Analysis of Scopolamine and Its Degradation Products by GLC and Liquid Partition Chromatography

JOHN J. WINDHEUSER▲, JOHN L. SUTTER, and AWNI SARRIF

Abstract □ A procedure for the determination of aqueous solutions of scopolamine and its acidic and basic degradation products is given. Liquid–liquid partition chromatography is used to remove the compounds quantitatively from aqueous solution. After preparation of trimethylsilyl derivatives, the individual compounds are determined by dual-column, temperature-programmed GLC. The sensitivity and specificity of the method are such that traces of any of the compounds may be accurately determined in the presence of relatively large amounts of any or all of the others.

Keyphrases □ Scopolamine and degradation products—analysis, GLC and liquid–liquid partition chromatography □ GLC—analysis, scopolamine and degradation products □ Liquid–liquid partition chromatography—analysis, scopolamine and degradation products

Scopolamine has been used therapeutically for almost 150 years and has found wide application as an anticholinergic agent in the treatment of various conditions.

Although studies of ester linkage cleavage and optical isomerism in the compound have indicated that degradation occurs in acidic and basic solutions, relatively little has been done to investigate the kinetics of degradation. A review of the literature (1) indicates that scopolamine degradation proceeds by a multiplicity of routes as illustrated in Scheme I.

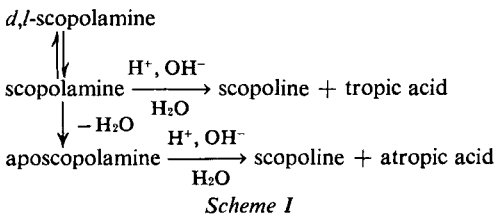
It is apparent that aside from the isomerization reaction, the degradation products fall into two groups, with basic and acidic structures. The reactions and relative importance of each route of degradation appear to be governed by pH. To establish clearly the pH dependence of these reactions, a highly specific method of analysis, both for scopolamine and its degradation products, is required.

The assay for scopolamine is classically done by extraction and titration of the basic nitrogen under aqueous or nonaqueous conditions. The latter procedure was used in USP XVII (2). Another method, which is specific only for the ester linkage, involves reaction with hydroxylamine (3). Similarly, UV spectrophotometry lacks the specificity to determine quantitatively mixtures of scopolamine hydrobromide and its degradation products.

Recognizing that these approaches are highly nonspecific, USP XVIII (4) introduced a procedure involving isothermal GLC following extraction from aqueous solution into methylene chloride. The procedure is based upon the method published by Zimmerer and Grady (5).

Although the USP XVIII method appears to be suitable for intact scopolamine and some of its degradation products, investigation has shown that it is not directly useful in studies where all degradation products must be quantitatively determined.

This reports deals with a method that can successfully separate and quantitatively determine the drug and its degradation products.



Scheme I

MATERIALS AND METHODS

Reagents—All reagents were analytical grade unless otherwise noted. Scopolamine was recrystallized from water and acetone. Aposcopolamine was prepared from scopolamine according to the procedure of King (6). Tropic acid was recrystallized from benzene, and atropic acid was recrystallized from water and ethanol. Scopoline was obtained from a commercial source[1].

Assay Solution—The assay solution consisted of scopolamine and potential degradation products dissolved in distilled water.

Standard Solution—A solution in *N,N*-dimethylformamide, containing 3.75 mg./ml. of scopolamine hydrobromide and 1.25 mg./ml. of each of the degradation products, was used.

Liquid–Liquid Partition Chromatography—Glass columns, 400 × 15 mm., were equipped with Teflon stopcocks and glass wool plugs. The solid support was diatomaceous earth, acid washed[2]. The stationary phases were: acidic column, 0.15 *N* H_2SO_4; and basic column, pH 9.4 borate buffer (USP XVIII, p. 939).

GLC[3]—The glass columns were 4 mm. × 2.4 m. (8 ft.), phenyl methyl silicone gum 1%[4] on acid–base washed, silane-treated, flux-calcined diatomaceous earth[5], 80–100 mesh, conditioned at 300° and treated with 50-μl. injections of column conditioner[6] at 250°.

The carrier gas was nitrogen at a flow rate of 70 ml./min. The injector temperature was 240°, and the detector temperature was 340°. The temperature program was: isothermal at 100° for 13 min., then 10°/min. rise up to 220°, and then isothermal at 220° until elution of scopolamine was complete.

ASSAY PROCEDURE

Preliminary Extraction by Liquid–Liquid Partition Chromatography—*Acidic Column*—Mix 2.0 ml. of assay solution plus 3.0 ml. of 0.25 *N* H_2SO_4 in a 150-ml. beaker; add 7.5 g. diatomaceous earth[2] and mix thoroughly. Pack into a column containing approximately 20 ml. water-saturated chloroform, adding the diatomaceous earth to the column through a powder funnel in small increments and packing firmly with a glass tamping rod after each addition. After quantitative transfer of the diatomaceous earth to the column, place a small glass-wool plug on top of the column.

