# Unit-Dose Assay of Tropine Alkaloids and Their Synthetic Analogs

CAMELIA GOMAA and ALY TAHA ˣ

**Abstract** □ A charge-transfer spectrophotometric method was developed for unit-dose assay of the tropine alkaloids and some of their synthetic analogs. The high molar absorptivities of the charge-transfer bands of the alkaloids with iodine in ethylene dichloride resulted in improved recoveries and good precision, particularly at the low dose levels of pediatric and hypodermic tablets.

**Keyphrases** □ Tropine alkaloids and synthetic analogs—unit-dose analysis, charge-transfer UV spectrophotometry □ UV spectrophotometry—analysis, tropine alkaloids, and synthetic analogs, unit-dose sensitivity

Quantitative methods for microamounts of the solanaceous alkaloids are few, and those depending on simple and convenient direct measurement in the UV range are lacking. The absorption bands of the tropine alkaloids, which have molar absorptivities of less than 150 (1), are not suitable for analytical purposes. The low sensitivity of the direct UV assay precludes its adoption in unit-dose determinations where amounts of the alkaloids as small as 0.3 mg/single dose are handled (2).

Accordingly, various approaches to the single-dose analysis of the tropine alkaloids were reported (3–13) which do not include direct UV measurement. They include dye–complex methods (3, 4), colorimetry *via* nitration and reduction followed by diazotization and coupling (5, 6), colorimetry as ferric hydroxamates (7), fluorometry (8), indirect spectrophotometry *via* cerimetric oxidation to benzaldehyde (9), TLC with densitometry (10), and GLC procedures (11–13).

With the possible exception of the last approach, most procedures suffer from some drawbacks. Acid–dye methods were reported to yield erratic results (14) owing to extreme sensitivity to pH variations. The colorimetric method (5) is not specific and is quite cumbersome. The ferric hydroxamate procedure requires a prolonged reaction time at low temperature, the colors are not very stable, and, more significant, no great improvement in sensitivity is achieved since reported molar absorptivities are in the 350–600 range (7). Photodensitometry on thin-layer chromatograms gave low recoveries of hyoscyamine (10).

The purpose of the present study was to develop a UV spectrophotometric procedure, for the analysis of the tropine alkaloids and some of their synthetic analogs, of sufficient sensitivity to allow single-dose assay.

Recently, Taha *et al.* (15) reported that about a 50-fold increase in the values of molar absorptivities of the tropine alkaloids could be achieved *via* charge-transfer complex formation with iodine in chlorinated solvents. This method, being of sufficient sensitivity, was adopted in the analytical technique described here.

## EXPERIMENTAL[1]

**Material**—Pharmaceutical grade atropine base, atropine sulfate, scopolamine hydrobromide, hyoscyamine hydrobromide, homatropine hydrobromide, eucatropine hydrochloride, and *N*-butylscopolammonium bromide were utilized as working standards and in the construction of calibration curves.

Atropine sulfate tablets containing 0.3, 0.4, and 0.6 mg alkaloidal salt/100-mg tablet (prepared by direct compression using microcrystalline cellulose[2] as vehicle), atropine sulfate injection[3], tablets of total belladonna alkaloids as malates with phenobarbital[4], atropine sulfate ophthalmic solution[5], and *N*-butylscopolammonium bromide tablets[6] were subjected to the analytical procedure.

**Reagents**—*Iodine Solution ($10^{-3}$ M)*—Dissolve 25.5 mg resublimed iodine in spectrograde ethylene dichloride in a 100-ml volumetric flask to obtain a solution of 0.255 mg/ml. The solution is stable for 1 week at 4°.

*Buffer*—Prepare 0.2 *M*, pH 9.0 buffer, standardized against the glass electrode, by dissolving 34.8 g of dibasic potassium phosphate in 900 ml of water. Adjust to pH 9.0 and make to 1 liter with water.

**Standard Preparation**—*Alkaloidal Salts*—Dissolve an accurately weighed amount of the appropriate working standard in water and dilute the solution quantitatively and stepwise to obtain a concentration of the salt equivalent to 1.0 mg of base/ml. Pipet 1.0 ml of this solution into a 30-ml separator containing 5 ml of buffer. Extract with 10 ml of ethylene dichloride, passing the separated organic layer through 2 g of anhydrous sodium sulfate supported by glass wool in a small funnel. Collect the filtrate in a 10-ml volumetric flask, wash the filter with a few drops of ethylene dichloride, and dilute to volume with the same solvent.

