Colorimetric and Gas Chromatographic Analyses of Arecoline in Capsule Preparations

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Abstract □ Two procedures have been developed for the quantitative determination of arecoline hydrobromide in capsule preparations. In the colorimetric assay, the arecoline salt was converted to the base with aqueous sodium bicarbonate, extracted into chloroform, and reacted with methyl orange. The absorbance was determined at 527 mμ. With the GLC method, the arecoline salt was converted to the base in a 5:100 triethylamine–chloroform solution and directly injected onto the column, using nicotine as the internal standard. The GLC method was found to be faster and more accurate.

Keyphrases □ Arecoline capsules—analysis □ Colorimetric analysis—spectrophotometry □ GLC—analysis

Arecoline hydrobromide, methyl 1,2,5,6-tetrahydro-1-methylnicotinate hydrobromide, is a parasympathomimetic agent. Its major pharmaceutical uses are as a cathartic for horses and a teniacide for dogs.

The only officially recognized assay pertaining to this material has its major application with the raw material (1). Until 1960, when Stainier and Gloesener (2) published their somewhat laborious gas chromatographic procedure, the compound had been virtually ignored in light of advanced analytical instrumentation.

The presence of arecoline hydrobromide in many soft gelatin capsule preparations necessitated these investigations. First a colorimetric assay was developed and subsequently a faster and more convenient gas chromatographic assay.

EXPERIMENTAL

Colorimetric Assay—*Preparation of Sample and Standard*—Fifty milligrams of arecoline hydrobromide dissolved in 500 ml. of water served as the standard. Capsules sufficient to yield a sample of 10 mg. of arecoline hydrobromide were cut into a 200-ml. centrifuge bottle; 25 ml. of chloroform was added, and the sample was swirled to disperse the fill material. To the solution, 0.1 *N* H_2SO_4 (100 ml.) was added; then the sample was shaken for 10 min. and centrifuged to separate the biphase mixture.

Assay Procedure—A 5-ml. aliquot of the aqueous phase and the standard were each transferred to 200-ml. centrifuge bottles. Five milliliters of water and 1 g. of sodium bicarbonate were then added to the solutions. The samples (*i.e.*, the prepared sample and standard) were heated on a steam bath until effervescence subsided and then cooled to room temperature. Chloroform (100 ml.) was added to the samples, which were then shaken for 10 min. The samples were centrifuged and the aqueous phase was discarded.

A 40-ml. portion of each sample was transferred to a 50-ml. glass-stoppered centrifuge tube along with a 40-ml. portion of chloroform as a reagent blank. One milliliter of a saturated aqueous solution of boric acid and 1.0 ml. of a saturated aqueous solution of methyl orange were added to each sample and the reagent blank. The resulting solutions were shaken for 20 min., centrifuged, and the water layer discarded. A 15-ml. aliquot of each was then transferred to 50-ml. glass-stoppered tubes, and 1.0 ml. of a 2% H_2SO_4 solution in absolute ethanol was added. The absorbance was read at 527 mμ against the reagent blank.

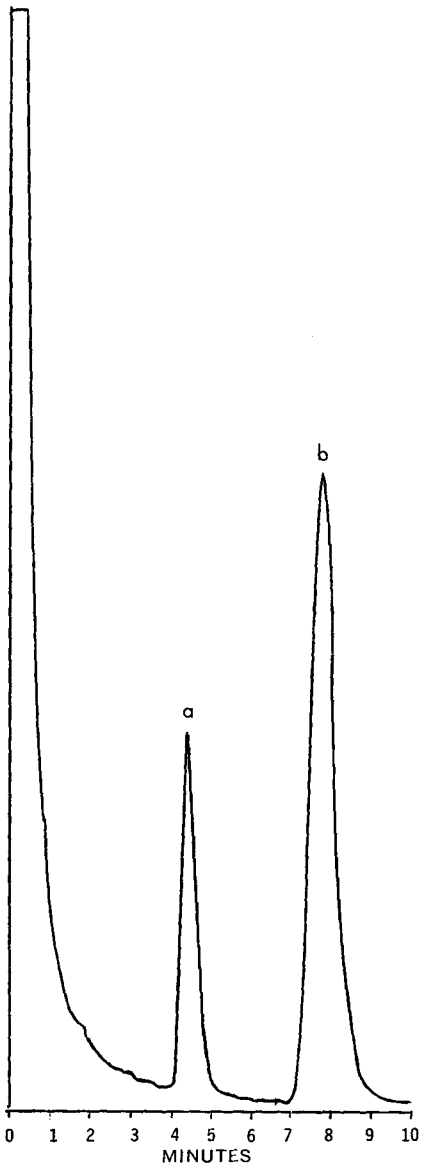

Figure 1—*Representative chromatogram: a, arecoline (4.5 min.); and b, nicotine (8 min.).*

Calculations—The potency of arecoline hydrobromide in milligrams per capsule was calculated using the following formula:

$$\frac{A_u}{A_s} \times \frac{10}{\text{number of capsules}} = \text{mg. arecoline HBr/capsule} \quad \text{(Eq. 1)}$$

where A_u is the absorbance of assay sample solution, and A_s is the absorbance of standard solution.

