Table II—Analysis of Reserpine–Chlorothiazide in Known Mixture

Mixture	Added, mg	Amount Found[a], mg	Accuracy, %
Reserpine	0.125	0.120 ± 0.013[b]	3.8
Chlorothiazide	250.000	260.600 ± 4.886	4.2

[a] Based on five replicate determinations of known mixture. [b] Confidence limits at $p = 0.05$.

both drugs in the simulated dosage form were unsuccessful. Solvents such as acetonitrile and methanol solubilized both reserpine and chlorothiazide, but the ratio of chlorothiazide to reserpine was so large that the two components could not be resolved completely. This difficulty was resolved by the sequential use of the two solvents, chloroform and methanol, in the analytical scheme. Reserpine was very soluble in chloroform whereas chlorothiazide was essentially insoluble. Peak overlap of the two drugs was avoided, thus enabling determination of microgram quantities of reserpine in the presence of milligram concentrations of chlorothiazide. In addition, the volume of chloroform could be reduced, thus enhancing the detectability of reserpine in a more concentrated solution. Methanol was then added to solubilize the undissolved chlorothiazide and the analysis was completed.

The ratios of drug peak areas/internal standard peak areas (D/IS) were calculated for each drug. The constants (slope and intercept) for the linear regression equation shown in Table I were used to solve for drug concentration [D/IS = slope (concentration) + intercept]. The calculations were performed on a programmable calculator[7].

The data in Table II demonstrate the quantitative results obtained for the simulated dosage form. The utility of HPLC in the analysis of the reserpine–chlorothiazide mixture is clearly demonstrated, with an accuracy of 3–5% (14).

[7] Olivetti-Underwood programma 101.

REFERENCES

(1) I. L. Honigberg, J. T. Stewart, and A. P. Smith, *J. Pharm. Sci.*, **63**, 766(1974).

(2) I. L. Honigberg, J. T. Stewart, A. P. Smith, and D. W. Hester, *ibid.*, in press.

(3) T. Urbanyi and A. O'Connell, *Anal. Chem.*, **44**, 565(1972).

(4) R. P. Haycock, P. B. Sheth, and W. J. Mader, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 479(1959).

(5) E. B. Dechene, *ibid.*, **44**, 657(1955).

(6) C. R. Szalkowski and W. J. Mader, *ibid.*, **45**, 613(1956).

(7) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 587.

(8) R. Chu, *J. Ass. Offic. Anal. Chem.*, **54**, 603(1971).

(9) P. Kertesz, *Acta Pharm. Hung.*, **39**, 127(1969); through *Chem. Abstr.*, **71**, 33475c(1969).

(10) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 160.

(11) K. Weclawska, *Gdansk. Tow. Nauk. Rozpr. Wydz. 3*, **5**, 211(1968); through *Chem. Abstr.*, **71**, 128808(1969).

(12) J. F. Magalhaes and M. G. Prios, *Rev. Farm. Bioguim. Univ. Sao Paulo*, **8**, 273(1971); through *Chem. Abstr.*, **75**, 121466(1971).

(13) D. D. Phillips, *Org. Syn.*, coll. vol. IV, 313(1963).

(14) W. F. Pickering, "Modern Analytical Chemistry," Dekker, New York, N.Y., 1971, p. 38.

ACKNOWLEDGMENTS AND ADDRESSES

Received April 12, 1974, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication June 11, 1974.

The authors express their appreciation to Mr. Donald E. Maner for technical assistance.

[x] To whom inquiries should be directed.

Ion-Pair Extraction and Precipitation Methods for Ethambutol Determination

E. H. GIRGIS [x], Z. GAD, and M. MARAM

Abstract □ A sensitive method for determining ethambutol (20–100 μg) in aqueous solutions and tablets is described, using bromthymol blue as a complex-forming agent. Extraction of the complex in chloroform as well as in methylene chloride or ethylene dichloride is accomplished readily at an optimum pH of 7. A stoichiometric relationship of 1:2 between ethambutol and the acid dye is proved. Cycloserine, isoniazid, and sodium aminosalicylate do not interfere with the assay. The reineckate precipitation method for ethambutol determination is compared and its direineckate derivative is identified.

