the presence of degradation products was performed. The method may also find application in kinetic studies of cycloserine degradation.

REFERENCES

(1) P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz, and H. R. Sullivan, *J. Amer. Chem. Soc.*, 77, 2345(1955).

(2) M. Y. Karpeskii, Y. N. Brensov, R. M. Khomutov, E. S. Severin, and O. L. Polyanovskii, *Biochemistry (USSR)*, 28, 280 (1963).

(3) J. L. Miller, F. C. Neuhaus, F. O. Lassen, and C. H. Stammer, *J. Org. Chem.*, 33, 3908(1968).

(4) F. O. Lassen and C. H. Stammer, *ibid.*, 36, 2631(1971).

(5) L. J. Jones, *Anal. Chem.*, 28, 39(1956).

(6) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, p. 280.

(7) Q. N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," Wiley-Interscience, New York, N. Y., 1971, p. 518.

ACKNOWLEDGMENTS AND ADDRESSES

Selective Determination of Belladonna Alkaloids by GLC

RALPH S. SANTORO▲, PATRICIA P. PROGNER, EDWARD A. AMBUSH, and DAVID E. GUTTMAN

Abstract □ Atropine/hyoscyamine and scopolamine, in dosage forms in which the scopolamine represented less than 9% of the total belladonna alkaloids, were isolated as free bases from phenylpropanolamine, chlorpheniramine, and interfering excipients by a series of pH- and solvent-controlled extractions and were quantitated by GLC. Homatropine was used as the internal standard. Precision and accuracy data are represented, along with assay results on three commercial capsule lots.

Keyphrases □ Belladonna alkaloid mixtures with phenylpropanolamine and chlorpheniramine, low scopolamine content—GLC analysis, atropine/hyoscyamine and scopolamine □ Atropine/hyoscyamine and low-content scopolamine—GLC analysis in mixtures with phenylpropanolamine and chlorpheniramine □ Scopolamine (low content) and atropine/hyoscyamine—GLC analysis in mixtures with phenylpropanolamine and chlorpheniramine □ GLC—analysis, atropine/hyoscyamine and scopolamine in mixtures with phenylpropanolamine and chlorpheniramine

Because of its specificity, sensitivity, and resolving properties, GLC has been increasingly used for belladonna alkaloid analysis (1–6). The USP collaborative study for the assay of atropine and scopolamine dosage forms by GC (7) indicated that these two substances, when found individually in tablet dosage forms, could be assayed in this manner with an acceptable degree of precision.

However, the case becomes less clear, even with the high resolving power of GC, when belladonna is found in the presence of a preponderant concentration of other amines and/or in dosage forms with high background contributions. The problem is further complicated when both atropine and scopolamine must be determined together with, as is many times the case, the scopolamine only a small fraction of the atropine.

This paper describes a GLC procedure in which hyoscyamine sulfate, atropine sulfate, and scopolamine hydrobromide in dosage forms are determined in the presence of chlorpheniramine maleate and phenylpropanolamine hydrochloride, and also where the scopolamine content is less than 9% of the total belladonna present.

EXPERIMENTAL

Materials—*Buffer*—Dissolve 34.8 g. of dibasic potassium phosphate in 900 ml. of water, adjust to pH 9.0 with 1 N hydrochloric acid or sodium hydroxide, and dilute to 1 l. with water.

Methylene Chloride—Distill reagent grade methylene chloride and store in a tightly closed container.

Atropine Sulfate Standard Solutions—Prepare solutions of USP Atropine Sulfate Reference Standard (or NF Hyoscyamine Sulfate Reference Standard), dried at 105° for 4 hr., in 0.01 N sulfuric acid with concentrations of 0.70, 0.65, and 0.60 mg./ml.

Scopolamine Hydrobromide Standard Solutions—Prepare solutions of USP Scopolamine Hydrobromide Reference Standard, dried at 105° for 4 hr., in 0.01 N sulfuric acid with concentrations of 0.070, 0.065, and 0 060 mg./ml.

