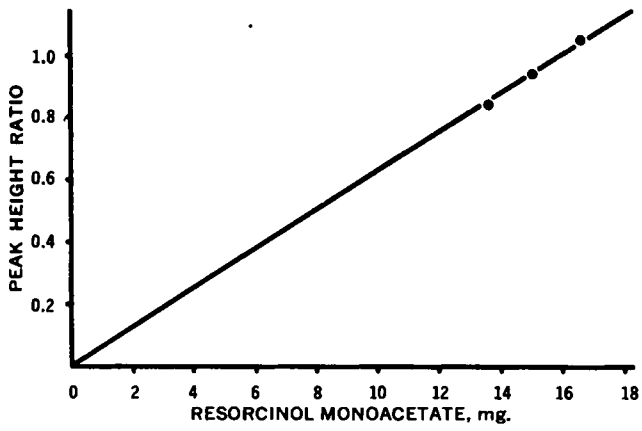

Figure 3—*Standard curve of resorcinol monoacetate concentration* versus *peak height ratio.*

statistical data are shown in Table I. The sample cream base formulated was composed of sorbitan monolaurate, glycerin, polyoxyethylene (20), glyceryl monostearate, hexachlorophene, hydrocortisone acetate, propylene glycol, cetyl alcohol, and colloidal sulfur. The statistical evaluation of the results indicates that the method had no bias at any of the three levels of resorcinol monoacetate. The average coefficient of variation was 1.40%. For 16 replicate samples, the average recovery was 99.76%.

A typical chromatograph of the standard mixture is shown in Fig. 1, and a chromatograph obtained from the extract of the dermatological preparation is shown in Fig. 2. The correlation coefficient for concentration of resorcinol monoacetate *versus* peak height ratio (resorcinol diacetate/orcinol diacetate) was found to be 1.025. A calibration curve for milligrams of resorcinol monoacetate *versus* peak height ratio is shown in Fig. 3.

The statistical results indicate that the method is accurate and reproducible for quality control of resorcinol monoacetate in dermatological preparations.

Table II—Determination of Resorcinol Monoacetate in Commercial Preparations

Sample	Number of Samples	Label Claim, %	Found, % ($\bar{x}$)
Cream I	20	3.0	3.03
Lotion I	12	3.0	3.09
Cream II	16	3.0	3.04
Lotion II	9	3.0	2.88

This method has been used in these quality control laboratories for over a year. The results obtained during this period are shown in Table II.

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GLC Analysis of Homatropine Methylbromide in Tablets and Elixirs

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Abstract □ A GLC assay was developed for the determination of homatropine methylbromide in both tablets and elixirs. The method allows for assaying the drug in the presence of other tropine derivatives and the usual constituents of tablets and elixirs. An aqueous suspension of tablets or a sample of elixir was adjusted to pH 2.2 with hydrochloric acid buffer USP and extracted with ether. This preliminary extraction of the buffered sample removes interfering substances and excludes any hydrolyzed material in the sample from analysis. The homatropine methylbromide was then hydrolyzed by adding 10% sodium hydroxide solution to pH 10–11 and boiling the solution for 20 min. After acidification with hydrochloric acid buffer, the resulting mandelic acid was extracted with ether. The trimethylsilyl derivative of the mandelic acid resulting from hydrolysis was then chromatographed, with the trimethylsilyl derivative of 2-naphthol as the chromatographic standard. A blank elixir preparation with added homatropine methylbromide (0.12 mg./ml.) assayed with an accuracy of 99.12% of the calculated value. This procedure was applied to various commercial preparations containing homatropine methylbromide, with reproducible results ranging from 97.59 to 100.86% of the labeled amount of homatropine methylbromide.

Keyphrases □ Homatropine methylbromide tablets and elixir—GLC analysis □ GLC—analysis, homatropine methylbromide tablets and elixir

Many esters of the amino alcohol tropine exhibit strong anticholinergic activity (1) and, as a result, are found in several pharmaceutical preparations. Analysis of both naturally occurring and semisynthetic derivatives, particularly those contained in pharmaceutical preparations, has been of interest to the pharmaceutical chemist for many years (2). Several of the official procedures (3) for the analysis of these compounds, how-

ever, lack both sensitivity and specificity (4, 5). Because of these deficiencies, many procedures for the analysis of tropine derivatives have been developed (6, 7) and one of these (8) has been adopted as the official analysis procedure for atropine sulfate in various dosage forms (9).