Keyphrases □ Ethambutol hydrochloride—analysis, ion-pair extraction and precipitation methods □ Ion-pair extraction—bromthymol blue–ethambutol complex, precipitation and analysis □ Acid-dye technique—ion-pair extraction of ethambutol–bromthymol blue complex, precipitation and analysis □ Bromthymol blue—ion-pair extraction of ethambutol and analysis

Oral administration of ethambutol hydrochloride, (+)-2,2′-(ethylenediimino)-di-1-butanol dihydrochloride, currently marketed as the dextro form, is used in the treatment of tuberculosis (1, 2). Colorimetric methods for determination of ethambutol as the copper complex (3, 4) and as the reineckate derivative (5) were reported previously. The chelation of ethambutol with copper required either a nonaqueous medium or a critical amount of excess alkali to produce a stable color with low absorptivity, and small amounts could not be determined with sufficient accuracy. In the present study the acid-dye technique formerly adopted for the microdetermination of various pharmaceutical amines (6–12) was applied to assay ethambutol and compared with the reineckate assay method after being optimized.

Table I—Effect of Different Solvents on Extraction of Ethambutol–Dye Complex at pH 7

Solvent	Absorbance (1 cm)[a]
Chloroform	0.63
Methylene chloride	0.63
Ethylene dichloride	0.63
Benzene	0.33
Carbon tetrachloride	0.11
Hexane	0.00

[a] Mean value of three experiments obtained from 5-ml aliquots of dilute ethambutol hydrochloride in 20 ml of solvent.

EXPERIMENTAL

Materials—The following were used: ethambutol, crystalline commercial product[1]; bromthymol blue[2]; chloroform; methylene chloride; ethylene dichloride; benzene; carbon tetrachloride; and hexane. All were BP or analytical reagent grade.

Reagents—The following were used: buffers, pH 2–10 (BP); solutions of bromthymol blue (6.4×10^{-4} *M*) and of dilute ethambutol hydrochloride (0.72×10^{-4} *M*) in the required buffers; solutions of ethambutol hydrochloride (6.4×10^{-4} *M*) in the buffers (pH 4.8 and 7); and a saturated solution of ammonium reineckate and a diluted 1% solution of the reagent, freshly prepared.

Preparation of Test Sample Solutions—Twenty ethambutol tablets were weighed and finely powdered. In the acid-dye assay, an aliquot of the powder, accurately weighed, was dissolved, filtered, and diluted with the pH 7 buffer to make approximately 20 μg of ethambutol/ml. In the reineckate assay, an aliquot of the powder, accurately weighed, was dissolved, filtered, and diluted with 0.1 *N* HCl to give approximately 1–2 mg of ethambutol/ml.

Acid-Dye Assay Procedure—The prepared test sample solution of ethambutol (3 ml, ~60 μg) was added to 4 ml of bromthymol blue in the pH 7 buffer contained in a 125-ml separator and diluted to 14 ml with the pH 7 buffer solution. The aqueous solution was shaken gently for 2 min with 25 ml of chloroform, and the separated chloroform layer was centrifuged. A blank experiment was carried out using 3 ml of the pH 7 buffer in place of the test sample solution. The absorbance of the yellow-colored extract was measured at 410 nm against chloroform from the blank experiment. The mean value of three experiments was taken, and the exact amount of ethambutol hydrochloride was plotted from a calibration curve.

Determination of Stoichiometric Relationship—Aliquots (3 ml) of variable proportions of ethambutol and bromthymol blue (6.4×10^{-4} *M*) in the buffered solutions (pH 4.8 or 7) were delivered into six dry separators and diluted with 4-ml aliquots of the pH 4.8 or 7 buffer. Each solution was shaken thoroughly for 2 min with 25 ml of chloroform. The separated organic phases were centrifuged and measured at 410 nm against chloroform from the blank experiment in the absence of ethambutol.