Internal Standard Solution—Prepare a solution of USP Homatropine Hydrobromide Reference Standard, dried at 105° for 4 hr., in 0.01 N sulfuric acid with a concentration of 0.50 mg./ml.

Procedure—For this study, the contents of six capsules, each containing 50 mg. of phenylpropanolamine hydrochloride, 4 mg. of chlorpheniramine maleate, and 0.25 mg. of combined belladonna alkaloids (0.0375 mg. atropine sulfate, 0.0219 mg. scopolamine hydrobromide, and 0.1906 mg. hyoscyamine sulfate) were triturated with 0.01 N sulfuric acid. The slurry was quantitatively transferred to a 50-ml. volumetric flask, 2.0 ml. of internal standard was added, and it was diluted to volume with 0.1 N sulfuric acid. The contents of the flask were transferred to a 50-ml. centrifuge bottle and centrifuged at 2000 r.p.m. for 15 min., and 25.0 ml. of clear supernatant solution was transferred to a 150-ml. separator.

The solution was extracted twice with 25-ml. portions of chloroform, and the chloroform extracts along with any emulsion were discarded. Vigorous shaking was avoided in this step to prevent excessive emulsion formation, so losses of belladonna alkaloids and internal standard were minimized. The pH of the solution was adjusted to 8.0 ± 0.1 by the addition of 25 ml. of pH 9.0 buffer and the dropwise addition of 1 N sodium hydroxide; then the solution was extracted with three 25-ml. portions of cyclohexane, and the cyclohexane extracts were discarded. The solution was further
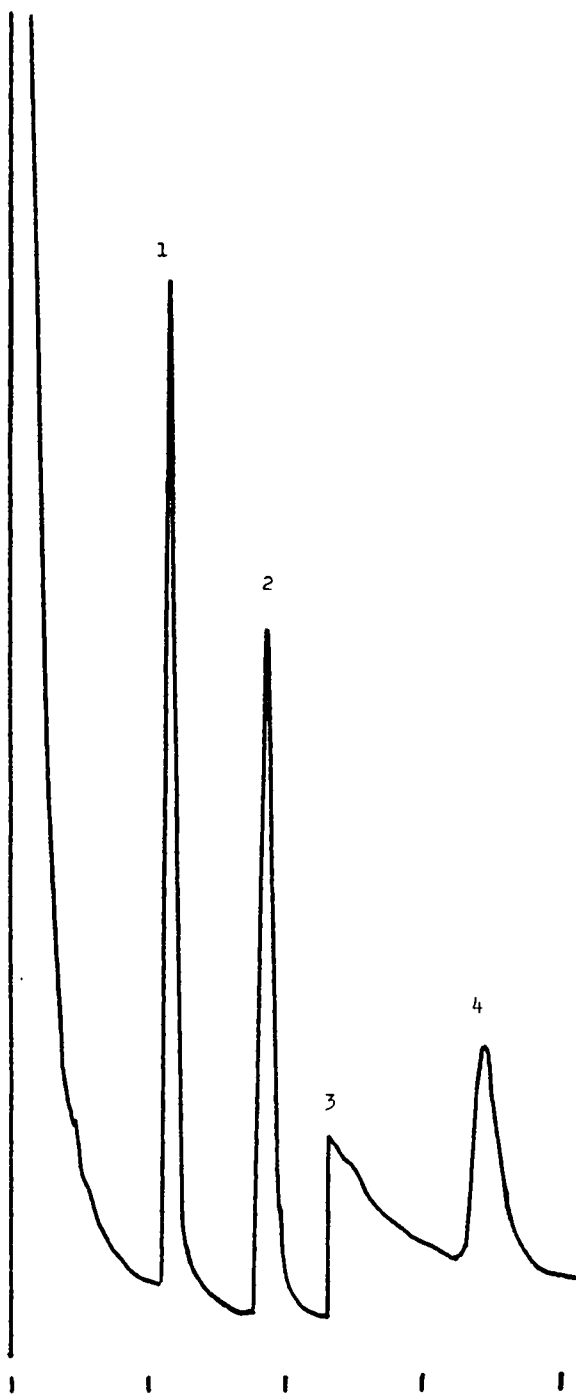
Figure 1—*Typical chromatogram (standard). Key: 1, homatropine; 2, atropine/hyoscyamine; 3, attenuation change; and 4, scopolamine.*

carried through the procedure as already described, beginning with: "The solution was extracted twice with 25-ml. portions of chloroform...."

