mixture (Fig. 2).

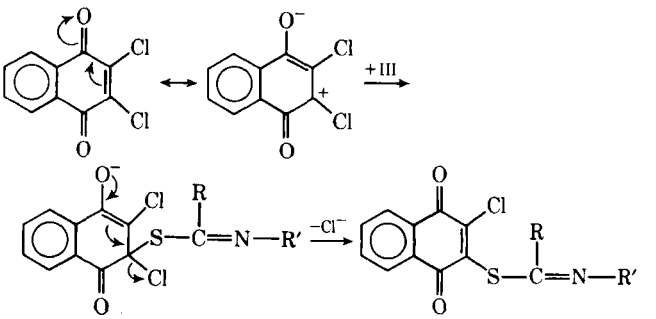
DISCUSSION

Thioamides are known to exist mainly in the thione form (I) (33). The reaction seems to be specific for compounds represented by a general formula R—C(=S)—NH—R′, provided the thioketo compound is able to enolize. In a nonpolar medium, the thio–thiol equilibrium shifts toward the thiol form (II) (34). In the presence of ammonia, thioenol (II) is converted into thioenolate ion (III) which, being a strong nucleophile, helps to remove one chlorine atom of 2,3-dichloro-1,4-naphthoquinone (Scheme I). The reaction shown in Scheme II is thereby initiated.

The color develops within 5 min after the reactants are mixed. Maximum color intensity is obtained after 20 min but decreases slowly on further standing. The absorption maxima of the colored products obtained on reacting various thioamides with the 2,3-dichloro-1,4-naphthoquinone reagent lie between 530 and 540 nm (Table I). The absorption curve for ethionamide is shown in Fig. 1. The corresponding amides such as acetamide, benzamide, and nicotinamide do not give any appreciable color even at a concentration 10-fold that used for thioamides.

Various substituents on the thioamide moiety seem to influence the color intensity of the reaction products: pyridyl > phenyl > methyl. This can be explained by the fact that an electron-withdrawing group like pyridyl or phenyl increases the acidity of the thiol group (II) and thus facilitates the formation of thioenolate ion (III). On the other hand, an electron-donating group like methyl decreases the acidity due to its positive inductive effect.

Ethionamide samples and tablets were assayed by the proposed procedure. The percent recovery and the standard deviation calculated from a series of experiments are given in Tables II and III.



Scheme II

Table II—Analysis of Ethionamide Samples

Sample	Recovery[a], %	
	Official BP Method (12)	Proposed Method
A	99.6 ± 0.852	99.7 ± 0.706
B	99.3 ± 0.856	99.8 ± 0.610

[a] Standard deviations were determined from the results of 10 experiments.

Table III—Analysis of Ethionamide Tablet

Sample	Labeled Amount, mg/Tablet	Recovery[a], mg/Tablet	
		Official BP Method (12)	Proposed Method
A	125.0	120.2	120.7
B	125.0	124.7	124.9

[a] Average result of three determinations.

The results compare favorably with those obtained by the official BP method (12). The usual tablet diluents and lubricants do not interfere in the proposed procedure. The method is simple, rapid, and accurate.

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