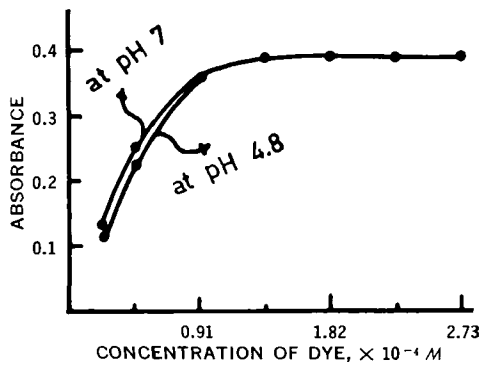

Figure 1—*Effect of dye concentration on color formation at pH 4.8 and 7 at fixed ethambutol hydrochloride concentration of 1.54 × 10⁻⁵ M.*

[1] Assayed by nonaqueous titration against standard perchloric acid in acetic acid to give 99.53% result.
[2] Merck.

Table II—Effect of Buffer Concentration on Extraction of Ethambutol–Dye Complex

Buffer Concentration, *M*	Absorbance (1 cm) of	
	Blank/Chloroform	Complex[a]/Blank
0.05	0.025	0.39
0.10	0.040	0.39
0.15	0.060	0.39
0.20	0.110	0.39

[a] Results obtained from 3-ml aliquots of dilute ethambutol solution at pH 7 in 20 ml of solvent.

Reineckate Assay Procedure—The prepared test sample solution of ethambutol (25 ml, ~50 mg) was precipitated by adding, with continuous stirring, 25 ml of saturated ammonium reineckate solution. The mixture was set aside for 30 min and then filtered through a previously dried and tared sintered-glass crucible (porosity 4). The precipitated reineckate was completely transferred onto the filter and washed successively with two 5-ml portions of the diluted reagent of ammonium reineckate and 5 ml of ice-cold water.

In the gravimetric determination, each gram of the dried precipitate at 105° is equivalent to 0.3292 g of ethambutol hydrochloride. For the colorimetric determination, the washed precipitate was dissolved in an appropriate volume of methanol and the absorbance was read at 525 nm. The exact amount of ethambutol hydrochloride was determined from a calibration curve.

RESULTS AND DISCUSSION

Ion-Pair Extraction of Ethambutol—Maximum extraction of the dye complex in chloroform was achieved at pH 4.8–7, and not more than gentle shaking was needed to extract the complex readily in chloroform at a pH value approximating 7. Table I demonstrates that methylene chloride and ethylene dichloride are equally effective in extracting the complex. A spectrophotometric scanning of the yellow color in the visible region showed a maximum absorbance at 410 nm, and the color was stable for 2 hr at 20 and 40°. An increase in buffer concentration from 0.05 to 0.2 *M* at pH 7 (Table II) did not influence extraction of the complex, although high blank values were produced with the higher buffer concentrations.

Effect of Dye Concentrations on Absorbance of Complex—Different dye concentrations were made from bromthymol blue (6.4×10^{-4} *M*) solutions in pH 4.8 or 7 buffer; 3-ml aliquots of the dilute ethambutol solution at the same pH were added to each and they were diluted to 14 ml with the buffer used. Extraction was

