# Assay of Hyoscyamine, Atropine, Scopolamine, and Phenobarbital in Unit Doses of Tablets and Elixirs

RUPERT O. ZIMMERER, Jr. and LEE T. GRADY

**Abstract** □ A gas chromatographic assay for unit dose quantities of scopolamine and atropine-hyoscyamine was developed for tablets and elixirs containing phenobarbital. The method allows the complete assay of unit dose forms. Standards assayed with precisions of 4.8% for scopolamine, 6 mcg./unit dose, 2.5% for atropine-hyoscyamine at 100 mcg./dose, and 1.0% for phenobarbital (spectrophotometric) at 16 mg./dose. Homatropine served as extracted internal standard. Derivatives of the alkaloids were unnecessary using properly cured columns.

**Keyphrases** □ Hyoscyamine, atropine, scopolamine, phenobarbital tablets, elixirs—analysis □ Internal standard, "extracted"—homatropine □ GLC—analysis □ UV spectrophotometry—analysis

Quantitative methods for small amounts of belladonna alkaloids are few. No method has been reported which is suitable for the complete assay of hyoscyamine sulfate, atropine sulfate, scopolamine hydrobromide, and phenobarbital tablets and elixir.[1] This paper reports the development of a procedure which allows the complete assay of these formulations on unit dose forms.

Das Gupta and Ferguson (1) noted recently the need for determination of small amounts of atropine in elixirs and tablets and applied a dye-complex method. A separation of phenobarbital by partition column and subsequent colorimetric determination of total belladonna alkaloids was reported by Koch *et al.* (2). This sequence was adapted to kaolin-pectin suspensions by Bracey and Selzer (3). None of these methods determined scopolamine.

Sterescŭ and Popovici (4) determined atropine and scopolamine individually by paper chromatography in the presence of papaverine and sparteine. Schill and Agren separated scopolamine from hyoscyamine by partition chromatography on kieselguhr columns (5). Countercurrent distribution (6) also separates these alkaloids. Paper chromatographic separation of scopolamine from atropine-hyoscyamine was effective, and Reichelt (7) was able to separate as much as 50-fold ratios. Thin-layer chromatographic separation of scopolamine from the other belladonna alkaloids has been particularly successful, for example Wartman-Hafner's system (8).

Some workers have explored the gas chromatography of belladonna alkaloids. Kazyak and Knoblock (9) used silicone gums on silanized supports to separate atropine and scopolamine, but reported no satisfactory system. Brochmann-Hanssen and Fontan (10) reported separation of these from morphine and homatropine, most usably with a cyanosilicone liquid phase. The identification of alkaloids, including atropine, in blood was studied by Jain and Kirk (11) who recommended a polyester for general use. Most recently, Solomon *et al.* (12) used methylsilicone gum on silanized diatomite in assaying atropine and scopolamine in plant extracts; however, their system decomposed the alkaloids.

[1] Provisionally admitted to NF XIII.

## MATERIALS AND METHODS

Chromatographic grade methylene chloride (Matheson, Coleman & Bell). Borate buffer, 0.05 *M*, was prepared from boric acid and sodium hydroxide. Dibasic potassium phosphate and sodium hydroxide were used to prepare 0.2 *M* phosphate pH 10.5 buffer. Both buffers were standardized against the glass electrode. Homatropine hydrobromide, atropine sulfate, and scopolamine hydrobromide (Merck & Co., Inc.) and hyoscyamine sulfate and the tablet and elixir (A. H. Robins & Co., Inc., Richmond, Va.) were also used.

**Internal Standard Solution**—Dissolve 20 mg. homatropine hydrobromide in 250 ml. 0.005 *N* sulfuric acid and mix well.

**Alkaloids Standard Solution**—Dissolve 16.2 mg. scopolamine hydrobromide in 100 ml. 0.005 *N* sulfuric acid. Dissolve 30.8 mg. atropine sulfate in 200 ml. 0.005 *N* sulfuric acid, add 10.0 ml. of the scopolamine solution, and make to 250 ml. with acid. Dilute 20.0 ml. of this solution to 100 ml. with the acid. All solutions were prepared fresh daily.

**Gas Chromatography**[2]—Analyses were performed using an 0.6-m. × 3-mm. i.d. glass column packed with 3% methylphenylsilicone gum on 80/100 mesh silanized, acid-washed, flux-calcined diatomite.[3] Column temperature was 210°, with the injection port at 235°. Helium flow was about 60 ml./min. Samples, 1–2 μl., were injected on-column. Flame ionization detectors were used.