Analytical methods utilizing spectrophotometry (10), TLC (11), and GLC (12) appear to provide useful procedures for the analysis of these compounds. Of these methods, the latter would be the most useful for the analysis of tropine derivatives since it has the potential advantages of speed, sensitivity, and specificity.

Quaternary ammonium salts of tropine esters would, however, present difficulties in GLC analysis. Analysis of quaternary ammonium salts *via* GLC is limited by the fact that chromatographic characteristics of permanent cations are unsuitable for precise quantification (13). Additionally, because of the polarity of these salts, their solubility in organic solvents is low and, therefore, both their preliminary extraction from pharmaceutical dosage forms and their purification are difficult. These problems would be applicable to the GLC analysis of homatropine methylbromide, which is contained in a number of pharmaceutical preparations.

Since homatropine methylbromide is a semisynthetic product, it would be desirable to assay for the drug in the presence of closely related natural products, *e.g.*, atropine and scopolamine, and the usual constituents of the pharmaceutical dosage forms in which it is contained. Furthermore, the assay should be specific for the intact drug molecule and be independent of the hydrolysis products of homatropine methylbromide, *i.e.*, tropine methylbromide and mandelic acid.

With these considerations in mind, a GLC procedure for the assay of homatropine methylbromide was developed which is based upon the amount of mandelic acid produced upon its hydrolysis. This procedure was applied, with satisfactory results, to several commercial tablet and elixir preparations containing homatropine methylbromide.

EXPERIMENTAL

Materials—*N,O*-Bis(trimethylsilyl)acetamide[1] was used as both the silylating solvent and the silylating agent. Hydrochloric acid buffer (pH 2.2) was prepared according to the procedure outlined in USP XVIII (14). Homatropine methylbromide[2], *d,l*-mandelic acid[3], and 2-naphthol[4] were used (the latter two melted at reported temperatures after recrystallization). Standard solutions used in analyses were prepared by diluting 60.0 mg. homatropine methylbromide to 100 ml. with distilled water and by diluting 20.7 mg. *d,l*-mandelic acid and 60.0 mg. 2-naphthol, respectively, to 100.0 ml. with anhydrous ether[5]. Both tablets[6] and elixirs[7] containing homatropine methylbromide were used for analysis.

Apparatus—A gas chromatograph[8] with isothermal control, flame-ionization detector, and an electronic digital integrator[9] was used. The columns were 1.52 m. long, spiral-shaped, borosilicate glass tubing (3.2-mm. i.d.) packed with 15% phenylmethyl silicone

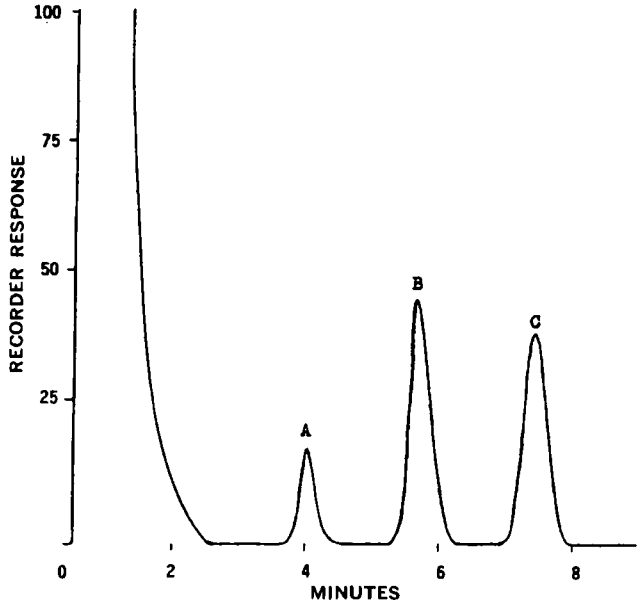

Figure 1—*Chromatogram trace of the silyl derivatives of: A, mandelic acid, 0.207 mg./ml.; B, tropic acid, 0.60 mg./ml.; and C, 2-naphthol, 0.6 mg./ml. Conditions were as listed in the text.*

on 60–80-mesh silanized, acid-washed, flux-calcined diatomite[10] which, prior to use, was conditioned at 275° for 24 hr. The injector port (fitted with a glass injection sleeve), column, and detector temperatures were 195, 160, and 210°, respectively. The gas flow rates were: hydrogen, 40 ml./min.; compressed air, 180 ml./min.; and nitrogen as the carrier gas, 50 ml./min.