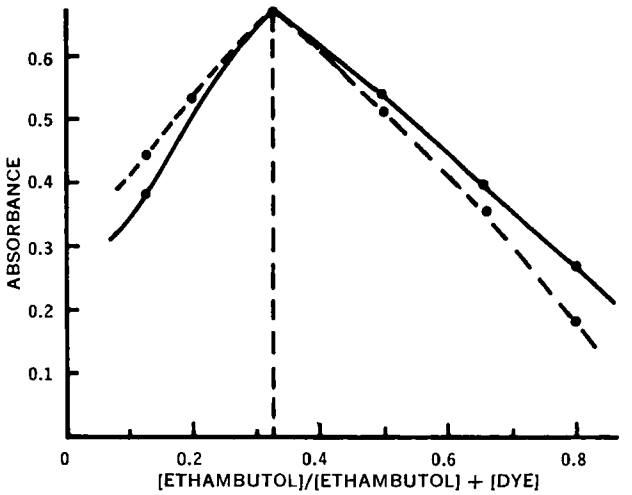

Figure 2—*Continuous variation curve obtained from solutions of ethambutol hydrochloride and bromthymol blue (6.4×10^{-4} M) at pH 4.8 (---) and 7 (——).*

Table III—Acid-Dye and Reineckate Assay Results of Ethambutol Hydrochloride Tablets (250 mg)

Acid-Dye Assay		Reineckate Assay			
		Gravimetric		Colorimetric	
Found per Tablet, mg	Found, %	Found per Tablet, mg	Found, %	Found per Tablet, mg	Found, %
252.15	100.86	252.00	100.80	253.22	101.28
253.82	101.53	252.00	100.80	253.22	101.28
253.82	101.53	259.00	103.60	253.22	101.28
253.82	101.53	251.95	100.78	250.00	100.00
250.00	100.00	250.85	100.34	250.00	100.00
250.00	100.00	—	—	—	—
Mean	100.90		101.26		100.73
% *SD*	±0.75		±1.30		±0.64

Table IV—Weight Ratios between Assayed Ethambutol Hydrochloride and Reineckate Derivatives

Weight of Ethambutol Hydrochloride, mg	Weight of Reineckate Derivative, mg, Produced by Adding Excess Reagent and Leaving for		Ratio between Ethambutol Hydrochloride and Reineckate Derivative
	30 min	60 min	
21.0	63.77	—	1:3.037
51.6	—	157.7	1:3.056
50.0	151.50	—	1:3.030
100.4	—	304.6	1:3.033

Table V—Reineckate Assay of Ethambutol Hydrochloride in Bulk Material

Sample, mg	Amount of Ethambutol Hydrochloride			
	Gravimetric		Colorimetric	
	Found, mg	Recovery, %	Found, mg	Recovery, %
40	40.10	100.25	40	100
50	49.90	99.80	50	100
60.3	60.60	100.49	60	99.5
100	100.08	100.08	100	100
101	100.70	99.70	101.2	100.19
	Average	100.064	Average	99.938
	% *SD*	±0.336	% *SD*	±0.24

then effected by thorough shaking with 20 ml of chloroform for 2 min, and absorbances of the yellow color were measured against their blanks. Figure 1 shows that a 1.82×10^{-4} *M* dye concentration is sufficient to bring maximum absorbance at a constant ethambutol hydrochloride concentration of 1.54×10^{-5} *M* in the aqueous phase.

Stoichiometric Balance—Figure 2 represents the stoichiometric relationship of 1:2 between ethambutol and the acid dye attributed to the presence of two basic centers in ethambutol.

Adherence to Beer's Law—The yellow complex of ethambutol hydrochloride (20–100 μg) in chloroform, assayed by the acid-dye method, showed adherence to Beer's law.

Acid-Dye Assay of Ethambutol Tablets—Comparison of the acid-dye results with those of the reineckate method for ethambutol tablets (Table III) indicates good precision, agreement between results, and no interference from tablet excipients (6). Microamounts of ethambutol could be determined by the acid-dye technique with a fairly rapid and simple procedure.

Analysis of Ethambutol in Presence of Cycloserine, Isoniazid, or Sodium Aminosalicylate—A preliminary experiment carried out by the acid-dye method on a 5-ml aliquot solution of cycloserine, isoniazid, or sodium aminosalicylate (600 μg) showed no formation of a colored complex in chloroform. Consequently, on assaying ethambutol (60 μg) in the presence of either former solution, no interferences occurred and ethambutol was easily and quantitatively extracted.

