Colorimetric Determination of Atropine, Homatropine, Scopolamine, and Their Derivatives by the Ferric Hydroxamate Method

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Abstract ☐ The purpose of this study was to develop a procedure for the quantitative analysis of the salts of the solanaceous alkaloids in the presence of their hydrolysis products. The salts taken were atropine sulfate, homatropine hydrobromide, homatropine methylbromide, scopolamine hydrobromide, and scopolamine methylbromide. The ferric hydroxamate procedure was used. The conditions necessary for a reproducible reaction were established; a procedure of sufficient sensitivity was developed which will allow unit dose analysis; and an analysis was developed which will permit the salts of the solanaceous alkaloids to be quantitatively analyzed in the presence of their degradation products.

Keyphrases ☐ Atropine, homatropine, scopolamine dosage forms—analysis ☐ Solanaceous alkaloids with degradation products—analysis ☐ Ferric hydroxamate method—solanaceous alkaloid analysis ☐ Colorimetric analysis—spectrophotometer

Atropine and its synthetic homolog homatropine are, respectively, the tropic acid and mandelic acid esters of the aminoalcohol tropine, whereas scopolamine is the tropic acid ester of scopine. The official assay methods for these drugs have inadequate sensitivity; a single assay sample for Scopolamine Hydrobromide Injection requires a minimum of 40 ml. of the solution, and at least 100 tablets are needed for the USP XVII assay of Atropine Sulfate Tablets. Another limitation of the compendial assays is that they do not discriminate between the intact ester drugs and their inactive hydrolysis products. The USP XVII assays (1) for Atropine Sulfate Injection, Homatropine Hydrobromide Ophthalmic Solution, and Scopolamine Hydrobromide Injection provide for chloroform extraction of the bases from ammoniacal solution followed by evaporation of the extracts and acidimetric titration. Chafetz and Daly (2) recently observed that tropine is extracted along with homatropine base in the USP XVII assay, invalidating it as a stability-indicating method. They proposed a selective method for homatropine; however, their method is inapplicable to tropic acid esters. Separative techniques have also been applied to the solanaceous alkaloids (3). This report describes a method which: (*a*) is generally applicable to the solanaceous alkaloids and their derivatives; (*b*) is stability indicating; and (*c*) affords adequate sensitivity for unit-dose analysis.

Hydroxylaminolysis of esters and formation of colored complexes of the hydroxamic acids so derived with ferric ion afford convenient means for determination of esters in the presence of their alcohol and acid constituents. Siegel *et al.* (4) used this reaction to follow the hydrolysis rates of methylphenidate. Vincent and Schwal (5) evaluated a ferric hydroxamate colorimetric procedure for the analysis of alkaloid drugs with ester and lactone functions such as the veratrum alkaloids, cocaine, aconitine, and pilocarpine. However, they reported that atropine, homatropine, and scopolamine gave only very faint colors. Kinetic studies (6–8) have shown that these compounds are easily hydrolyzed in alkali; hydroxylaminolysis of esters is a base-catalyzed reaction. The inference derived from these observations was that conditions wherein formation of the hydroxamate was facilitated and the rate of the competing ester saponification inhibited would afford colors intense enough to be analytically useful. Such conditions were attained by use of a large excess of hydroxylamine at ice-water bath temperature. The method was applied to atropine sulfate, homatropine hydrobromide, homatropine methylbromide, scopolamine hydrobromide, and scopolamine methylbromide. The potential utility of the method in the analysis of dosage forms of these compounds is indicated.

Table I—Absorbance Concentration Relationships

mg./5 ml.	Absorbance at 540 mμ: Atropine Sulfate	Homatropine Hydrobromide	Homatropine Methylbromide	Scopolamine Hydrobromide	Scopolamine Methylbromide
0.5	0.060	0.062	0.060	0.070	0.092
1.0	0.140	0.115	0.121	0.130	0.180
2.0	0.260	0.258	0.240	0.280	0.360
3.0	0.410	0.340	0.360	0.410	0.535
4.0	0.540	0.490	0.472	0.530	0.640
5.0	0.650	0.600	0.540	0.650	0.890

EXPERIMENTAL

Reagents and Supplies—Saturated hydroxylamine HCl (83 g. in 100 ml.), 10.5 *M* potassium hydroxide, 4 *M* hydrochloric acid, and 0.37 *M* ferric chloride in 0.1 *M* hydrochloric acid were filtered before use if necessary and stored under refrigeration. Absorbance data were obtained with 11.67-mm. diameter cylindrical cells in a Bausch and Lomb Spectronic 20 spectrometer.

All chemicals used were USP, NF, or analytical reagent grade.

Standard Preparation—Dissolve an accurately weighed amount of the appropriate reference standard in water, and dilute the solution quantitatively and stepwise to obtain a concentration of about 600 mcg./ml.

Assay Preparation—*Tablets*—Weigh and finely powder not less than 20 tablets. Extract a weighed amount with water, filter the extract if necessary, and dilute it quantitatively to represent a concentration of about 600 mcg./ml.

Solutions—If the solution is preserved with chlorobutanol or parabens, extract it with ether as described by Brochmann-Hanssen

et al. (9). Transfer an appropriate volume of the injection or ophthalmic solution to a volumetric flask and dilute it to represent about 600 mcg./ml.