*Atropine Base and N-Butylscopolammonium Bromide*—Dissolve an accurately weighed amount of the appropriate standard in ethylene dichloride and dilute to obtain a concentration of 0.1 mg/ml.

**Assay Preparation**—*Atropine Sulfate Tablets*—Place one powdered tablet, or its equivalent from a composite of 20 tablets, in 5 ml of buffer in a 30-ml separator. Extract with two 10-ml fractions of ethylene dichloride, passing the separated organic layers through the same 2 g of anhydrous sodium sulfate suitably supported in a small funnel. Wash the filter with about 3 ml of ethylene dichloride and collect the filtrate and washings in a 25-ml volumetric flask. Dilute to volume with the same solvent.

This procedure is also applicable to tablets of total belladonna alkaloids with phenobarbital.

*N-Butylscopolammonium Bromide Tablets*—Place one powdered tablet, or its equivalent from a composite of 20 tablets, in a 30-ml beaker. Extract with two 10-ml portions of warm (40–50°) ethylene dichloride. Filter the fractions through a small piece of cotton wool, wash the filter with a small amount of warm solvent, and collect the filtrate and washings in a 25-ml volumetric flask. Cool to room temperature and dilute to volume with ethylene dichloride.

*Atropine Sulfate Injection*—Proceed as directed under *Atropine Sulfate Tablets*, substituting 1.0 ml, or the measured contents of a single-dose container of the injection, for the tablets.

*Atropine Sulfate Ophthalmic Solution*—Dilute an appropriate volume of the solution in a volumetric flask so as to represent 0.5 mg/ml. Proceed as directed under *Atropine Sulfate Tablets*, substituting 1.0 ml of the diluted ophthalmic solution for the tablets.

[1] Spectra were made on Spectromom 203 UV-VIS spectrophotometer, Mom, Budapest, Hungary.
[2] Avicel, American Viscose Corp., Marcus Hook, Pa.
[3] El-Nile Co., Cairo, Egypt.
[4] Belladenal, Sandoz, Basel, Switzerland.
[5] Isopto Atropine, Alcon, Fort Worth, Tex.
[6] Buscopan, Boehringer Ingelheim, Germany.

Table I—Peak Position and Intensity in Two Solvents

| Compound | Ethylene Dichloride | | Chloroform | |
|---|---|---|---|---|
| | $\lambda_{max}$, nm | $\epsilon_{max}$[a] | $\lambda_{max}$, nm | $\epsilon_{max}$[a] |
| Atropine | 295 | 15,900 | 270 | 8,200 |
| | 375 | 6,200 | 410 | 2,900 |
| Scopolamine | 295 | 8,100 | 270 | 1000 |
| | 375 | 4,800 | — | — |
| *N*-Butylscopolammonium bromide | 280 | 33,500 | 280 | 44,200 |
| Homatropine | 295 | 10,500 | —[b] | — |
| | 375 | 4,700 | | |
| Eucatropine | 295 | 18,500 | —[b] | — |
| | 375 | 10,200 | | |
| Hyoscyamine hydrobromide | —[c] | — | 275 | 856 |
| Scopolamine hydrobromide | —[c] | — | 270 | 613 |
| Homatropine hydrobromide | —[c] | — | 275 | 783 |

[a] Based on molecular weight of free base. [b] Undetermined. [c] Salts are insoluble in ethylene dichloride.

**Procedure**—Pipet 1.0 ml of the standard preparation and a volume of the assay preparation equivalent to 0.05–0.15 mg of alkaloid into separate 10-ml volumetric flasks. Add 1.0 ml of iodine reagent solution to each flask and dilute to volume with ethylene dichloride. Determine the absorbance of the assay and standard solutions at the proper $\lambda_{max}$ (295 nm for atropine, scopolamine, homatropine, and eucatropine and 280 nm for *N*-butylscopolammonium bromide) *versus* a blank prepared from 1.0 ml of iodine reagent solution diluted to 10.0 ml with ethylene dichloride.