Reineckate Precipitation of Ethambutol—An acid solution of ethambutol was required for an optimum precipitation of ethambutol at room temperature by the reineckate reagent. With the unacidified solution, the precipitated reineckate was not easily filtered; with solutions made slightly ammoniacal, no precipitation occurred. The separated crystals of ethambutol reineckate from an acid solution at room temperature showed the same melting point (177–180° dec.) as crystals separated at 70°. Washed and dried crystals of ethambutol reineckate showed no loss in weight when heated for 3 hr at 105°.

Table IV illustrates that 1 g of ethambutol hydrochloride is equivalent to nearly 3.037 g of ethambutol reineckate, indicating a direineckate formation. Unlike most dibasic compounds (13), ethambutol yielded the direineckate derivative in an acid solution at 70°.

A comparison between the gravimetric and colorimetric reineckate assay results of ethambutol bulk material (Table V) indicated good precision and recovery within the range of 40–100 mg of the material.

UV Absorbance of Ethambutol Direineckate—The absorbance of an ethambutol reineckate methanolic solution (1.6 mg/100 ml) in the UV region showed two maxima at 235 and 310 nm, attributed to the reineckate moiety (13) and independent of ethambutol itself which had no absorption in this region. It was found that a methanolic solution of ethambutol reineckate containing 0.6–1.6 mg in 100 ml of methanol obeyed Beer's law at 310 nm.

CONCLUSION

A sensitive acid-dye technique is applied for assaying ethambutol (20–100 μg) in aqueous solutions and in tablets through the formation of a complex with bromthymol blue, which is extracted readily in chloroform at pH 7. The method is more sensitive and rapid than the reineckate method. Moreover, it is adequate for selective extraction of ethambutol in the presence of cycloserine, isoniazid, or sodium aminosalicylate. The reineckate precipitation of ethambutol is optimized and the direineckate derivative (mp 177–180°) is heat stable at 105° and shows, in methanolic solution, the two typical maxima in the UV region ascribed to the reineckate moiety.

REFERENCES

(1) J. P. Thomas, C. O. Baughn, R. G. Wilkinson, and R. G. Shepherd, *Amer. Rev. Resp. Dis.*, **83**, 89(1961).

(2) "Martindale—The Extra Pharmacopoeia," 25th ed., The Pharmaceutical Press, London, England, 1967, p. 1341.

(3) J. M. Burger, F. D. Pisano, and R. A. Nash, *J. Pharm. Sci.*, **58**, 110(1969).

(4) A. Doadrio and A. Garcia, *An. Real Acad. Farm.*, **34**, 291(1968); through *Chem. Abstr.*, **71**, 42395d(1969).

(5) P. Tarli, F. Lattanzi, and S. Benocci, *Farmaco, Ed. Prat.*, **24**, 707(1969); through *Chem. Abstr.*, **72**, 47405n(1970).

(6) V. D. Gupta and D. E. Cadwallader, *J. Pharm. Sci.*, **57**, 112(1968).

(7) V. D. Gupta and L. A. Luzzi, *Amer. J. Hosp. Pharm.*, **25**, 360(1968).

(8) V. D. Gupta and N. M. Ferguson, *ibid.*, **26**, 168(1969).

(9) *Ibid.*, **26**, 408(1969).
(10) W. N. French and B. A. Riedel, *Can. J. Pharm. Sci.*, **1**, 80(1966).
(11) W. N. French, F. Matsui, and I. F. Truelove, *ibid.*, **3**, 33(1968).
(12) J. R. Waston, F. Matsui, and W. N. French, *J. Pharm. Sci.*, **59**, 391(1970).
(13) L. Kum-Tatt, *J. Pharm. Pharmacol.*, **12**, 668(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 22, 1972, from the *Stability Research Department, The Nile Company for Pharmaceuticals and Chemical Industries, El-Ameria, Cairo, A.R.E.*

Accepted for publication June 11, 1974.

