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Microdetermination of Hyoscyamine and Scopolamine in Mixtures

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Abstract □ Following the acid-dye technique, hyoscyamine (or atropine) could be selectively determined in the presence of scopolamine, using bromcresol purple at pH 6.6. Total alkaloids were determined using bromthymol blue at pH 5.6. By referring to calibration curves of the two alkaloids with the appropriate dye, the concentration of each alkaloid in the mixture could be computed. The suggested procedure was adopted to analyze the two alkaloids in synthetic mixtures. As low as 0.05 mg. of each alkaloid could be estimated, with average percentage recoveries of 98.6 and 101.5 for hyoscyamine and scopolamine, respectively. Tincture of belladonna was assayed following a modification of the suggested method. The results obtained were comparable with those obtained following the BP method.

Keyphrases □ Hyoscyamine mixtures with scopolamine—acid-dye analysis without prior separation, effect of pH and dye type □ Scopolamine mixtures with hyoscyamine—acid-dye analysis without prior separation, effect of pH and dye type □ Tropane alkaloid mixtures—acid-dye analysis of hyoscyamine and scopolamine □ Acid-dye technique—analysis of hyoscyamine and scopolamine in mixtures

Most reported methods for the determination of tropane alkaloids present in mixtures necessitate separation of the individual alkaloids (1–3). The acid-dye technique reported for the estimation of microquantities of atropine (4, 5) can be adopted to determine the individual alkaloids, without preliminary separation, if a proper choice is made regarding the dye used and the pH of the medium. Khalil and El-Masry (6) found that tropine did not interfere in the assay of atropine when using bromthymol blue at pH 3. The present article examines the effects of pH and selection of the dye on the assay of microquantities of hyoscyamine and scopolamine without prior separation.

EXPERIMENTAL

Materials—Both hyoscyamine sulfate[1] and scopolamine hydrobromide[1] were BP quality. For the dye solutions, bromcresol purple and bromthymol blue were separately dissolved in chloroform to produce 2×10^{-4} *M* solutions. For the buffer solutions, McIlvaine's buffer solutions of various pH values (3–7.5 ± 0.05) were used. Chloroform was reagent grade[1] and freshly distilled. Two lots[2] of belladonna tincture BP were used.

Method—The procedure described by Khalil and El-Masry (6) was followed. Into a 50-ml. separator containing 10 ml. of the buffer solution, a volume of the sample corresponding to about 0.05–0.1 mg. of either alkaloid was added. Then 10 ml. of the chloroformic dye solution was added. After manual shaking for 1 min., the layers were left for complete separation and the chloroformic layer was transferred into a 25-ml. volumetric flask. The aqueous layer was further extracted with 10 and 5 ml. of chloroform, and the color intensity of the combined chloroform extracts was measured at 420 nm. using a spectrophotometer[3] and a blank similarly prepared.

RESULTS AND DISCUSSION

Figure 1 shows the effect of pH on the extractability of the alkaloid–bromcresol purple complex. At all pH values studied, the hyoscyamine–dye complex gave comparatively higher absorbance values at 420 nm. compared with scopolamine. The latter was not assayable at pH values higher than 6.6 since the chloroformic phase was colorless. Due to the relatively high absorbance readings obtained at pH 6.6, hyoscyamine was thus selectively determined at this pH using bromcresol purple. A linear relationship was obtained over the concentration range used (Fig. 2). When using bromthymol blue, both alkaloids were complexed at all pH values studied. However, the calibration curve for hyoscyamine using bromthymol blue at pH 5.6 and that with bromcresol purple at pH 6.6 were almost identical. The slopes were 2.564 and 2.578, respectively (Fig. 2). Therefore, to simplify calculation, pH 5.6 was chosen for the