Procedure—*Chromatographic Standards*—Mix together 1.0 ml. each of the *d,l*-mandelic acid and 2-naphthol standard solutions in a cone-shaped vial[11] fitted with a Teflon septum. Evaporate the solution to dryness with a stream of dry nitrogen, and add 200-μl. of the silylating agent to the residue contained in the vial. Cover and allow to stand at room temperature for 20 min., with occasional shaking. Inject an appropriate volume of the solution (1–3 μl.), on column, into the chromatographic system. Obtain the peak areas A_m for the trimethylsilyl derivative of *d,l*-mandelic acid and A_n for the 2-naphthol derivative. Calculate R_s for the chromatogram as follows:

$$R_s = A_m/A_n \qquad \text{(Eq. 1)}$$

Tablets—Weigh and finely powder not less than 20 tablets containing homatropine methylbromide. Weigh accurately a portion of the powder, equivalent to about 0.60 mg. of homatropine methylbromide, and transfer with the aid of 10 ml. of the hydrochloric acid buffer to a separator. Add 10 ml. of hydrochloric acid buffer, and extract with three 20-ml. portions of anhydrous ether, discarding the ether extracts. Adjust the solution to pH 10–11 with 10% sodium hydroxide solution, and boil the solution on a hot plate for 20 min. Cool to room temperature, adjust the solution to pH 2.2 with hydrochloric acid buffer, and transfer to a separator. Extract the solution with three 20-ml. portions of anhydrous ether, receiving the extracts in a flask. Dry the combined ether extracts over anhydrous sodium sulfate and filter. Transfer the combined ether extracts to a cone-shaped vial, and evaporate to dryness with a stream of dry nitrogen. Add 200 μl. of the silylating agent to the residue contained in the vial, and proceed as directed under *Chromatographic Standards* beginning with: "Cover and allow to stand. . . ." Calculate R_u for the chromatogram as follows:

$$R_u = A_m/A_n \qquad \text{(Eq. 2)}$$

where A_m is the peak area for the trimethylsilyl derivative of mandelic acid and A_n is the peak area for the derivative of 2-naphthol.

[1] Silylation grade, Pierce Chemical Co., Rockford, Ill.
[2] Reference standard, NF XIII.
[3] Baker grade, J. T. Baker Chemical Co., Phillipsburg, N. J.
[4] Certified, Fisher Scientific Co., Fair Lawn, N. J.
[5] Ether anhydrous, Fisher Scientific Co., Fair Lawn, N. J.
[6] Commercial products.
[7] Dia-Quel elixir and placebo elixir, supplied by Marion Laboratories, Kansas City, Mo.
[8] Varian model 575.
[9] Varian model 475.
[10] QF-1 on Gas Chrom Q, Applied Science Labs., Walnut Creek, Calif.
[11] Reacti-Vials, Pierce Chemical Co., Rockford, Ill.

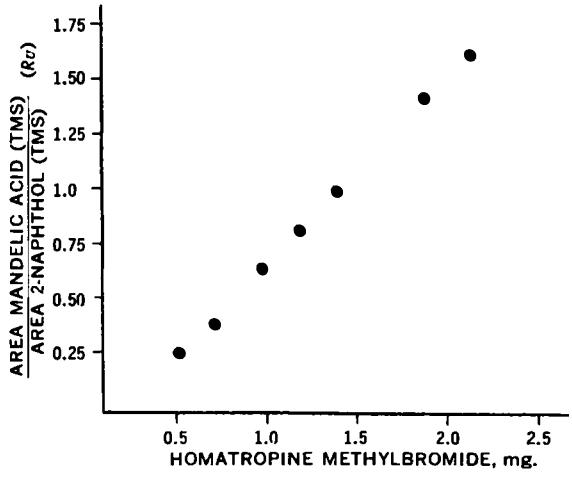

Figure 2—*Standard curve.* R_U versus *concentration.*

Calculate the quantity, in milligrams, of homatropine methylbromide in the sample as follows:

$$W_u = W_s \frac{R_s}{R_u} 2.43 \qquad \text{(Eq. 3)}$$

where W_u is the weight, in milligrams, of homatropine methylbromide in the sample and W_s is the weight, in milligrams, of *d,l*-mandelic acid in the standard solution.

Elixir—Transfer a volume of elixir equivalent to 0.60 mg. of homatropine methylbromide to a separator. Add 20 ml. of the hydrochloric acid buffer, and extract with three 20-ml. portions of anhydrous ether, discarding the ether extracts. Proceed as directed under *Tablets*, beginning with: "Adjust the solution. . . ."