GLC—The analysis was performed on a gas chromatograph[1] equipped with a 1.22-m. (4-ft.) × 4-mm. glass column packed with 3% (w/w) methyl phenyl silicone oil on 100–120-mesh silanized, acid-washed, flux-calcined diatomite[2] and also equipped with a flame-ionization detector. The column was conditioned at 250° for 1 hr. with helium flowing to remove oxygen and solvents, stopping the flow of helium and heating at about 340° for 4 hr., and finally lowering the temperature to 250° and conditioning with helium flowing until a stable baseline was obtained (7, 8). Weekly injections[3]
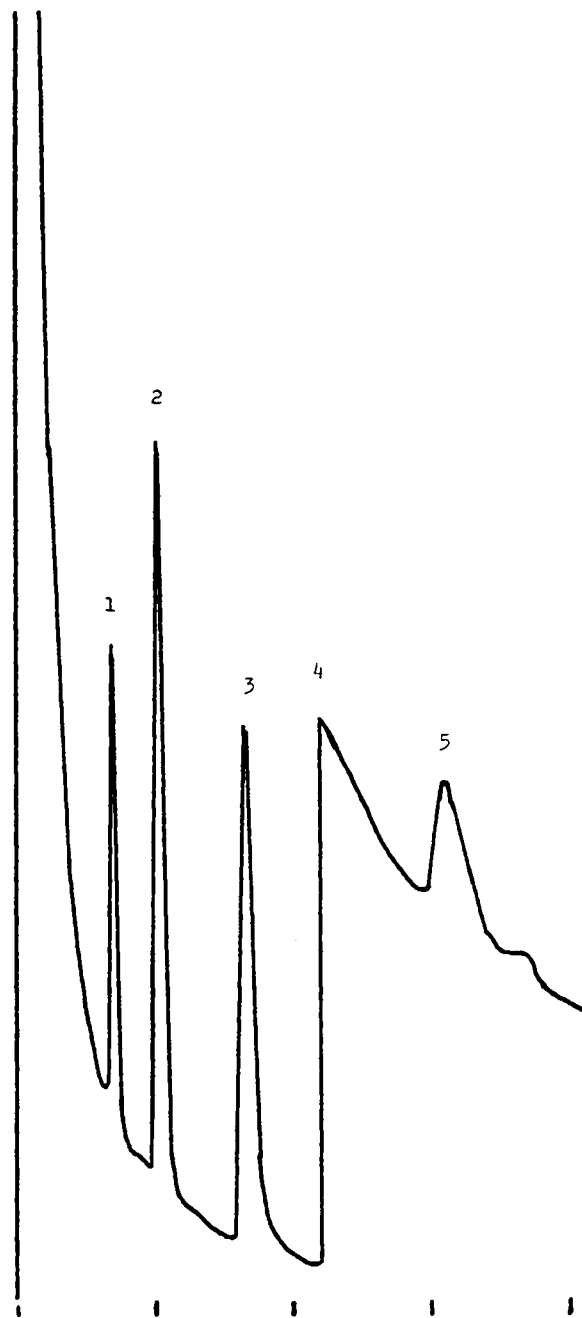