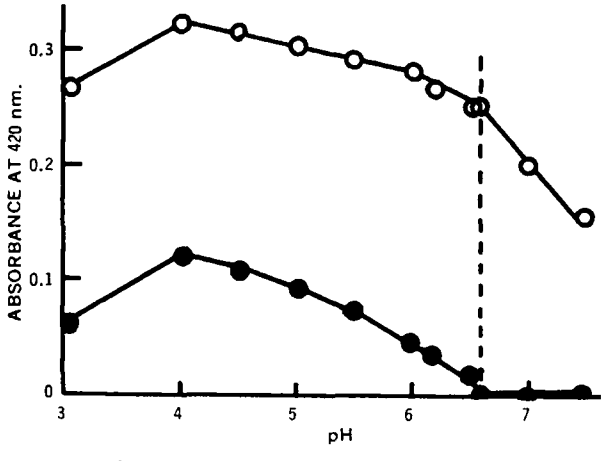

Figure 1—*Effect of pH on extraction of the alkaloid–bromcresol purple complex. Key: ○, hyoscyamine base; and ●, scopolamine base. The amount of the alkaloidal base used was 0.1 mg.*

[1] British Drug Houses Ltd., Poole, England.
[2] William Ransom & Son Ltd., Hitchen, Hartfordshire, England.
[3] Unicam SP 500.

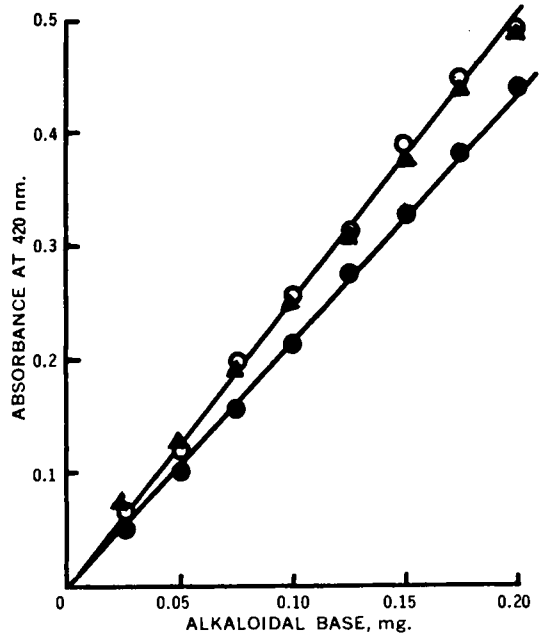

Figure 2—*Calibration curves of hyoscyamine (▲) and scopolamine (●) using bromthymol blue at pH 5.6 and of hyoscyamine (○) using bromcresol purple at pH 6.6.*

analysis of both alkaloids using bromthymol blue. From the slopes of the calibration curves shown in Fig. 2 for the two alkaloids, and since the absorbance values obtained for hyoscyamine complex were almost identical when using either dye, the following equations can be derived:

$$\text{milligrams hyoscyamine in mixture} = A/2.57 \quad \text{(Eq. 1)}$$

$$\text{milligrams scopolamine in mixture} = B - (A/2.18) \quad \text{(Eq. 2)}$$

where:

A = absorbance at 420 nm. of hyoscyamine-bromcresol purple complex at pH 6.6

B = absorbance at 420 nm. of complexes of both alkaloids with bromthymol blue at pH 5.6; 2.57 and 2.18 are the slopes of the curves (Fig. 2) for hyoscyamine and scopolamine, respectively, as determined by the method of least squares

The validity of Eqs. 1 and 2 was tested by assaying synthetic mixtures containing known amounts of hyoscyamine sulfate and scopolamine hydrobromide (Table I). As low as 0.05 mg. of either alkaloid could be determined, with percentage recoveries of 98.6 and 101.5 for hyoscyamine and scopolamine, respectively.

Determination of Hyoscyamine Content of Tincture of Belladonna—An improved procedure, suitable for the determination of hyoscyamine content in tincture of belladonna, was found. About 0.1–0.2 ml. of the tincture was added to a 50-ml. glass-stoppered separator containing 10 ml. of buffer solution (pH 6.6). A 10-ml.