Calculate the amount of drug, in milligrams, in the sample taken from the formula: $(f)C(A_u/A_s)$, in which $f$ is a conversion factor derived from the ratio of the formula weight of the alkaloid salt to the molecular weight of its free base, $C$ is the concentration in milligrams per milliliter of the standard preparation, and $A_u$ and $A_s$ are the absorbances of the solutions from the assay preparation and the standard preparation, respectively.

**Ferric Hydroxamate Colorimetric Method**—The basic procedure of Feldman and Robb (7) was followed.

## RESULTS AND DISCUSSION

**Peak Position and Intensity**—In the preliminary report (15), which included the theoretical background of the method and the determination of the basic thermodynamic constants of the complexes, chloroform and carbon tetrachloride were examined as solvents for charge-transfer complex formation. The present investigation revealed the superior qualities of ethylene dichloride as the assay solvent. It has a favorable boiling point and a low cutoff point. More important, the dilution effects previously reported for chloroform and carbon tetrachloride (15), which resulted in a decrease in peak intensity, were not observed with ethylene dichloride. This finding was probably due to the decreased tendency of ethylene dichloride to form contact charge-transfer pairs (16) with the nitrogen of the alkaloid because of the lower ratio of chlorine to carbon atoms in its molecule.

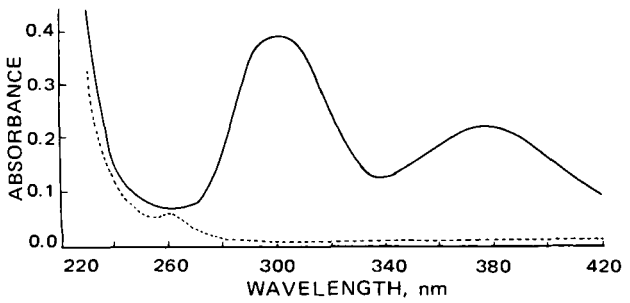


**Figure 1**—*Spectra of uncomplexed and iodine-complexed atropine in ethylene dichloride. Key: —, atropine (7 μg/ml) with iodine (25.5 μg/ml); and - - - -, atropine (70 μg/ml).*

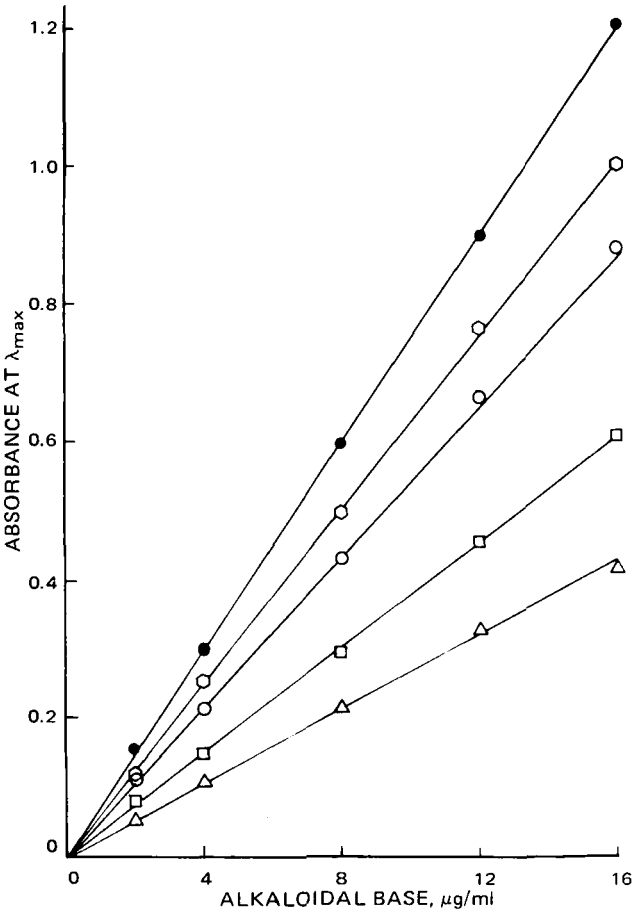


**Figure 2**—*Calibration curves of* N*-butylscopolammonium bromide (●), eucatropine (○), atropine (○), homatropine (□), and scopolamine (△). The $\lambda_{max}$ of* N*-butylscopolammonium bromide is at 280 nm; it is at 295 nm for all other compounds.*

Peak positions of the complexes in ethylene dichloride are also different from those in chloroform (Table I). The spectra of complexed and uncomplexed atropine are presented in Fig. 1 as typical examples.

