Determination of Hyoscyamine Hydrobromide in a Multicomponent Tablet

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The quantitative assay of hyoscyamine hydrobromide in a multicomponent tablet formulation is described. The method consists of tablet treatment with sulfuric acid, an ether extraction from an alkaline aqueous solution, followed by quantitative column chromatography to separate the alkaloid from the nonalkaloidal tablet components.

THE DETERMINATION of the parasympatholytic alkaloid, hyoscyamine hydrobromide, in a urinary analgesic, antispasmodic, sedative tablet combination[1] proved to be a formidable problem. The problem was amplified by the minimal dosage per tablet of 0.3 mg. of the alkaloid to 150 mg. of phenazopyridine hydrochloride.[2] No problems were encountered due to the presence of 15 mg. of butabarbital.

Phenazopyridine hydrochloride is measured at its ultraviolet absorption peak at 240 mμ in aqueous acid without prior separation. Butabarbital can be assayed by triturating the tablets with methanol, extracting an acid solution with chloroform, re-extracting the chloroform with sodium hydroxide, and finally determining the ultraviolet absorption at 240 mμ. The assay procedures for butabarbital and phenazopyridine hydrochloride were precise and accurate.

Separation of hyoscyamine hydrobromide and phenazopyridine hydrochloride by liquid-liquid extraction proved difficult because the phenazopyridine hydrochloride distributed itself between both phases. Phenazopyridine hydrochloride also interfered with the usual methods of alkaloid quantitation. Thus, simple acid-base titration methods (1, 2), titration with picric acid (3), extraction and colorimetry of the picrate salt (4), precipitation with ammonium reineckate (5), and estimation by ultraviolet absorption spectroscopy yielded invalid results because of interference due to phenazopyridine hydrochloride. Attempts at reducing the azo linkage in phenazopyridine hydrochloride with $TiCl_3$ (6) or $Na_2S_2O_4$ similarly proved unsatisfactory.

It became apparent that a complete separation of the two compounds was required to assure an accurate assay for the solanaceous alkaloid. Chromatographic methods of analysis were investigated and limited success was achieved using a unidimensional multisolvent paper technique. This method consisted of chromatographing the mixture in an ethyl acetate–acetic acid–water solvent system in which hyoscyamine hydrobromide has an R_f value of 0.00 and most of the phenazopyridine hydrochloride migrates down the paper. The papergram is then placed in *n*-butanol–acetic acid–water where the alkaloid has an R_f value of 0.65. This method gave incomplete recoveries because of the difficulty in preparing a sufficiently concentrated extract of the tablet to allow for proper spotting on the paper chromatogram.

A liquid-liquid partition chromatographic column method was evolved based upon experience gained with paper chromatographic methods. A description of this method along with results obtained follows.

Received November 9, 1963, from the Applied Analytical Research Department, Warner-Lambert Research Institute, Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.
Accepted for publication December 11, 1963.

[1] Pyridium (150 mg.), 0.3 mg. of hyoscyamine hydrobromide, and 15 mg. of butabarbital. Marketed as Dolonil by the Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.

[2] Marketed as Pyridium by Warner-Chilcott Laboratories, Division of Warner-Lambert Pharmaceutical Co.

PROCEDURE

Reagents.—*Buffer pH 7.*—Dissolve 10 Gm. of KH_2PO_4 in 500 ml. of distilled water, adjust to pH 7.0 with 1 *N* NaOH, and dilute to 1 L. with distilled water.

Buffer pH 11.2.—Dissolve 1 Gm. of Na_3PO_4 in 1 L. of distilled water and adjust to pH 11.2.

Ethyl Acetate/Acetic Acid/Water Chromatographic Solvent.—Equilibrate 990 ml. of ethyl acetate, 10 ml. of glacial acetic acid, and 50 ml. of water in a separator and allow the phases to separate. Save both phases; the upper layer is used as the mobile phase and the lower layer as the stationary phase.

Sodium Picrate Reagent.—Dissolve 1 Gm. of picric acid in 50 ml. of distilled water, adjust pH to 7.0 with 1 *N* NaOH, and dilute to 100 ml. with water.

Sample Preparation.—Determine the average weight of not less than 15 tablets and reduce them to a fine powder. Weigh accurately a portion of the powder equivalent to about 3 mg. of hyoscyamine hydrobromide and transfer it to a 100-ml. centrifuge tube. Add 60 ml. of 1 *N* H_2SO_4 and heat the sample on the steam bath for 1 hour with occasional agitation. Centrifuge the suspension and filter the supernatant liquid through a medium porosity sintered-glass funnel into a 100-ml. volumetric flask. Wash the centrifuge tube with an additional 20 ml. of 1 *N* H_2SO_4, resuspend the solids, and repeat the centrifugation. Filter the supernatant liquid through the same funnel into the volumetric flask and dilute to volume with 1 *N* H_2SO_4. Pipet a 25-ml. aliquot into a separator, make alkaline (about pH 9) with 10% NH_4OH, and extract with 1 × 75 and 2 × 50 ml. of ether. Evaporate the combined ether extracts to about 20 ml. on the steam bath with a current of air directed above the solution.

