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ACKNOWLEDGMENTS AND ADDRESSES

Received January 3, 1975, from *CSIRO, Division of Chemical Physics, P.O. Box 160, Clayton, Victoria 3168, Australia.*

Accepted for publication July 1, 1975.

The author is grateful to the following companies for providing samples: Ciba Laboratories (guanethidine and the morpholino compound) and Pfizer Ltd. (guanoclor). The help of M. E. Hughes, J. L. Little, and R. I. Willing in recording spectra is also gratefully acknowledged.

Determination of Hyoscyamine in BPC Mixtures

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Abstract □ The hyoscyamine contents of four BPC mixtures (containing either belladonna or hyoscyamus tincture) were determined using the acid-dye technique. A sample size of 10 ml was required. The mean percentage recovery of hyoscyamine ranged from 99.73 to 101.03 from three mixtures; from the magnesium trisilicate and belladonna mixture, it was 94.8. The effects of pH and adsorption on the extraction of the alkaloid–dye complex from the mixtures examined are discussed.

Keyphrases □ Hyoscyamine—colorimetric determination using acid-dye technique, mixtures containing belladonna or hyoscyamus tincture □ Colorimetry—determination, hyoscyamine in mixtures, acid-dye technique

The current edition of the British Pharmaceutical Codex (BPC) lacks a quantitative method for the estimation of hyoscyamine content in mixtures containing either belladonna or hyoscyamus tincture. Instead, a TLC method is specified for testing the presence of tropane alkaloids in these mixtures (1). Recently, a colorimetric method based on the acid-dye technique was suggested for the microdetermination of hyoscyamine and scopolamine in synthetic mixtures (2).

The present work reports the suitability of the suggested method for the determination of hyoscyamine content in some BPC mixtures. The following mixtures were examined: aluminum hydroxide and belladonna; belladonna mixture, pediatric; magnesium trisilicate and belladonna; and potassium citrate and hyoscyamus.

EXPERIMENTAL

Materials—The materials used in preparation of the BPC mixtures were of either BP or BPC grade[1]. Belladonna and hyoscyamus tinctures BP[2], bromcresol purple[1], and reagent grade chloroform[1] were used.

Procedures—Hyoscyamine determination was carried out on the freshly prepared mixtures as follows:

Aluminum Hydroxide and Belladonna Mixture—Measure 10 ml of the mixture, add 10 ml of water (used to rinse the pipet), and centrifuge the diluted mixture for 3 min at 4000 rpm. To a 5-ml aliquot of the clear supernate, add 10 ml of McIlvaine buffer solution (pH 6.60 ± 0.05) and 10 ml of chloroform solution of bromcresol purple (4×10^{-4} *M*). Shake the mixture for 1 min and allow the layers to separate for 10 min.

Separate the chloroform layer containing the hyoscyamine–dye complex and reextract the aqueous layer with 3 × 10 ml of chloroform. To the combined chloroform extracts, add 15 ml of 0.1 *N* NaOH and shake to liberate the combined dye. Dilute the aqueous phase to 25 ml with 0.1 *N* NaOH and measure the absorbance at 580 nm[3].

Carry out a standard run under the same conditions using 0.25 ml of the standard hyoscyamine sulfate solution in 70% alcohol (containing the equivalent of 30 mg % of hyoscyamine base). Compare using a blank similarly prepared.

Belladonna Mixture, Pediatric—Measure 10 ml of the mixture and add about 0.5 ml of 0.1 *N* NaOH to adjust the pH to 6.6. Follow the procedure for the aluminum hydroxide and belladonna mixture, starting from "... add 10 ml of McIlvaine buffer solution...." Use 0.3 ml of the standard hyoscyamine sulfate solution containing the equivalent of 30 mg % of hyoscyamine base.

Potassium Citrate and Hyoscyamus Mixture—Measure 10 ml of the mixture, add 10 ml of water and 5 ml of ammonia T.S., and extract with 4 × 10 ml of chloroform. Evaporate the combined chloroform extracts and dissolve the residue in the 10 ml of McIlvaine buffer solution used in the assay. Follow the procedure for the aluminum hydroxide and belladonna mixture; use the 3 × 10-ml chloroform aliquots to rinse the container previously washed with the buffer.

For the standard run, use 2 ml of the standard hyoscyamine sulfate solution in 70% alcohol (containing the equivalent of 5 mg % of hyoscyamine base).

Magnesium Trisilicate and Belladonna Mixture—Measure 10 ml of the mixture, add 40 ml of 0.5 *N* HCl, and heat at 50° for 30 min with shaking. Cool and then centrifuge for 3 min at 4000 rpm. To a 10-ml aliquot of the supernate, add a few drops of 0.1 *N* NaOH to adjust the pH to 6.6. Follow the procedure for the aluminum hydroxide and belladonna mixture, starting from "... add 10 ml of McIlvaine buffer solution ...." Use 0.1 ml of the standard

[1] British Drug Houses Ltd., Poole, England.
[2] William Ransom and Son Ltd., Hitchin, Hertfordshire, England.
[3] Unicam SP 500 Series 2.