Figure 2—*Typical chromatogram (capsule). Key: 1, chlorpheniramine; 2, homatropine; 3, atropine/hyoscyamine; 4, attenuation change; and 5, scopolamine.*

adjusted to pH 9.0 ± 0.1 with 1 N sodium hydroxide and extracted with two 10-ml. portions of methylene chloride. Then the methylene chloride extracts were combined and allowed to stand to permit as much of the emulsion to break as practical. The clear extract was filtered through a cotton–anhydrous sodium sulfate filter, previously wetted with methylene chloride into a 25-ml. graduated cylinder, transferred to a 19 × 36-mm. glass vial equipped with a plastic liner cap, and evaporated to dryness under nitrogen and mild heat. The residue was dissolved in 0.9 ml. of methylene chloride and used for GLC. A series of combined standards was prepared by pipeting 1.0 ml. of each atropine and scopolamine standard solution into each of three 150-ml. separators containing 25 ml. of 0.1 N sulfuric acid, so that combined standards of 0.70 and 0.070, 0.65 and 0.065, and 0.60 and 0.060 mg. of atropine and scopolamine, respectively, were obtained. A 1.0-ml. of aliquot of the internal standard solution was pipeted into each separator, and the combined standards were then

[1] Perkin-Elmer 900.
[2] 3% OV-17 on 100–200-mesh Gas Chrom Q, Applied Science Laboratories.
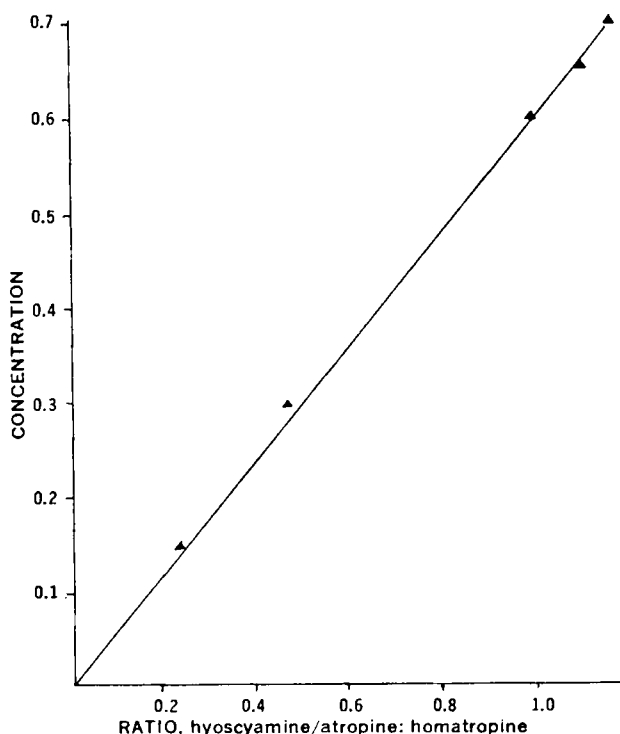[3] Silyl-8 GLC column conditioner, Pierce Chemical Co.

Figure 3— *Representative standard curve for atropine/hyoscyamine dissolution studies.*

maintained column efficiency. The analysis was carried out isothermally at 210°, with the helium carrier gas flow rate at about 50 ml./min. and the temperature of the injection port not more than 25° above that of the column.

Appropriate volumes of the standard and sample preparations were injected into the chromatograph, and the peaks were obtained at a convenient attenuation. The attenuation was decreased by a factor of 8 about halfway between the hyoscyamine/atropine peak and the scopolamine peak to increase the height of the scopolamine peak. The heights were measured for the first (homatropine), second (hyoscyamine and atropine), and third (scopolamine) components for each standard injection, and the values were recorded as P_1, P_2, and P_3, respectively. The standard hyoscyamine/atropine ratio, R_a, and standard scopolamine ratio, R_s, were calculated by:

$$R_a = P_2/P_1 \qquad \text{(Eq. 1)}$$

$$R_s = P_3/P_1 \qquad \text{(Eq. 2)}$$

The average factors for hyoscyamine/atropine, F_a, and scopolamine, F_s, were determined by dividing each R_a and R_s into its respective standard concentration and averaging the individual factors obtained. The peak heights were measured for the sample preparations in the same manner as above and recorded as p_1, p_2, and p_3, respec-

Table I— Belladonna Alkaloid Recovery

Sample	—Percent Recovered[a]— Hyoscyamine/ Atropine	Scopolamine
1	99	95
2	101	102
3	100	102
4	100	105
5	101	105
6	100	100
Average	100	102
Sigma	0.8	3.7
Standard error	0.3	1.5

[a] Each recovery sample contained 150 mg. phenylpropanolamine hydrochloride, 12 mg. chlorpheniramine maleate, and 0.75 mg. belladonna alkaloids (atropine sulfate, 0.1125 mg.; hyoscyamine sulfate, 0.5700 mg.; and scopolamine hydrobromide, 0.0675 mg.) plus placebo.