Standard Preparation.—Dissolve 60 mg. of hyoscyamine hydrobromide N.F. in 200 ml. of 1 *N* H_2SO_4 and dilute a 10-ml. aliquot to 100 ml. with 1 *N* H_2SO_4. Pipet a 25-ml. aliquot into a separator, make alkaline (about pH 9) with 10% NH_4OH, and proceed as directed for the sample.

Column Preparation.—Mechanically stir 10 Gm. of Solka-Floc BW-40 (Gurntly Brown Co.) with 200 ml. of the mobile phase for about 15 minutes; then add 5 ml. of stationary phase and stir for an additional 30 minutes. Place a pledget of glass wool into a chromatographic column (150 × 25 mm.) and pack the column with the adsorbant to a height of 50 mm. using positive pressure. Place a pledget of glass wool on top of the column bed and wash the column with 20–30 ml. of mobile phase.

Chromatographic Development.—Quantitatively transfer the prepared sample and standard to

TABLE I.—RESULTS OF ASSAY OF SYNTHETIC MIXTURES

Analysis No.	% Recovery	Analysis No.	% Recovery
1	98.3	9	102.8
2	100.3	10	104.3
3	99.1	11	103.5
4	99.5	12	101.4
5	100.3	13	96.5
6	100.7	14	103.3
7	99.0	15	93.9
8	104.3		
$\bar{X}$ =	100.4%	$(X - \bar{X})^2$ =	121.6
σ =	3.0%	3σ =	9.0%

TABLE II.—RESULTS OF TRIPLICATE ASSAY[a] FOR HYOSCYAMINE HYDROBROMIDE

Analysis No.	Assay, mg./Tablet	Recovery, % of Theoretical
	Tablet *A*	
1	0.300	100.0
2	0.290	96.7
3	0.306	102.0
	Tablet *B*	
1	0.293	97.6
2	0.295	98.4
3	0.300	100.0
	Tablet *C*	
1	0.296	98.8
2	0.304	101.5
3	0.294	98.0

[a] The average assay is 99.2% ± 1.8% of the theoretical value.

separate columns, washing the vessel with small increments of mobile phase and collect the eluate at a flow rate of approximately 3 ml./minute. When the vessel has been washed free of phenazopyridine hydrochloride (absence of yellow color), wash the column with an additional 100 ml. of mobile phase. Discard these washings. Elute the column with 0.1 *N* HCl and collect in a 100-ml. volumetric flask until just up to volume, remove the organic phase with a syringe, and dilute to volume with an additional portion of 0.1 *N* HCl which has passed through the column.

Color Development.—Adjust a 25-ml. aliquot of each sample and standard to pH 7 with 1% NH_4OH and transfer each solution quantitatively to separators using 25 ml. of pH 7 buffer. Add 3 ml. of sodium picrate solution to each separator and extract with 3 × 25 ml. of $CHCl_3$, filtering each $CHCl_3$ layer carefully through glass wool. Extract the combined $CHCl_3$ layers with 2 × 35 ml. of pH 11.2 buffer collecting the buffer solution in 100-ml. volumetric flasks. Dilute to volume with pH 11.2 buffer.

Determine the absorbance of the sample and standard solutions *versus* pH 11.2 buffer at lambda maximum about 355 mμ in a suitable spectrophotometer using matched 10-cm. cells. These values are inserted into

$$\frac{A}{A_s} \times \frac{\text{Av. tablet wt.}}{\text{sample wt.}} \times \frac{\text{std. wt.}}{20} = \text{mg. hyoscyamine HBr/tablet} \quad \text{(Eq. 1)}$$

where A and A_s are the absorbances at 355 mμ of the sample and standard, respectively.

DISCUSSION

To evaluate the precision and accuracy of the method, synthetic mixtures of hyoscyamine hydrobromide and tablet formulation mixtures were prepared and assayed. The results of this study are shown in Table I.

Three individual lots of tablets were assayed in triplicate for hyoscyamine hydrobromide. The results of this study are shown in Table II.

RESULTS

A precise and accurate method has been developed for the determination of hyoscyamine hydrobromide in a tablet formulation. The method is generally applicable to other combinations of alkaloids and amines. The method circumvents the interference due to azo substances which exhibit atypical liquid-liquid partition characteristics.

The method can be easily manipulated and lends itself quite readily to routine control measurements.

SUMMARY

A method has been presented for the separation and assay of small amounts of hyoscyamine hydrobromide in complex tablet mixtures by partition column chromatography. The method has accuracy and precision well within the limits required for good quality control and stability testing.

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