**Column Preparation**—Maintain the column at 250° for 1 hr. with helium flowing to remove oxygen and solvents, stop the flow of helium and heat at about 340° 4 hr., lower temperature to 250°, and condition with helium flowing until stable. A suitable initial test for support inertness, necessary with these low polarity liquid phases, is the delivery of a single, symmetric peak for injected cholesterol with no evidence of decomposition.

## PROCEDURE

**Standards**—Weigh 16.2 mg. phenobarbital USP reference standard into a separator and add 5.0 ml. of the alkaloids standard Solution. Add 1.00 ml. of the internal standard solution and

[2] F & M model 810, Avondale, Pa.
[3] OV-17 on Gas Chrom Q, Applied Science Laboratories, State College, Pa.

Table I—Assay of Commercial Preparations, Percent of Labeled Amounts

| Sample[a] | | Hyoscyamine-Atropine | Scopolamine | Phenobarbital |
|---|---|---|---|---|
| Elixir | | 103 Av. = 103% | 94 Av. = 96% | 102 Av. = 102 |
| | | 105 | 90 | 101 |
| | | 102 | 103 | 103 |
| Tablet | Lot A | 101 Av. = 101% | 93 Av. = 97% | 100 Av. = 101% |
| | | 101 | 100 | 102 |
| | Lot B | 102 | 98 | 101 |
| | | 95 Av. = 96% | 109 Av. = 111% | 100 Av. = 99% |
| | Lot C | 97 | 112 | 98 |
| | | 95 Av. = 96% | 112 Av. = 114% | 101 Av. = 100% |
| | | 96 | 116 | 99 |

[a] 103.7 mcg. Hyoscyamine sulfate and 19.4 mcg. atropine sulfate, 6.5 mcg. scopolamine hydrobromide, 16.2 and mg. phenobarbital in each tablet or 5 ml. of elixir.

extract with two 25-ml. portions of methylene chloride, filtering through anhydrous sodium sulfate into a 100-ml. volumetric flask. Wash the sodium sulfate with 25 ml. methylene chloride, dilute to volume, and mix well. Pipet 10 ml. of the solution into a 150-ml. beaker and evaporate to dryness on a steam bath. Add 1 ml. alcohol and, using pH 9.5, 0.05 *M* borate buffer, quantitatively transfer the residue to a 100-ml. volumetric flask. Dilute to volume with buffer and determine the absorbance at 240 mμ using 1-cm. silica cells.

To the aqueous phase in the separator remaining after extraction of phenobarbital add 4.0 ml. of pH 10.5, 0.2 *M* phosphate buffer and extract twice with 10 ml. of methylene chloride, filtering through anhydrous sodium sulfate. Wash the sodium sulfate with 5 ml. of the methylene chloride. Evaporate[4] the combined extracts to about 0.1 ml. at reduced pressure and inject an appropriate volume into the chromatograph. Record the chromatogram, decreasing the amplifier attenuation by a factor of about 16 about halfway between the hyoscyamine-atropine peak and the scopolamine peak to increase the measured peak height of scopolamine. Obtain the peak heights, *H*, of scopolamine, hyoscyamine-atropine, and homatropine and determine *Rs* for each drug, where *Rs* is = *H* alkaloid/*H* homatropine. *Ru* values are determined similarly for the assay preparations. The amount of each alkaloid in each dosage form may be calculated[5] from the formula $Wu = Ws\,(Ru/Rs)$, where *Wu* = weight of unknown, *Ws* = exact weight of standard.

When using a column for the first time prepare additional standards by adding 4.0 and 6.0 ml. of alkaloid solution and carrying out the assay. A plot of *Ru versus* amount yields a straight line. Assay values should be read directly from this calibration curve if the line does not pass through zero.

The amount of phenobarbital in each dosage form may be calculated from the formula $Wu = Ws\,(Au/As)$, where *Wu* = weight of unknown, *Ws* = exact weight of standard, *Au* = absorbance of unknown, and *As* = absorbance of standard.

**Tablet Composite**—Weigh and finely powder not less than 20 tablets. Transfer the equivalent of one tablet to a 60-ml. separator, add 1.0 ml. of the internal standard solution, 5 ml. water, mix, and adjust the pH to a value not greater than 3 with 1 *N* $H_2SO_4$ (about 0.5 ml. for those used in this report). Proceed as directed in the standard assay beginning with "Extract with two 25-ml. portions..."

If necessary, the aqueous phase in the separator, after adding 4 ml. pH 10.5 buffer, should have the pH adjusted to greater than 9 using 1 *N* NaOH (about 0.5 ml. for those used in this report).