Table II—Belladonna Alkaloid Assay of Commercial Capsule Lots

Lot	—————Percent Claim[a]————— Hyoscyamine/ Atropine	Scopolamine
1	97	97
2	100	97
3	100	102

[a] Claim per capsule: 50 mg. phenylpropanolamine hydrochloride, 4 mg. chlorpheniramine maleate, and 0.25 mg. belladonna alkaloids (atropine sulfate, 0.0375 mg.; hyoscyamine sulfate, 0.1906 mg.; and scopolamine hydrobromide, 0.0219 mg.).

tively. The hyoscyamine/atropine ratio, r_a, and scopolamine ratio, r_s, were calculated by:

$$r_a = p_2/p_1 \qquad \text{(Eq. 3)}$$

$$r_s = p_3/p_1 \qquad \text{(Eq. 4)}$$

The hyoscyamine/atropine (A_1) and scopolamine (A_2) assay was calculated by:

$$A_1 = \frac{F_A \times r_a \times 1.023}{3} \qquad \text{(Eq. 5)}$$

$$A_2 = \frac{F_s \times r_s \times 1.12}{3} \qquad \text{(Eq. 6)}$$

The factors 1.023 and 1.12 convert anhydrous hyoscyamine/atropine and scopolamine to the USP hydrated form.

RESULTS AND DISCUSSION

Figures 1 and 2 are typical chromatograms obtained from standards and samples with a 10-μl. injection, preliminary attenuation of 256, and a final attenuation for scopolamine of 32. The system does not differentiate the isomers, hyoscyamine and atropine, which are eluted as one peak. The peak before the internal standard, homatropine, in the sample chromatogram (about 3 4 min.) is a trace of chlorpheniramine not removed by the cyclohexane extraction at pH 8.0. The dissolution of atropine/hyoscyamine in some dosage forms was followed, and Fig. 3 is a representative standard curve obtained in these experiments. The curve shows good linearity throughout the required range.

Table I lists recoveries of known amounts of the belladonna alkaloids mixed with placebo, which contained phenylpropanolamine hydrochloride and chlorpheniramine maleate in the ratio of 200 and 16 times, respectively, the concentration of the total belladonna content. Recoveries were quantitative, with the standard deviation for scopolamine significantly higher than that for hyoscyamine/atropine, reflecting the fact that its peak was measured at a much lower attenuation because it represented less than 9% of the total belladonna present. Furthermore, a small error in measurement produced a proportionately greater error in the final calculation.

Three different commercial lots of capsules were assayed (Table II). The reproducibility was checked in Lot 1, and the standard deviations were found to be 1.4 and 3.4 atropine/hyoscyamine and scopolamine, respectively. These values were of the same order of magnitude as reported in the recovery experiments.

Control of pH was important for obtaining correct results and acceptable precision limits. The initial extraction with chloroform from acid was to remove interfering wax excipients that chromatograph with similar retention times as the internal standard and belladonna alkaloids. The adjustment to pH 8.0 for the cyclohexane extraction was carefully controlled, since a higher pH would entail significant loss of belladonna while a lower value would leave too much chlorpheniramine behind. The chlorpheniramine would be extracted in the next step and interfere with the internal standard peak. Similarly, careful adjustment to pH 9.0 for the methylene chloride extraction of the belladonna was required because phenylpropanolamine would begin to partition at a higher pH. Distilled methylene chloride was necessary for extraction because reagent grade and spectrograde material contained additives that chromatographed with a similar retention time as that of scopolamine.

Throughout the extractions, vigorous shaking was avoided to control emulsion formation.