Procedure—Transfer 5-ml. portions of the assay preparation, the standard preparation, and water to separate 50-ml. conical flasks chilled in an ice-water bath. Successively pipet 1 ml. of hydroxylamine HCl reagent and 1 ml. of potassium hydroxide solution into each flask, and mix. After 1 hr., add 2.0 ml. of 4 *M* hydrochloric acid (pH 1.2–1.4) and 1.0 ml. of ferric chloride reagent to each flask, mixing after each addition. Remove the flasks from the ice-water bath, allow the gas evolution to subside, and determine the absorbance of the assay and standard solutions *versus* the blank at the wavelength of maximum absorbance at about 540 mμ in a suitable spectrometer. Calculate the amount of drug, in milligrams, in the sample taken from the formula: $0.001\ C(A_U/A_S)$, in which C is the concentration in micrograms per milliliter of the standard preparation, and A_U and A_S are the absorbances of the solutions from the assay preparation and from the standard preparation, respectively.

RESULTS AND DISCUSSION

Color Intensity—The absorbance values found in trials of this procedure are presented in Table I, from which conformance to Beer's law is evident. According to Aksnes (10), the color results from a 1:1 complex of ferric ion and hydroxamic acid.

Since tropohydroxamic acid is formed by three of the compounds and mandelohydroxamic acid by the homatropine derivatives, the molar absorptivities for atropine and the scopolamine derivatives, on the one hand, and for the other two compounds, on the other, should be identical if ester saponification or other side reactions do not occur. It is evident that scopolamine methylbromide, although it has the highest molecular weight, is converted to the hydroxamate most efficiently. Since reference standards are reacted concomitantly, the differences in molar absorptivities are of little practical consequence. The molar absorptivities, ε, in liters/mole/centimeter for atropine sulfate, homatropine hydrobromide, homatropine methylbromide, scopolamine hydrobromide, and scopolamine methylbromide are, respectively, 420, 350, 385, 490, and 615. The color intensity obtained in this procedure is about 10 times that reported by Vincent and Schwal (5) for atropine, homatropine, and scopolamine, from which one may calculate ε-values of 41, 35, and 92, respectively. The increase in absorptivity effected by the procedure reported here affords a useful analytical method.

Two precautions must be taken to achieve a procedure that produces absorbance values conforming to Beer's law. First, the volume taken of the assay preparation must be constant (5 ml. in the procedure proposed). Second, the pH of the solution prior to the addition of the ferric chloride reagent must be adjusted to a value of 1.2–1.4.

The main advantage of this analysis, in addition to its sensitivity, is the fact that the analysis reaction is specific for esters and will not react with the degradation products of ester hydrolysis, the acids and aminoalcohols. To verify this, the procedure was performed on several of the degradation products which might be formed by the hydrolysis of the esters involved. Tropic acid, mandelic acid, tropine, and scopoline were tested alone in solution and in combinations. These solutions did not absorb any energy at 540 mμ.

Instrumental reproducibility, according to the manufacturers, will account for a relative error of 10% at a concentration of 0.5 mg./5 ml. However, by taking proportionate quantities of reaction solutions, unit-dose solutions containing concentrations of 0.5 mg./ml. (2.5 mg./5 ml.) can be analyzed at a relative instrumental error of 4%.

The procedure was also applied to a typical scopolamine hydrobromide injection formulation which contained 0.4 mg./ml. of scopolamine hydrobromide, mannitol, acetate buffer, and a non-ester preservative. A control was run which contained everything but the scopolamine hydrobromide, and no interference from the formulation was found. The lack of absorbance by the blank, while the absorbance of the formulation containing the scopolamine hydrobromide reflected the proper concentration of the compound, signifies that the procedure is applicable to the analysis of formulations containing esters and ester salts of solanaceous alkaloids.

CONCLUSION

A procedure that allows for the quantitative analysis of several of the salts of the solanaceous alkaloids was developed. The conditions necessary for reproducible reaction were established; a procedure of sufficient sensitivity was developed which will allow for unit-dose analysis; and an analysis was developed which will permit the salts of the solanaceous alkaloids to be selectively and quantitatively analyzed in the presence of their degradation products. Official USP and NF procedures leave much to be desired as far as analyzing for these compounds in the presence of their degradation products. It is hoped that the proposed procedure will offer a better means of analysis for the salts of the solanaceous alkaloids.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 53, 284, 577.

(2) L. Chafetz and R. E. Daly, *J. Pharm. Sci.*, **57**, 1977(1968).

(3) H. A. Lloyd, H. M. Fales, P. F. Highet, W. J. A. Vandenheuver, and J. C. Wildman, *J. Amer. Chem. Soc.*, **82**, 3791(1960).

(4) S. Siegel, L. Lachman, and L. J. Malspeis, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 431(1959).

(5) D. Vincent and H. Schwal, *Ann. Pharm. Franc.*, **19**, 6(1961).

(6) E. R. Garrett, *J. Amer. Chem. Soc.*, **79**, 1071(1957).

(7) P. Zvirblis, I. Socholitsky, and A. A. Kondritzer, *J. Amer. Pharm. Ass., Sci. Ed.*, **45**, 450(1956).

(8) J. L. Patel and A. P. Lemberger, *ibid.*, **47**, 878(1958).

(9) E. Brochmann-Hanssen, P. Schmid, and J. D. Benmaman, *J. Pharm. Sci.*, **54**, 783(1965).

(10) G. Aksnes, *Acta Chem. Scand.*, **11**, 710(1957).

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