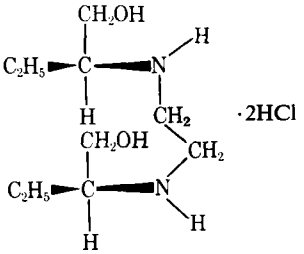
Colorimetric Determination of Ethambutol

J. M. BURGER, F. D. PISANO,* and R. A. NASH

Abstract □ A method has been developed for the separation and determination of ethambutol using cupric ion as a chelating agent. The method is sensitive for routine assay and simple to carry out. The effect of time on color stability, the reproducibility of the method, and the determination of the complexation ratio have been investigated.

Keyphrases □ Ethambutol—analysis □ Cupric ion chelation—ethambutol □ Column chromatography—separation □ Colorimetric analysis—spectrophotometer

Ethambutol dihydrochloride[1] is an oral chemotherapeutic agent which is specifically effective against actively-growing microorganisms of the genus *Mycobacterium*, including *M. tuberculosis* (1). The structural formula for the drug is given (I) (+)-2, 2′(ethylenediimino)-di-*l*-butanol dihydrochloride



I

In this communication a simple and rapid assay method for the drug is reported. The method is based on the color produced by the complexation of ethambutol free base with cupric ion in a nonaqueous medium. Hershenson and Hume (2) have described a nonaqueous colorimetric assay for aliphatic amines using cupric ion as a chelating agent. Windheuser and Chu (3) have reported a similar colorimetric procedure for the determination of iodochlorhydroxyquin and diiodohydroxyquin.

In general, the procedure consists of converting ethambutol dihydrochloride to the free base by the addition of excess alkali. The free base is subsequently isolated by partitioning into chloroform and then reacted with a methanolic solution of cupric chloride to produce a vivid greenish-blue color. In the presence of excess cupric ion reagent the color produced is proportional to the concentration of ethambutol and obeys the Beer's law relationship. Although the absorptivity of the colored complex is low ($a = 0.515$), based on the concentration of ethambutol free base, the method is sufficiently sensitive to permit the assay of ethambutol dihydrochloride tablets. However, the method is not stereospecific for the pharmacologically active *d*-isomer of the drug.

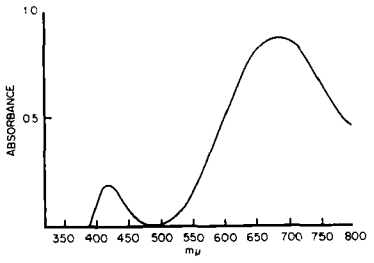


Figure 1—*Absorption spectrum of ethambutol-copper (II) chelate in methanol–chloroform. Cary model 11 recording spectrophotometer.*

EXPERIMENTAL

Chromatographic Column—A suitable column (1.5 × 40 cm.) is prepared by cutting off the delivery end of a 50-ml. serological pipet at about the 30-ml. mark. The tamping rod is made of glass and is about 1 mm. diameter less than the column. A filter paper disk about 12 mm. in diameter is used as the support in the base of the column.

Disodium Edetate-Washed Diatomaceous Earth—Prepare a slurry of diatomaceous earth[2] in 0.1 *M* disodium edetate USP solution and mix for 15 min. Filter the washed diatomaceous earth on a large Büchner funnel, wash repeatedly with purified water, and dry for 1 hr. under vacuum. A final washing with reagent grade acetone is carried out. Dry the diatomaceous earth for 4 hr. at 100°F. in a steam-heated oven, or spread thinly on trays and air-dry overnight.

Reagents—*Cupric Chloride Solution*—Dissolve accurately 10,000 g. of cupric chloride dihydrate (reagent grade) in enough absolute methanol (reagent grade) to make exactly 1 l.

10 M Sodium Hydroxide Solution—Dissolve cautiously 40.0 g. sodium hydroxide pellets (reagent grade) in enough purified water to make 100 ml.

Chloroform—Reagent grade.

Preparation of Tablets—Obtain the average weight of one tablet. Triturate three or four tablets to a fine powder in a glass mortar. Accurately weigh a portion of this powder, representing 95–120 mg. of ethambutol dihydrochloride, and transfer to a 250-ml. beaker. Add 2.0 ml. of purified water and agitate gently to dissolve the powder. The undissolved fillers can be ignored. Add 0.5 ml.

[1] Marketed as Myambutol by Lederle Laboratories, Division of American Cyanamid Co., Pearl River, NY 10965

[2] Celite 545, Johns-Manville Corp., New York, N. Y.