Gas Chromatographic Assay—*Preparation of Sample and Standard*—A 52.0-mg. sample of arecoline hydrobromide was weighed into a 250-ml. centrifuge bottle to use as the standard. Capsules comprising a 52.0-mg. sample of arecoline hydrobromide were cut open and dispersed in a 250-ml. centrifuge bottle. To both sample and standard, a 5:100 triethylamine solution in chloroform (100 ml.) was added, and the samples were shaken for 10 min. A 50-ml. portion of water was added to extract any compound

Table I—Comparison of the Two Assay Procedures

Product Lot	Capsule Claim, mg.	Methyl Orange	Recovery, %	GLC	Recovery, %
I	25.92	25.5	98.3	25.8	99.5
II	25.92	25.0	96.5	25.1	96.8
III	4.14	4.1	97.6	4.2	103.0
IV	34.56	31.8	92.0	33.4	96.8
V	2.70	2.7	100.0	3.3	121.1
VI	35.5	29.4	85.0	33.8	97.8

from the shell surfaces. The biphase mixture was shaken, centrifuged, and the water layer discarded.

The standard and sample solutions were then diluted 1:1 with a 0.5 mg./ml. solution of nicotine in chloroform, the internal standard. Two microliters of each solution was injected onto the column.

Gas Chromatograph—A Varian 2100 chromatograph with a hydrogen-flame ionization detector was used with a model 480 electronic digital integrator equipped with a Victor Digit-matic printout.

Column and Conditions—A 1.52 m. × 0.63 cm. × 2 mm. (5 ft. × 0.25 in. × 2 mm.) Pyrex column was packed with 5% polyethylene glycol 20 M[1] and 0.2% KOH on 60/80 diatomaceous earth.[2] The column was preconditioned overnight at 170° in a stream of helium. The operating conditions were as follows: column temperature, 120°; injector temperature, 235°; detector temperature, 250°; carrier gas, helium, 57 ml./min.; detector gas, hydrogen, 25 ml./min.; air, 300 ml./min.; and sensitivity, 124 × 10^{-12} amp./mv.

Identification of Constituents—The relative retention times were arecoline, 4.5 min., and nicotine, 8 min.

Calculations—The potency of arecoline hydrobromide in milligrams per capsule was calculated according to the following formula:

$$As \cdot \frac{Ca}{Ar} \cdot X = \text{mg. arecoline HBr/capsule} \qquad \text{(Eq. 2)}$$

where As is the area of the arecoline peak in sample/area of internal standard peak, Ca is the concentration of arecoline hydrobromide in mg./ml. in the standard solution, Ar is the area of arecoline peak in prepared standard/area of internal standard peak, and X is the dilution factor.

RESULTS AND DISCUSSION

Several gelatin capsule preparations were assayed by the method proposed in the "Official Methods of Analysis" (1). The method was found inadequate for capsules, because the average dosage level of arecoline hydrobromide is 1–8 mg. The number of capsules needed to procure the necessary 100.0-mg. sample created severe emulsion problems. The overabundance of gelatin made it virtually impossible to separate the two phases required for the assay. The methyl orange colorimetric method proposed here, although sensitive to any trace of amines or ammonia compounds on the glassware, did suffice as an analytical tool for assaying low concentrations of arecoline hydrobromide.

In an attempt to conserve the relatively large amount of time necessary for running the assay colorimetrically, the investigation then turned to developing a quicker, more precise instrumental method. A perusal of the literature revealed Stainier and Gloesener's (2) chromatographic assay of arecoline. Their samples of arecoline were hydrolyzed with KOH, and the corresponding alcohols were assayed quantitatively. Since the method was rather indirect, it was felt that arecoline could be chromatographed directly by merely converting the hydrobromide salt to its base in an appropriate solvent and injecting the arecoline directly onto the proper column.

A typical chromatogram is shown in Fig. 1. The peaks are well resolved and symmetrical; absorption effects appear to be absent.

Table I shows a comparison of values for each of the proposed methods, which were each run on six capsule lot preparations of varying concentrations of arecoline hydrobromide. In each case, a greater part of the theoretical claim was recovered with the gas chromatographic method, which also took less time to complete. Nine samples, each containing 54.5 mg. of arecoline hydrobromide, showed a mean percent recovery of 99.4 with a relative standard deviation from the mean of ±3.0 (Table II). The precision of the colorimetric assay was found in experimental studies to be of the magnitude of ±6.0%.

Table II—Statistical Treatment of GLC Procedure

Sample No.	Claim, mg.	Amount Recovered, mg.	Recovery, %
1A	54.5	52.0	95.4
1B	54.5	56.1	102.9
IC	54.5	52.6	96.5
IIA	54.5	55.5	101.8
IIB	54.5	54.6	100.2
IIC	54.5	52.9	97.1
IIIA	54.5	52.5	96.3
IIIB	54.5	55.5	101.9
IIIC	54.5	55.8	102.3
Mean		54.2	99.4
SD			3.0

REFERENCES

(1) "Official Methods of Analysis," 10th ed., Association of Official Agricultural Chemists, Washington, D. C., Section 32.008, 1965, p. 535.

(2) C. Stainier and E. Gloesener, *Farmaco Ed. Prat.*, **15**, 721 (1960).

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[1] Carbowax, Union Carbide Corp., New York, N. Y.

[2] Gas-Chrom W, Applied Science Laboratories, Inc., State College, Pa.