Table I—Results of Analysis of Synthetic Mixtures of Hyoscyamine and Scopolamine[a]

Mixture Number	Compositions, mg. %[b] Hyoscyamine	Scopolamine	Found, mg. % Hyoscyamine	Found, mg. % Scopolamine
1	0	75	0	74.30
2	5	50	4.93	49.29
3	20	20	19.60	19.71
4	40	15	40.81	15.21
5	60	10	59.30	9.89
6	80	5	80.72	5.05
7	100	5	98.84	5.10
8	100	0	99.11	0

[a] Average of four replicates (±3%). [b] As alkaloidal base.

Table II—Results of Analysis of Two Batches of Belladonna Tincture BP by the BP Procedure and the Suggested Method

Batch	BP Method[a], mg. %	Suggested Method, Hyoscyamine, mg. %
A	30.1	29.6
B	28.8	28.2

[a] Total alkaloids calculated as hyoscyamine.

portion of chloroformic solution of bromcresol purple was added, and the mixture was manually shaken for 1 min. and left for complete separation (15 min.). After separation of the chloroformic phase, the aqueous layer was further extracted with 10 and 5 ml. of chloroform. The combined chloroformic extracts were treated with 0.1 *N* NaOH, and the liberated dye was measured at 580 nm. In this way, sensitivity was increased by a factor of about 2.4 (Fig. 3). Interference due to chlorophyll was also eliminated through the measurement of the violet color of the liberated dye. Tropine, one of the hydrolytic products of hyoscyamine, did not interfere in the assay following the above-mentioned procedure. This is an advantage over the procedure of Durick *et al.* (7), who used bromcresol purple at pH 5.3. Under this condition, it was found that tropine (6) and scopolamine (Fig. 1) interfered.

Two batches of belladonna tincture BP were assayed following the suggested method. The results obtained are compared in Table II with the data obtained following the BP procedure for the assay of belladonna tincture. The hyoscyamine contents of both batches, as determined by the suggested method, are in good agreement with the results obtained following the BP procedure for the total alkaloidal content. The application of Eqs. 1 and 2 failed to show any scopolamine in the tinctures, apparently because of the relatively minute amounts of scopolamine in the two tinctures studied; TLC testing[4] of the tinctures revealed no spots corresponding to scopolamine. The suggested method for the determination of hyoscyamine content in tincture of belladonna couples both simplicity and sensitivity, and only a small volume (0.1–0.2 ml.) is required.

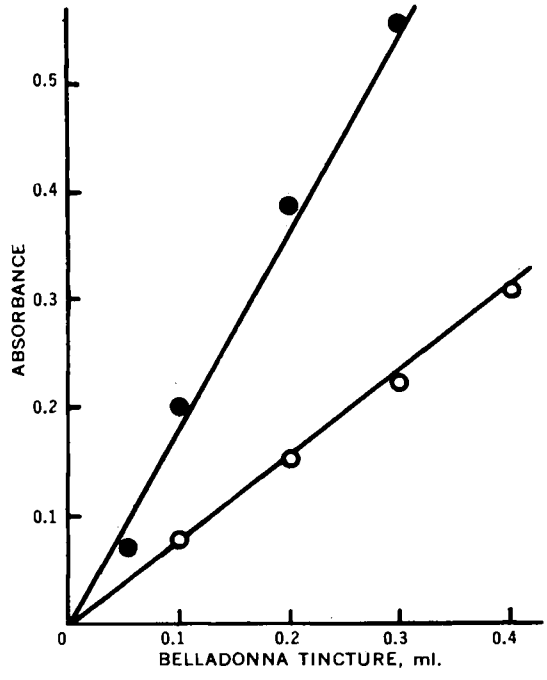

Figure 3—*Calibration curves of belladonna tincture (Batch A): (○) absorbance at 420 nm. of the yellow complex, and (●) absorbance at 580 nm. of the liberated dye after treatment with 0.1 N NaOH. The dye used was bromcresol purple at pH 6.6.*

[4] Using silica gel H as the adsorbent and a system of methanol (100 ml.) and ammonia 0.88 (1 ml.).

SUMMARY

1. Bromcresol blue at pH 6.6 forms a chloroform-extractable complex with hyoscyamine (atropine); tropine and scopolamine do not interfere.