**Tablet Content Uniformity**—Finely powder one tablet and transfer to a 60-ml. separator and proceed as in tablet assay above. Discard the phenobarbital extracts. Determine hyoscyamine-atropine only after evaporation of the extract to about 1 ml.

**Elixir**—Pipet 5.0 ml. of the elixir into a 60-ml. separator and add 1.0 ml. of the internal standard solution. Proceed as in the tablet assay, beginning with "Add 1.00 ml. internal standard solution...."

## RESULTS AND DISCUSSION

The small amounts and similar structures of the alkaloids in these formulations recommended gas chromatographic analysis. Moreover, a method was desired that would allow the entire assay to be performed on individual dose units.

Ten complete standards were taken through the procedure. The coefficients of variation in the hyoscyamine-atropine and scopolamine assay ratios were 2.5 and 4.8%, respectively. Phenobarbital precision was 1.0%. Applicability of the procedures to commercial formulations was checked (See Table I). The elixir was assayed in triplicate. Tablet composites of three lots were assayed. Placebos were not available.

In summary, this procedure allows assay of individual dose units of hyoscyamine sulfate, atropine sulfate, scopolamine hydrobromide, and phenobarbital tablets and elixir. The method is specific, rapid, and highly sensitive. Precisions are typical for gas chromatography, and using duplicates where possible, particularly for scopolamine, is recommended for accuracy.

This procedure also applies to other, simpler official tablets and solutions of belladonna alkaloids after appropriate change in the amount of internal standard and, usually, elimination of the acid extraction step. This has been done with hyoscyamine sulfate tablets NF and morphine sulfate and atropine sulfate tablets NF.

Applicability to content uniformity determination is inherent in single dose procedures, so only an estimate was prepared in a single lot. Content uniformity is based on hyoscyamine-atropine rather than scopolamine (the least abundant active ingredient) because of the better precision for that peak and to eliminate some of the time for concentration. This decision assumes that the ratio of scopolamine to the other alkaloids is fixed prior to manufacture of the dosage forms.

**Comments on Procedure**—Homatropine was chosen as the "extracted" internal standard prior to selection and acceptance of the column. It differs from these alkaloids only in a methylene group and the nature of substitution of the carbinol so chromatographic characteristics are nearly identical. Because of functional group similarities, minor alkaline ester cleavage, or amine degradation are controlled. Mechanical losses of various layers and volumetric considerations along the way are rendered insignificant as the partition behaviors are the same. Finally, purified material is readily available.

Phenobarbital must be eliminated in any event prior to the gas chromatographic work-up, so the acid extraction conveniently was adopted to determine phenobarbital. Methylene chloride was chosen because it is easily evaporated and distribution data indicated that the double extraction removes all but 0.2% of the phenobarbital. Spectrophotometric assay was chosen for speed and precision and to avoid introduction of a second column. The spectra of extracted standard and those from elixir and tablets showed no differences and all were identical to directly prepared standards. pH limits for the two extractions are specified in the procedure so that different product formulations will not interfere with alkaloid, particularly scopolamine, recovery. Phosphate buffer was added so that the final pH of the aqueous phase was 9.1 and was used instead of alkali to minimize ester cleavage. At this pH distribution data indicate that the two extractions remove 99.6% of the atropine and all scopolamine. Heat and air during the subsequent concentration should be avoided to prevent cleavage or oxidation of the small amounts of the alkaloids. The additional problem of racemization is not considered in this report.

The final volume, 0.1 ml., was chosen so that almost any flame ionization-equipped gas chromatograph would have the necessary sensitivity. The chromatograph in this laboratory was used at one-

[4] Rotary Evapo-mix, Büchler Instruments, Fort Lee, N. J.
[5] For the case of a linear curve "passing through zero."