RESULTS AND DISCUSSION

Studies of the hydrolysis of homatropine methylbromide indicated both the conditions and minimum time necessary to assure its complete hydrolysis. The present studies of the hydrolysis of homatropine methylbromide in 10% sodium hydroxide solution at various saponification times showed that complete hydrolysis is achieved after 8 min. Solutions of homatropine methylbromide in the hydrochloric acid buffer (pH 2.2) were also studied. After 30 min., no mandelic acid was detected in the buffer solution, which suggests that hydrolysis of the salt during the preliminary extraction process is negligible. These results support the work of Patel and Lemberger (15) on the stability of tropine esters in alkaline and acidic media.

Ether extraction of the acidic solution prior to hydrolysis removes many potentially interfering substances. Mandelic acid, which would be liberated as a result of any prior hydrolysis of homatropine methylbromide in the dosage form, does not interfere with the results of the assay since it is removed by ether extraction. This assay, therefore, can also be used to study the hydrolytic stability of the drug. In addition, other possible components of the dosage forms analyzed, such as benzoic acid and *p*-hydroxybenzoate esters, are also removed by this ether extraction.

Other possible contaminants, such as the methylbromide derivatives of atropine and scopolamine, are quantified in the chromatographic scheme. For example, solutions containing both atropinium methylbromide and homatropine methylbromide were analyzed by this procedure. The results indicate that the retention time of the trimethylsilyl derivative of tropic acid relative to that of the trimethylsilyl derivative of *d,l*-mandelic acid in this system is 1.54, and the resolution factor, calculated according to the method of Gadzinowicz (16), is 4.60.

Silylation rates of *d,l*-mandelic acid and 2-naphthol at room temperature were also studied. The results indicate that the maximum quantity of the trimethylsilyl derivative is obtained after 9.5 min. These derivatives were also found to be stable at room temperature for a least 24 hr. when protected from light and moisture.

Studies of liquid stationary phases on the column packing material indicated that a relatively high charge (15%) of the liquid stationary phase was necessary to obtain proper separation of the trimethylsilyl derivatives of tropic and *d,l*-mandelic acids. While this charge leads to less than optimal chromatographic parameters (asymmetry: 1.20; tailing: 1.20) (17), the resulting baseline separation (Fig. 1) allows for accurate peak area determinations.

Table I—Assay of Commercial Homatropine Methylbromide Preparations

Sample	Other Known Components	Homatropine[a] Methylbromide	*N*	Standard Deviation
Elixir A	Opium tincture, pectin, preservatives	98.64	10	2.94
Elixir B	Phenobarbital, preservatives	97.59	8	2.86
Tablet A	Phenobarbital	99.46	9	1.80
Tablet B	—	100.86	8	2.10

[a] Expressed as percent of labeled amount.

A plot of R_u and concentration of homatropine methylbromide, in milligrams, for the analysis of placebo elixir containing added amounts of homatropine methylbromide was constructed and was found to be linear (Fig. 2). This calibration curve should be constructed and used to determine the amount of homatropine methylbromide analyzed by this procedure.

Samples of placebo elixir containing added amounts of homatropine methylbromide were analyzed, with reproducible results, on successive days by the procedure outlined here. The mean calculated value of the results was 99.12% of the added homatropine methylbromide, with a coefficient of variation of 2.6%. Several commercial tablet and elixir preparations were also assayed by this procedure, and the results are reported in Table I. In all cases, the trimethylsilyl derivative of tropic acid was absent, and there were no interfering peaks in the chromatogram.

In conclusion, a GLC determination of the mandelic acid liberated by the saponification of homatropine methylbromide was developed and is proposed as an assay for the drug contained in both tablets and elixirs. The assay is specific for the determination of the mandelic acid esters of quaternary ammonium salts, and the usual components of tablets and elixirs do not interfere. This procedure was applied, with satisfactory results, to several tablet and elixir preparations containing homatropine methylbromide

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Analysis of Trisulfapyrimidines by High-Pressure Liquid Chromatography

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Abstract □ The application of high-pressure liquid chromatography to the separation and analysis of trisulfapyrimidines in pharmaceutical dosage forms is demonstrated. The preparation of samples of both tablet and suspension dosage forms is simple and rapid. The chromatographic conditions chosen optimize the separation of sulfadiazine, sulfamerazine, and sulfamethazine and allow quantitative analysis of these trisulfapyrimidines in a reasonable time.