2. Both hyoscyamine and scopolamine form chloroform-extractable complexes using bromthymol blue at pH 5.6. In this way, mixtures of both alkaloids can be determined without preliminary separation.

3. The hyoscyamine content in belladonna tincture can be accurately and selectively determined using as little as 0.1–0.2 ml. of the tincture. Bromcresol purple is used at pH 6.6 and the complex formed is treated with 0.1 *N* NaOH and measured at 580 nm. Neither tropine nor scopolamine interferes.

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NMR Quantitative Analysis of Quinidine in Mixtures of Quinidine and Hydroquinidine

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Abstract □ A new procedure for the rapid quantitative analysis of quinidine in mixtures of quinidine and hydroquinidine is described. The method is based upon measurement of the NMR spectrum of these compounds in deuterated chloroform, using 2,3,5-tribromothiophene as an internal standard. The signal chosen is from the vinyl group of quinidine. The great advantage of this procedure is that it permits the determination of the percentage of hydroquinidine in commercial bulk quinidine.

Keyphrases □ Quinidine in mixtures with hydroquinidine—NMR analysis □ Hydroquinidine as impurity in quinidine bulk—NMR analysis □ NMR spectroscopy—analysis, quinidine and hydroquinidine mixtures

Commercial bulk quinidine, the base and its salts, contains variable amounts of hydroquinidine. The USP identification test for quinidine by TLC (1) does not separate these two cinchona alkaloids (2). Many analytical procedures for quinidine have been reported, including UV spectrophotometric (3, 4), colorimetric (5), fluorometric (6), titrimetric (1), and gravimetric (7, 8) determinations. All of these procedures determine the total mixture of alkaloids: quinidine and hydroquinidine. Work in this laboratory indicates that the UV and visible absorptivity and the quantum efficiency of fluorescence are the same for quinidine and hydroquinidine.

A method for their separation and identification by TLC was described previously (2, 9). NMR spectroscopy affords a suitable method of distinguishing between the $-CH{=}CH_2$ and $-CH_2{-}CH_3$ groups present in quinidine and hydroquinidine, respectively. The quinidine spectrum (10) in deuterated chloroform possesses signals that are absent in the hydroquinidine spectrum and are thus available for use in quantitative analysis. A specific NMR quantitative analytical method for quinidine is described here.

EXPERIMENTAL

Spectra were obtained at 60 MHz. using an analytical NMR spectrometer[1]. All spectra were taken at 8 Hz., and a chart width of 72 Hz. was used for all integrals. Tetramethylsilane in deuterated chloroform[2] was used as an internal reference to measure chemical shifts.

Assay Procedure—Pure quinidine base was obtained from quinidine sulfate[3] by eliminating hydroquinidine by the method of Thron and Dirscherl (11).

Approximately 60 mg. of quinidine and 65 mg. of the internal reference 2,3,5-tribromothiophene[4] were accurately weighed and dissolved in a volumetric flask of 1 ml. of deuterated chloroform containing tetramethylsilane. The NMR spectrum was obtained and integrated five times through the region of interest. The mean of the integrated values was used for quantitative calculations.

Calculation—

$$W_{quin} = \frac{EW_{quin}}{EW_{std}} \times \frac{A_{quin}}{A_{std}} \times W_{std} \times F \qquad \text{(Eq. 1)}$$

where:

W_{quin} = weight of quinidine in milligrams
EW_{quin} = molecular weight of quinidine divided by the number of protons in the signal chosen, 324.45/1 = 324.45
EW_{std} = molecular weight of standard divided by the number of protons in the signal chosen, 320.84/1 = 320.84
A_{quin} = integral value of quinidine signal at 5.16 p.p.m.
A_{std} = integral value of standard signal at 6.93 p.p.m.

[1] Perkin-Elmer R-12B.
[2] Stohler Isotope Chemicals, Rutherford, N. J.
[3] Aldrich Chemical Co., Inc., Montréal, Québec, Canada.
[4] K & K Laboratories, Plainview, N. Y.