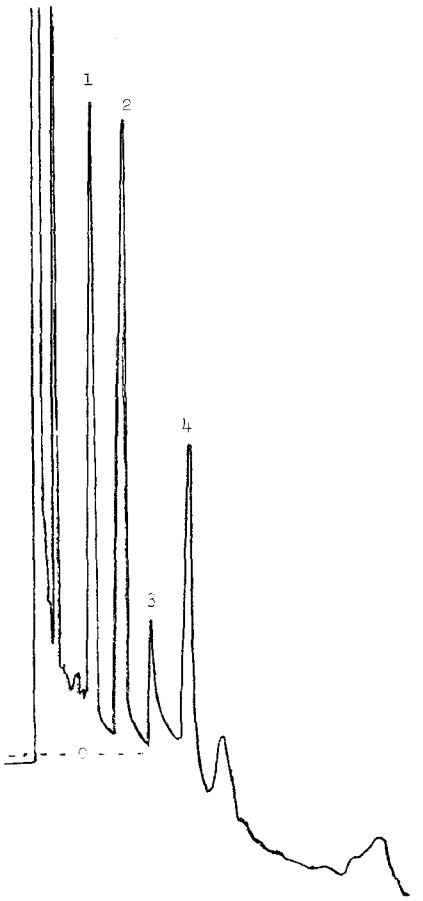


**Figure 1**—*Typical chromatogram (tablet). Key: 1, homatropine; 2, hyoscyamine-atropine; 3, attenuation change; 4, scopolamine.*

tenth of its maximum usable sensitivity range for scopolamine. The reagents must be pure as high boiling impurities are concentrated 200 times. Gas chromatographic grade methylene chloride is essential.

Reagent blanks were run to locate the origin of the additional peaks appearing in the chromatogram and see if there were interferences. Ten small peaks corresponding to those seen on the extracted chromatogram appeared. There were no peaks with retentions the same as or close enough to any of the drugs to interfere with the analysis (see Fig. 1). All these additional peaks were attributable to the reagent blank, mostly from methylene chloride. Of these, six emerged prior to homatropine. The others had relative (atropine = 1.0) retentions of 0.88, 1.18, 2.05, and 2.75. However, a less efficient column could allow minor species to bias the homatropine measurement. Additional standards, in triplicate, showed the analysis to be linear in the region of interest for both hyoscyamine-atropine and scopolamine. For this particular column the two lines obtained graphically terminated along the abscissa, so the assay data reported herein were taken from the calibration curves. The formula presented in the procedure would be accurate only where calibration curves have been demonstrated to "pass through zero." The column was rechecked (cholesterol, alkaloids, standard curves) at the end of the study and found intact.

**Chromatography**—The difficulties associated with the GLC of polar amines are well known. Improperly or partially-cured and conditioned columns often cause extensive tailing of such compounds and nonlinear recoveries of sample sizes. An additional problem is partial on-column dehydration of atropine and scopolamine as was reported by Solomon *et al.* (12). Although the preparation of less polar derivatives often allows successful chromatography, the additional steps and problems are appreciable. Current phases, supports, and column treatments have extended the range of molecules which can be chromatographed directly, without prior formation of less polar derivatives.

The peaks were symmetric and tailing was negligible (see Table II). Examination of the chromatograms of pure compounds gave peaks

**Table II**—Gas Chromatographic Data

| Parameter | Homatropine | Hyoscyamine-Atropine | Scopolamine |
|---|---|---|---|
| Retention[a] | 0.65 | (1.00) | 1.76 |
| Theor. plates (15) | 1000 | 1100 | 1200 |
| Asymmetry, *As* (15) | 1.10 | 1.02 | 1.08 |
| Tailing[b] | 1.06 | 1.06 | 1.04 |
| Response[c] | 0.97 | (1.00) | 0.99 |

[a] Actual retentions = × 2.95 min., see Fig. 1. [b] T. F. = $a + b/2a$ at 5% of peak height. [c] Equimolar amounts, ratio of peak areas.

of equal area (± 1% by planimeter). A mixture was prepared containing atropine and scopolamine in the same proportion (20:1) as that found in the commercial preparation: the scopolamine peak area was 97.4% of the value calculated based on equimolar amounts, showing that nonlinear adsorption losses are minor. Repetitive injections, $n = 12$, of this mixture in assay quantities gave coefficients of variation in the assay ratios of 1.0 and 4.0% for atropine and scopolamine, respectively. As seen from these results, most of the overall assay precision is attributable to the GLC step. Accuracy for scopolamine relative to atropine thus would be improved by duplicate injection of the same sample. Peaks in the procedural blank also were separated completely, so additional length of column was superfluous. Efficiencies of the order of a thousand plates appear sufficient for this assay.

The column preparation detailed in this procedure was essential to successful chromatography. Tailing values prior to curing were 1.4–1.7 with asymmetry factors of greater than 1.2. Resolution and recoveries were increased. Similar effects have been found with columns prepared from various samples of low (*W*) and medium (*G*) density silanized supports, both self-coated and supplier-coated. Street (13) reported recently his procedures for support treatment prior to coating and noted the importance of this for GLC of amines and alkaloids. Vessman (14) found that 315° conditioning of SE 30 on a silanized support did not eliminate tailing of high boiling amines, including scopolamine, and that alkali treatment of the support decreased tailing but decomposed the silicone.

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