SUMMARY

A GLC procedure was presented that is capable of determining atropine/hyoscyamine and scopolamine in the presence of phenylpropanolamine and chlorpheniramine, and also where the scopolamine is less than 9% of the belladonna present. Interfering excipients were removed with chloroform from acid, adjustment to pH 8.0 and extraction with cyclohexane removed chlorpheniramine, and further adjustment to pH 9.0 with methylene chloride extraction isolated the belladonna alkaloids. The final extract was concentrated and gas chromatographed. Homatropine was used as the internal standard. The method was sensitive, highly specific, and reasonably precise.

REFERENCES

(1) E. Brochmann-Hanssen and A. B. Svendsen, J. Pharm. Sci., 51, 1095(1962).

(2) B. Wu Chu and E. S. Mika, ibid., 59, 1508(1970).

(3) R. O. Zimmerer, Jr., and L. T. Grady, ibid., 59, 87(1970).

(4) J. L. Massingill, Jr., and J. E. Hodgkins, Anal. Chem., 37, 952(1965).

(5) H. V. Street, J. Chromatogr., 29, 68(1967).

(6) H. L. Tarlin and M. Batchelder, J. Pharm. Sci., 59, 1328 (1970).

(7) L. T. Grady and R. O. Zimmerer, Jr., ibid., 59, 1324(1970).

(8) "Gas-Chrom Newsletter," Applied Science Labs. Inc., vol. 10, no. 5, Nov.-Dec. 1969.

ACKNOWLEDGMENTS AND ADDRESSES

PHARMACEUTICAL TECHNOLOGY

Sensory Assessment of Spreadability of Hydrophilic Topical Preparations

B. W. BARRY▲ and M. C. MEYER

Abstract ☐ The rheological conditions operative during spreading of topical preparations on the skin were determined for series of aqueous gels and oil-in-water emulsions. Master curves were derived from the rheological data obtained using a sensory test with a panel of 24 members. Rates of shear varied from 350 to 10,000 sec.$^{-1}$, depending on the consistency and the type of preparation being spread. Two preference scoring techniques were used to assess the series of preparations in terms of spreadability. From the preference data, the shearing conditions with optimum patient acceptance potential were derived. An indication is given for the use of such master curves with their preferred regions for the determination of instrumental rheological conditions for use in routine and innovative industrial control procedures. Where relevant, all results were compared with previously reported work on lipophilic formulations.

Keyphrases ☐ Spreadability—sensory tests correlated to rheological conditions, hydrophilic topical preparations ☐ Rheological master curve—shear rate variation, spreadability of hydrophilic topical preparations, sensory tests ☐ Skin, sensory perception of spreadability of hydrophilic topical preparations—correlation of rheological conditions, master curves derived

In the pharmaceutical and cosmetic industries, it is important that formulations, besides possessing the correct physicochemical and biopharmaceutical properties, should have maximum patient and consumer acceptability. Spreadability plays an important role in the patient's assessment of a topical product. The correct consistency for such a preparation helps to ensure that a suitable dose is applied to the skin. This is particularly important with vehicles that incorporate potent drugs such as corticosteroids; excessive doses may lead to collagen atrophy and other undesirable side effects. Previous authors have assessed spreadability in terms of shear rate, either by a correlation with non-Newtonian viscosities (1–4) or by assuming that the spreading procedure can be likened to plane laminar flow between parallel plates (5–7) and thus can be described by the equation:

$$\dot{\gamma} = V/d \qquad \text{(Eq. 1)}$$

where $\dot{\gamma}$ is the rate of shear, V is the relative velocity of the plates or skin surfaces, and d is the distance between them. Estimates of V and d were used to find the shearing conditions operative during spreading.

In contrast, Wood (8) developed a method which involved comparing Newtonian liquids with pseudoplastic liquids to determine the approximate rate of shear developed in the mouth. This procedure, which has the advantage that V and d need not be known to determine the shearing conditions occurring in vivo, was modified by Barry and Grace (9) to investigate the rheological conditions that operate during the spreading of lipophilic preparations on the skin.

In the present work, the techniques used by Barry and Grace (9) were extended to investigate the shearing con-