[x] To whom inquiries should be directed.

Indirect Nonaqueous Titration of Hydrochlorides of Nitrogenous Bases

SOBHI A. SOLIMAN [x], HASSAN ABDINE, and NASHAAT A. ZAKHARI

Abstract □ An indirect nonaqueous titrimetric method was devised for the determination of the hydrochlorides of nitrogenous bases. The chloride-ion interference was prevented without the use of a mercuric acetate reagent. The method depends on the treatment of a solution of the hydrochloride of the organic base with an excess of standard aceteous perchloric acid solution and the hydrogen chloride displaced is removed by boiling. Residual excess perchloric acid is determined by titration against the basic titrant sodium acetate in glacial acetic acid, using either potentiometric or visual end-point detection with a crystal violet indicator. The proposed method was applied successfully to the determination of several hydrochlorides of nitrogenous bases. The mean percent recoveries obtained indicate that the proposed method is equivalent in accuracy and precision to the nonaqueous titrimetric method most commonly used by official compendia for this determination. Potentiometric titrations showed that the point of the maximum inflection in the titration curves coincided with the appearance of the violet color of the indicator.

Keyphrases □ Nitrogenous bases, hydrochlorides—indirect nonaqueous titration, method compared to compendial methods □ Nonaqueous titration, indirect—hydrochlorides of nitrogenous bases, compared to compendial methods □ Titrations, indirect nonaqueous—hydrochlorides of nitrogenous bases, compared to compendial methods

Many titrimetric methods have been developed for the determination of nitrogenous bases in aqueous solutions, and some have been accepted by various compendia. However, titrations of the nitrogenous bases in nonaqueous media allow much more accurate results and are quite simple. The nitrogenous bases may be extracted from an aqueous solution by a nonpolar solvent and determined directly without further isolation.

In the titration of the hydrochlorides of the nitrogenous bases in nonaqueous media, perchloric acid displaces hydrochloric acid from its salt. In an acetic acid medium, hydrogen chloride is only a little less acidic than perchloric acid, which makes the displacement reaction not quantitative and renders the method unsuitable for quantitative determination. Therefore, in the titration of the hydrochlorides of nitrogenous bases, there must be some specific technique to avoid the difficulty arising from the presence of the chloride ion.

Some procedures have been suggested to overcome this difficulty. An extraction titration method (1) was developed to determine a number of alkaloid hydrochlorides. Some hydrochlorides of nitrogenous bases were determined (2) by the titration of their acidic components against standard sodium methoxide solution in anhydrous pyridine.

A limited number of the hydrochlorides of organic bases and some alkali metals and ammonium halides were titrated directly in boiling glacial acetic acid against aceteous perchloric acid solution, using different indicators (3). The titrated solution had to be boiled to remove the hydrogen halides displaced during titration. However, no attempt was made to obtain extremely accurate quantitative results; rather, information was sought as to how some anions may influence analytical results.

An ingenious method was developed (4) for determining the hydrochlorides of nitrogenous bases in which the halide interference was prevented by using excess mercuric acetate reagent. The investigators used standard perchloric acid in dioxane as the titrant, with either potentiometric or visual end-point detection. This method has proved to be efficient for eliminating halide-ion interference and, therefore, has been adopted by many official compendia for the determination of the hydrochlorides of nitrogenous bases. The method has also been applied to the determination of some alkaloid halides (5, 6), using 0.01 *N* perchloric acid solution as the titrant and crystal violet as the indicator, and the hydrochlorides of several antihistamines (7) by potentiometric titration against standard tosylic acid solution in chloroform. The hydrochlorides of some phenothiazine derivatives were also determined (8) either in acetone using a mercuric acetate reagent or in a mixture of hexane and acetone after extracting the base from an aqueous potassium hydroxide solution.

In the present investigation, an indirect nonaque-