Table I—Colorimetric Determination of Potency of 100-mg. Ethambutol Dihydrochloride Tablets[a]

Tablet No.	Wt. of Sample Powder, mg.	Absorbance, 687 mμ	mg. Ethambutol Dihydrochloride/ Tablet
1	130.9	0.702	102.2
2	135.2	0.693	97.7
3	135.4	0.722	101.6
4	130.6	0.692	101.0
5	132.8	0.704	101.0
6	135.5	0.714	100.4
7	138.2	0.726	100.1
8	132.7	0.691	99.3
9	119.4	0.622	99.3
10	136.0	0.710	99.5
SD			1.3

[a] Average tablet wt. = 144.7 mg.

of 10 *M* sodium hydroxide solution to the mixture to convert the hydrochloride salt to the free base.

Procedure—Add 5 g. of the washed diatomaceous earth to the solution and mix thoroughly with a hard rubber spatula. Quantitatively transfer the wetted diatomaceous earth to the column and tamp lightly with the glass rod to insure a uniform mass. Elute the column with chloroform and collect the eluate to the mark in a 50-ml. volumetric flask to which 25.0 ml. of the cupric chloride solution was previously added. Prepare a blank column and elute in an identical manner using 2.0 ml. of purified water in place of the 2.0 ml. of ethambutol dihydrochloride sample solution. Read the absorbance of the sample solution *versus* the blank solution in 1-cm. cells at a wavelength of 687 mμ in a suitable analytical spectrophotometer. Calculate the amount of ethambutol dihydrochloride present in one average tablet with the use of the following expression[3]:

$$\frac{(\text{average tablet wt., mg.}) \times \text{absorbance (sample)} \times 10^3}{(\text{sample wt., mg.}) \times 7.59} = \text{mg. ethambutol dihydrochloride/average tablet}$$

DISCUSSION

The absorbance of the ethambutol-copper (II) complex is proportional to the concentration of ethambutol free base and hence obeys the Beer's law relationship. Figure 1 shows the visible absorption spectrum of the greenish-blue complex *versus* the pale green methanolic-chloroformic blank solution of cupric chloride dihydrate. The reproducibility of the analytical method is illustrated in Table I which lists assay results for ten 100-mg. ethambutol dihydrochloride tablets.

The effect of time on the color produced was studied by reading the absorbance of the greenish-blue solution at various time intervals. It was found that after 24 hr. the absorbance of the sample had not decreased.

[3] The value 7.59 is the product of the absorptivity (a) multiplied by the ratio of the dilution factor to a conversion factor. The conversion factor is the quotient of the molecular weight of ethambutol dihydrochloride divided by the molecular weight of the free base.

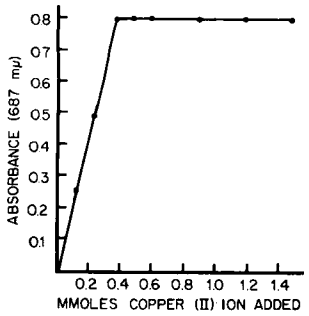


Figure 2—*Absorbance of ethambutol-copper (II) chelate at a constant ethambutol concentration of 3.61 × 10^{-1} mmole as a function of cupric ion concentration.*

A study was conducted at a constant ethambutol concentration to determine the molar ratio of ethambutol complexed by cupric ion. Into a series of volumetric flasks, each containing a chloroformic solution of 3.61 × 10^{-1} mmole ethambutol, increasing amounts of methanolic cupric chloride dihydrate solution were added. Each sample was brought to constant volume with methanol and read spectrophotometrically at 687 mμ *versus* a blank containing cupric ion at the same concentration as the sample. As seen in Fig. 2, the absorbance of the colored complex reaches a maximum and further addition of cupric ion does not increase absorbance. The point of maximum chelation was found to be equivalent to 3.61 × 10^{-1} mmole ethambutol and 3.52 × 10^{-1} mmole cupric ion. Assuming the reaction to be complete, the ratio of drug to cupric ion was in excellent agreement with the expected theoretical ratio of a 1:1 chelate.

The *l*-isomer of ethambutol dihydrochloride was indistinguishable from the *d*-isomer when subjected to the chromatographic separation and colorimetric assay.

Chromatographic separation of ethambutol free base was found to be more convenient than repeated extraction in separators. The columns are easy to prepare and allow for continuous elution directly into the flask containing reagent. In addition, a number of columns can be run at one time where extraction with separators is not feasible.

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