Table I—Results of Replicate Determinations of Hyoscyamine in Four BPC Mixtures by the Acid-Dye Technique Using Bromcresol Purple at pH 6.6

Number of Runs	Hyoscyamine Recovery, % in Mixtures			
	Aluminum Hydroxide and Belladonna	Belladonna, Pediatric	Potassium Citrate and Hyoscyamus	Magnesium Trisilicate and Belladonna
1	98.2	98.9	100.5	95.4
2	98.8	101.9	98.7	94.8
3	101.1	100.8	102.8	95.7
4	99.0	99.7	99.3	94.3
5	98.5	102.4	101.8	95.6
6	100.7	101.8	101.2	93.5
7	98.4	101.7	99.0	94.1
8	101.9	99.6	98.9	95.4
9	99.1	99.7	101.9	93.7
10	101.6	102.8	101.2	95.5
Mean	99.73	101.03	100.53	94.8
SD, %	1.43	1.46	1.47	0.84
CV, %	1.43	1.45	1.46	0.88

hyoscyamine sulfate solution containing the equivalent of 30 mg % of hyoscyamine base.

RESULTS AND DISCUSSION

At pH 6.6, bromcresol purple was found to complex selectively with hyoscyamine. Neither scopolamine nor tropine (one of the hydrolytic products of hyoscyamine) interfered. Interference due to chlorophyll was less than 2%.

To test for the possible interference from the volatile bases that may be present in the tinctures, standard curves were made using heated[4] and unheated tinctures. Almost identical results were obtained for both. The calculated slopes of the linear plots were 0.195 and 0.191 for heated and unheated belladonna tinctures, respectively, and 0.350 and 0.345 for heated and unheated hyoscyamus tinctures, respectively. This finding may suggest that the volatile bases, if present in the tinctures, did not interfere with the assay procedure.

It is concluded, therefore, that the recommended method is specific for the determination of hyoscyamine in belladonna and hyoscyamus tinctures. Compared with the BP methods of assay, which yielded 30.1 mg % for belladonna tincture and 5.3 mg % for hyoscyamus tincture, the recommended method gave the hyoscyamine contents as 29.6 and 5.1 mg % for the two tinctures, respectively.

Application of the suggested method for the determination of hyoscyamine in some BPC mixtures without pH adjustment, preextraction, or desorption steps was unsuccessful, perhaps due to the effect of pH, interference of the components of the mixture during the extraction of the alkaloid–dye complex, or adsorption of hyoscyamine onto the solid ingredient(s) of the mixture. In the aluminum hydroxide and belladonna mixture, however, direct estimation yielded a mean recovery of 99.73% (Table I). Aluminum hydroxide gel[5] BP had no apparent adsorptive effect when belladonna tincture was added and the mixture was stored for 7 days at 25 ± 0.2°.

The pH of the belladonna mixture, pediatric, was 3.6 owing to the presence of benzoic acid. Direct determination of hyoscyamine, without preadjustment of pH, gave high percentages, from 110.2 to 113.0 (eight determinations). This result was due mainly to the effect of pH since low values favor the partitioning of the excess dye in chloroform. After adjustment to pH 6.6, the mean recovery was 101.03% (Table I).

Direct determination of hyoscyamine in the potassium citrate and hyoscyamus mixture, without preextraction, failed possibly due to the acidic pH (5.9) and the high electrolyte concentration of this mixture (30% potassium citrate and 5% citric acid). The average recovery obtained by direct determination was 138.2% (±2.9%, eight determinations). Preadjustment to pH 6.6 using 1 *N* NaOH decreased the average recovery to 126.4%. Therefore, the relatively high electrolyte concentration of the mixture "salted out" the excess dye, thereby increasing its partitioning in chloroform; consequently, high results were obtained. Preextraction with chloroform from an alkaline medium (as outlined under *Experimental*) overcame this problem, the average recovery obtained being 100.53% (Table I).

TLC[6] confirmed that the supernate of the magnesium trisilicate and belladonna mixture was almost completely devoid of hyoscyamine. This finding was attributable to the rapid adsorption of hyoscyamine on magnesium trisilicate (3), which amounted to about 93% (4). When the BPC procedure for testing the presence of belladonna alkaloids (1) was adopted, the mean recovery was 54.1% (±5.7%, six determinations). The low result was probably due to the incomplete elution of the adsorbed hyoscyamine by the acetone–ammonia mixture used. With the procedure described under *Experimental*, the average recovery was 94.8% (Table I).

Attempts were made to improve the recovery by elevating the temperature of digestion (50–70°), prolonging the digestion time (0.5–3 hr), or boiling with concentrated hydrochloric acid as suggested by Gupta and Euler (3). No significant improvement was obtained; the results varied from 93.9 to 96.2% (mean 94.11%). The hydrated silica gel, produced from magnesium trisilicate in acid medium, probably had some adsorptive effect, so complete recovery of hyoscyamine could not be obtained.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 25, 1974, from the *Department of Pharmacy and Pharmacology, College of Medicine, University of Benin, Benin City, Nigeria.*

Accepted for publication July 2, 1975.

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[4] The tinctures were evaporated to dryness in the presence of a few drops of ammonia solution, and heating was continued for 15 min in the presence of a few drops of alcohol.

[5] Aludrox gel, Batch C.IG42, J. Wyeth & Brothers Ltd., Hants, England.

[6] Methanol–34.5% (w/w) ammonia (100:1) was the solvent system, and silica gel G (Merck) was the adsorbent.