[1] Pfaltz and Bauer, Inc.
[2] Celite 545, Johns-Manville, New York, N. Y.
[3] A Barber-Coleman Series 5000 gas chromatograph, with dual flame-ionization detectors and temperature-programmable column oven, was used.
[4] OV-17, Anspec Co., Inc., Ann Arbor, Mich.
[5] Gas Chrom Q, Anspec Co., Inc., Ann Arbor, Mich.
[6] Silyl-8, Pierce Chemical Co., Rockford, Ill.

Table I—Analyses of Synthetic Mixture of Scopolamine and Its Degradation Products

Compound	Assay Solution, mg./ml.	Percent Recovery—Sample I	Sample II	Sample III	Sample IV
Scopoline	0.25	97.1	97.0	97.6	97.3
Atropic acid	0.25	99.9	98.0	97.0	98.0
Tropic acid	0.25	102.4	101.4	101.9	102.1
Aposcopolamine	0.25	102.0	101.0	100.8	102.0
Scopolamine	0.75	99.9	97.8	97.2	97.8

Basic Column—Mix 2.0 ml. of assay solution plus 3.0 ml. of pH 9.4 borate buffer (USP XVIII, p. 939) in a 150-ml. beaker; add 7.5 g. diatomaceous earth and mix thoroughly. Pack into a column containing approximately 20 ml. water-saturated chloroform–ether (1:1) as in the acidic column.

Elution of Acidic Column—First elute the column with 125 ml. of water-saturated chloroform, collecting the eluate at a rate of approximately 2 drops/sec. in a flask with a ground-glass stopper. Stopper the flask (I) and set aside. Continue the elution with 250 ml. of water-saturated chloroform–ether (1:1), collecting in a separate flask. Stopper and set aside (II).

Elution of Basic Column—Elute with 250 ml. of water-saturated chloroform–ether (1:1), collecting the eluate in a round-bottom flask with a 24/40 ground-glass stopper at a rate of approximately 2 drops/sec. (III).

Evaporation of Eluates I, II, and III—First, evaporate Eluate III to dryness on a rotary evaporator under reduced pressure without heat. Next, quantitatively transfer Eluate II to the flask containing the residue from Eluate III and evaporate to dryness as already described. Finally, quantitatively transfer Eluate I to the flask, add 3 drops of 0.5 *N* methanolic HCl, and evaporate to dryness as described.

Quantitative Determination by GLC—*Silylation of Sample*—To the combined residues from the acidic and basic columns, add 400 μl. of *N,N*-dimethylformamide and 400 μl. of *N,O*-bis(trimethylsilyl)-acetamide[7], stopper tightly, and swirl until the residue is completely dissolved.

Silylation of Standard—Combine 400 μl. of the standard solution and 400 μl. of *N,O*-bis(trimethylsilyl)acetamide in a suitable container, stopper tightly, and mix.

Quantitation—Separate 4.0-μl. injections of the silylated standard and sample solutions are made onto the gas chromatograph, and the peak height of each compound is measured on the chromatograms. The concentration of each compound in the assay solution is then calculated as follows:

$$C_s = \frac{H_s \times C_{sT} \times V_{sT}}{H_{sT} \times V_s} \qquad \text{(Eq. 1)}$$

where H_s = peak height of sample; H_{sT} = peak height of standard; C_s = concentration in aqueous sample solution, milligrams per milliliter; C_{sT} = concentration in dimethylformamide standard solution, milligrams per milliliter; V_s = volume of aqueous sample solution taken for assay, milliliters; and V_{sT} = volume of dimethylformamide standard solution used, ml. = 0.400 ml.

RESULTS AND DISCUSSION

Recovery Data—Aqueous synthetic mixtures of the drug and degradation products were prepared and assayed, using as a standard a solution of the compounds in *N,N*-dimethylformamide. The percent recovery of each compound was calculated from the following formula:

$$\% \text{ recovery} = \frac{H_s \times C_{sT} \times V_{sT} \times 100}{H_{sT} \times C_s \times V_s} \qquad \text{(Eq. 2)}$$

where C_s is the concentration in milligrams per milliliter in the synthetic mixture, and the other symbols are as previously defined.

[7] BSA, Specially Purified Grade, Pierce Chemical Co., Rockford, Ill.

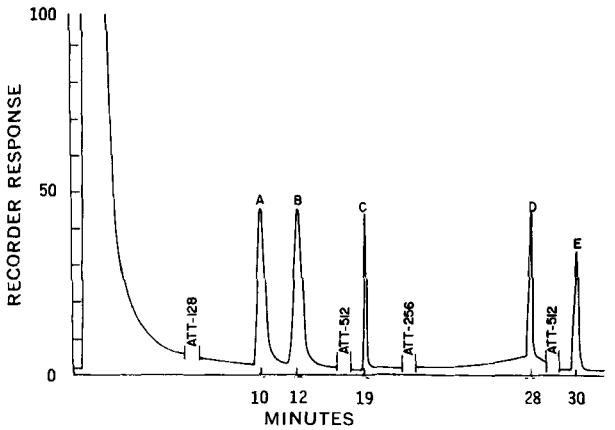


Figure 1—*Chromatogram obtained from analysis of Sample I (Table I) containing: A, scopoline, 0.25 mg./ml.; B, atropic acid, 0.25 mg./ml.; C, tropic acid, 0.25 mg./ml.; D, aposcopolamine, 0.25 mg./ml.; and E, scopolamine, 0.75 mg./ml. GC conditions were as listed in the text.*

Recoveries of all components were in the range 97.0–102.4%. Since the GC separations are excellent, the determination of trace components in the presence of the others is limited only by the sensitivity of the detector response of the gas chromatograph. Some typical results are shown in Table I.