Keyphrases □ Trisulfapyrimidine formulations—analysis, high-pressure liquid chromatography □ Sulfa drugs, sulfadiazine-sulfamerazine-sulfamethazine—separation, analysis, high-pressure liquid chromatography □ High-pressure liquid chromatography—analysis, trisulfapyrimidine formulations

The separation and quantitative analysis of sulfadiazine, sulfamerazine, and sulfamethazine in pharmaceutical dosage forms present a difficult problem to the pharmaceutical analyst. Current methods and proposed modifications of them are slow and tedious. The USP method (1) uses a paper chromatographic separation prior to colorimetric determination, by means of the Bratton–Marshall reaction, of the eluted individual sulfonamides. Modifications proposed by Kunze and coworkers (2, 3) require extreme care for satisfactory results. Banes and Riggleman (4) recently proposed a hybrid assay for trisulfapyrimidine preparations in which total sulfonamides are measured colorimetrically by the Bratton–Marshall procedure. Sulfadiazine is then measured colorimetrically by means of its specific reaction with thiobarbituric acid; sulfamethazine is separated from its homologs by column partition chromatography and is then determined by UV spectrophotometry. The third sulfonamide is obtained by difference. A qualitative chromatogram is used to confirm that only the three sulfonamides are present.

The determination of trisulfapyrimidines in dosage forms by high-pressure liquid chromatography was first reported by Poet and Pu (5). A recent paper by Kram (6) reported the conditions for the separation of a number of sulfapyrimidines. The determination of trisulfapyrimidines by high-pressure liquid chromatography overcomes or circumvents many shortcomings of the previously reported methods. The preparation of samples is simple and rapid, and separation and analysis times are reasonably short.

EXPERIMENTAL[1]

Mobile Phase and Reagents—The mobile phase, 0.2 *M* disodium phosphate solution adjusted to pH 6.0 with 85% phosphoric acid, was prepared fresh daily. Sodium hydroxide and sulfuric acid solutions (1 *N*) were required.

Internal Standard Solution—The stock internal standard solution, 1200 mcg./ml., was prepared by first dissolving 120 mg. of sulfadimethoxine in 5 ml. of 1 *N* sodium hydroxide solution and then diluting to 100 ml. with distilled water.

Trisulfapyrimidine Stock Standard Solution—The trisulfapyrimidine stock standard solution was prepared by first dissolving 120 mg. each of sulfadiazine, sulfamerazine, and sulfamethazine in 5 ml. of 1 *N* sodium hydroxide solution and then diluting to 100 ml. with distilled water. This solution contained each of the trisulfapyrimidines at a concentration of 1200 mcg./ml.

Standard Curve Solutions—These solutions were prepared by suitable dilution of the stock standard solution with distilled water. They contained each of the trisulfapyrimidines at a concentration between 108 and 132 mcg./ml. and the internal standard at a concentration of 120 mcg./ml.

Preparation of Tablet Sample—For a single-tablet analysis, the weight of a single tablet was determined. For a batch analysis, the weight of a pool of several tablets was determined and the average tablet weight was calculated. The sample was ground to a fine powder. An accurately weighed portion of the powdered sample, equivalent to 36 mg. of total trisulfapyrimidines, was transferred to a 100-ml. volumetric flask containing 10 ml. of 1 *N* sodium hydroxide solution. The stoppered flask was shaken on a mechanical reciprocal shaker for 15 min. A total of 9 ml. of 1 *N* sulfuric acid was added while the flask contents were swirled. Some distilled water was added, followed by 10 ml. of internal standard solution and enough additional distilled water to make 100 ml. of solution. A portion of the well-shaken extract, transferred to a glass-stoppered test tube, was centrifuged for 10 min. at 2000 r.p.m., and the supernate was used for analysis.

Preparation of Suspension Sample—A weight of well-shaken trisulfapyrimidine suspension, equivalent to 36 mg. of total trisulfapyrimidines, was transferred to a 100-ml. volumetric flask, and 10 ml. of 1 *N* sodium hydroxide solution was added. Preparation of the sample was continued as described earlier for tablet samples.

Conditions for Chromatographic Separation—The important features of the liquid chromatograph used were described in detail elsewhere (7, 8). The degassed mobile phase was passed through the cation-exchange column under a pressure of 1000 psig., to obtain a flow rate of 0.7–0.8 ml./min. at room temperature, until a stable

[1] A DuPont liquid chromatograph (model 820) equipped with a UV monitor, an Infotronics integrator (Model 10-AB-2), with digital printout, and a DuPont packed "Zipax" SCX cation-exchange column, 1 m. long, 6.35-mm. (0.25-in.) o.d. and 2.1-mm. i.d., was used. The column contained approximately 6 g. of "Zipax" support, having about a 1% loading of the cation-exchange polymer.