**Linearity of Beer's Plots, Accuracy, and Precision**—Standard curves for the different compounds were constructed by plotting observed absorbance readings *versus* the concentration of the alkaloid base in micrograms per milliliter of the final dilution. The results are presented in Fig. 2; conformance to Beer's law is evident. The slopes of the curves, determined by the method of least squares, were 0.076, 0.0635, 0.055, 0.0375, and 0.027 for *N*-butylscopolammonium bromide, eucatropine, atropine, homatropine, and scopolamine, respectively (Fig. 2). The slopes may be utilized for computation of unknown alkaloid concentration by applying the relation:

$$\text{concentration of base } (\mu g/ml) = A/\alpha \qquad \text{(Eq. 1)}$$

where $A$ = absorbance of the solution from the assay preparation, and $\alpha$ = slope of the calibration curve of the corresponding alkaloid.

The validity of Eq. 1 was tested by taking 10 standards of atropine and of scopolamine through the procedure. As low as 0.01 mg of atropine and 0.02 mg of scopolamine could be determined, with recoveries of 99.4 and 98.7% and coefficients of variation in the assays of 1.14 and 2.13%, respectively.

**Application to Dosage Forms**—The charge-transfer method was applied to specially prepared lots of atropine sulfate tablets, conforming to the specifications of the USP XVIII (2), at three dose levels of 0.3, 0.4, and 0.6 mg of alkaloid salt/100-mg tablet. A tablet composite from each lot was assayed by the charge-transfer

Table II—Comparison of Two Assay Methods for Atropine Sulfate

| Tablet, mg | Charge Transfer | | | Ferric Hydroxamate | | |
|---|---|---|---|---|---|---|
| | Found[a], mg | Recovery, % | SD, ±% | Found[a], mg | Recovery, % | SD, ±% |
| 0.3 | 0.308 | 102.6 | 2.83 | 0.263 | 87.6 | 7.19 |
| 0.4 | 0.406 | 101.5 | 2.24 | 0.369 | 92.3 | 5.30 |
| 0.6 | 0.593 | 98.8 | 1.59 | 0.576 | 96.0 | 6.34 |

[a] Average of six determinations of tablet composite.

and ferric hydroxamate methods (Table II). Placebos were utilized in the preparation of blanks.

Data of Table II suggest that the presented spectrophotometric method could be applied to assay individual dose units of the tropine alkaloids with good accuracy and precision. The poorer recoveries of the colorimetric method reflect differences in molar absorptivities of the absorbing chromogens in the two methods, *e.g.*, $\epsilon$ of 15,900 for the charge-transfer band of the atropine–iodine complex compared to $\epsilon$ of 420 for the ferric hydroxamate chromogen reported for atropine sulfate (7).

The applicability of the procedure to commercial dosage forms and to content uniformity determination was checked (Table III). Since placebos of commercial products were not available, the method of standard addition was adopted. The results of Table III further confirm the suitability of the charge-transfer spectrophotometric method for control analysis and unit-dose assay of the tropine alkaloids and some of their synthetic analogs.

**Comments on Procedure**—The charge-transfer method was unaffected by the presence of phenobarbital, commonly prescribed with the belladonna alkaloids. The single partition at the alkaline pH of the procedure effectively separated the acidic barbiturate from the basic alkaloids.

Chlorobutanol and esters of *p*-hydroxybenzoic acid, frequently present as preservatives in ophthalmic solutions, are known to interfere in the ferric hydroxamate method (7, 17). No interference of these compounds could be observed in the presented procedure.

However, the charge-transfer method must be considered nonspecific with regard to differentiation between members of the tropine alkaloids, and it is not directly applicable as a stability-indicating assay since tropine, the major product of degradation of the belladonna alkaloids, was found to give a strong charge-transfer band.

Table III—Assay of Commercial Dosage Forms

| Preparation | Label Claim | Found[a], % of Label Claim | Standard | |
|---|---|---|---|---|
| | | | Added[b] | Recovered[c], % |
| Tablets of total alkaloids with phenobarbital | 0.25 mg/tablet | 104.8<br>106.5<br>97.6 | 0.25 mg/tablet | 98.2 |
| Atropine sulfate injection | 1.0 mg/ampul | 101.6<br>102.0<br>101.3 | — | — |
| Atropine sulfate ophthalmic solution | 0.50% | 97.5[c] | 0.25% | 101.5 |
| *N*-Butylscopolammonium bromide tablets | 10.0 mg/tablet | 100.8<br>96.6<br>99.1 | 5.0 mg/tablet | 99.3 |

[a] Single-dose determination. [b] Added to tablet composite or ophthalmic solution. [c] Average of three determinations.

These shortcomings do not affect the utility of the method in routine analysis and content uniformity determination of singly prescribed alkaloids. The drawbacks may also be overcome by coupling the charge-transfer method to a separation procedure reported for the tropine alkaloids such as paper chromatography (18, 19), partition column chromatography (5, 6, 20), countercurrent distribution (21), or TLC (22–24). The enhanced sensitivity of the method allows for additional handling without fear of increasing error.

## REFERENCES


(1) "Isolation and Identification of Drugs," E. G. C. Clarke, Ed., The Pharmaceutical Press, London, England, 1969.

(2) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 56–58.

(3) V. Das Gupta and N. M. Ferguson, *Amer. J. Hosp. Pharm.*, **26,** 168(1969).

(4) S. El-Masry and S. Khalil, *J. Pharm. Sci.*, **62,** 1332(1973).

(5) S. A. Koch, J. Levine, and N. Zenker, *ibid.*, **54,** 1046(1965).

(6) A. Bracey and G. Selzer, *ibid.*, **57,** 464(1968).

(7) J. A. Feldman and B. J. Robb, *ibid.*, **59,** 1646(1970).

(8) L. A. Roberts, *ibid.*, **58,** 1015(1969).

(9) F. F. Cantwell, M. Domjan, and C. F. Hiskey, *ibid.*, **63,** 599(1974).

(10) B. L. W. Chu, E. S. Mika, M. J. Solomon, and F. A. Crane, *ibid.*, **58,** 1073(1969).

(11) M. J. Solomon, F. A. Crane, B. L. W. Chu, and E. S. Mika, *ibid.*, **58,** 264(1969).

(12) R. O. Zimmerer, Jr., and L. T. Grady, *ibid.*, **59,** 87(1970).

(13) L. T. Grady and R. O. Zimmerer, Jr., *ibid.*, **59,** 1324(1970).

(14) D. Spiegel, S. Ahuja, and F. R. Brofazi, *ibid.*, **61,** 1630(1972).

(15) A. M. Taha, A. K. S. Ahmad, C. S. Gomaa, and H. El-Fatatry, *ibid.*, **63,** 1853(1974).

(16) K. M. C. Davis and M. F. Farmer, *J. Chem. Soc. B*, **1967,** 28.

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

(18) J. Reichelt, *Pharmazie*, **9,** 968(1954).

(19) M. Sterescŭ and N. Popovici, *ibid.*, **14,** 313(1959).

(20) G. Schill and A. Agren, *Sv. Farm. Tidskr.*, **56,** 55(1952); through *Chem. Abstr.*, **46,** 6324b(1952).

(21) H. Brauniger and G. Borgwordt, *Pharmazie*, **10,** 591(1955).

(22) F. Wartman-Hafner, *Pharm. Acta Helv.*, **41,** 406(1966); through *Chem. Abstr.*, **65,** 19925g(1966).

(23) J. Polesuk and T. S. Ma, *Mikrochim. Acta*, **1970** (4), 670; through *Anal. Abstr.*, **21,** 1441(1971).

(24) W. Debska and S. Czyzewska, *Farm. Pol.*, **27,** 365(1971); through *Anal. Abstr.*, **23,** 1819(1972).


## ACKNOWLEDGMENTS AND ADDRESSES


Received October 30, 1974, from the *Faculty of Pharmacy, University of Assiut, Assiut, Egypt.*

Accepted for publication December 18, 1974.

The authors thank Dr. A. Sakr for the preparation of atropine sulfate tablets.



[x] To whom inquiries should be directed.