Liquid–Liquid Chromatography—The preliminary experiments showed that even repeated extractions in separators did not allow complete recovery of all the degradation products from aqueous solution; it was, therefore, decided to utilize the much greater efficiency of columns to accomplish the extractions. It was found that elution of the acidic compounds was quantitative from a column using 1 *N* HCl as the stationary phase; however, some aposcopolamine was also eluted, probably due to ion-pair formation. Since this prevented combining the eluates from the acidic and basic columns prior to GC, 0.15 *N* H_2SO_4 was used as the stationary phase on the acidic column. Elution of the acidic compounds from this column was quantitative, and no basic compounds were eluted.

The pH of the stationary phase of the alkaline column was found to be critical. At pH values above 10, there was significant degradation of scopolamine on the column; whereas at a pH below 7, recovery was incomplete. Use of pH 9.4 borate buffer as the stationary phase on the alkaline column permitted quantitative elution of the basic compounds without elution of any acidic compounds. Significant losses of atropic acid occurred on evaporating chloroform–ether solutions of it but did not occur on evaporating chloroform solutions of the compound; consequently, atropic acid was eluted first with chloroform and evaporated from the combined residues only after evaporation of the chloroform–ether eluates. The chloroform–ether elution of the acidic column was found to be necessary for complete recovery of tropic acid, which is relatively insoluble in chloroform.

GLC—Figure 1 shows a typical gas chromatogram of scopolamine and its hydrolytic degradation products obtained from an aqueous synthetic mixture. A linear relation beween peak height and concentration was found for all compounds.

Solomon *et al.* (7) determined scopolamine by GLC without prior silylation and observed some on-column degradation at the higher temperature used. In the present study, no evidence was observed of degradation of silylated scopolamine or any of the other compounds on injecting each of them separately onto the gas chromatograph under the conditions noted.

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TECHNICAL ARTICLES

Simulation Device for Preliminary Tablet Compression Studies

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Abstract □ A system was designed to simulate the double-acting compression effect of rotary tableting machines commonly used to prepare pharmaceutical compressed tablets. The device was constructed for use with a universal mechanical testing instrument, but the principle could also be applied to other types of compressing equipment including a modified reciprocating tablet machine. The double-acting compression is achieved by controlled downward movement of the die at a slower rate than the simultaneous downward movement of the upper punch. Adjustable components also allow control of: (*a*) the rate of loading, (*b*) the relative rate of movement of the die and upper punch, (*c*) the time at which movement of the die commences, (*d*) the depth of compression in the die, and (*e*) precompression of the powder bed. These controls permit simulation of a range of machine settings for different types of rotary tablet presses.

Keyphrases □ Tablet compression—preliminary testing, simulation of double-acting compression cycle of a rotary tableting machine □ Rotary tableting machine—simulation device for preliminary tablet compression testing □ Compression, tablets—preliminary testing, rotary tableting machine simulation device

Since the investigations of Shotton *et al.* (1) and Knoechel *et al.* (2, 3), there has been much interest in the instrumentation of rotary compression machines used for the manufacture of pharmaceutical compressed tablets.

In preformulation studies and during the early stages of development of tablets, the compression behavior of substances is often insufficiently well defined to compress the material on precision equipment such as a rotary machine. Also, sufficient quantities of a new drug substance may not be available at the appropriate time for such large-scale investigations. Preliminary studies, therefore, are often carried out using a single-acting press or a reciprocating tableting machine in which the compression conditions differ from those of a rotary machine with respect to factors such as friction effects at the die wall and stress distribution in a compact. It is often difficult to relate the results of these preliminary trials to the subsequent behavior of the material on a rotary machine.

This report describes a system (4) which is used with a single-acting mechanical press to simulate the double-acting compression cycle of a rotary tableting machine.

OPERATING PRINCIPLE

A diagram of the simulator is shown in Fig. 1. It was designed principally for use with a universal mechanical testing instrument[1] as in Figs. 2 and 3, but many of the design features allow the system to be used in conjunction with other types of mechanical and hydraulic compressing equipment or with a modified reciprocating tablet press.

A rotary tablet machine is double acting; consolidation of the compacted material occurs in a die between upper and lower punches, which move toward each other between compression wheels (Fig. 4). To simulate this effect using a single-acting press in which movement of only one component occurs, such as the upper punch, this movement must be translated also to a second component of the system, such as the lower punch. However, since the principal load-bearing components of such a compression train are the upper and lower punches, it is mechanically simpler to fix the punches to the upper and lower platens of the compressing equipment. For this reason, in the device described the double-acting compression is achieved by controlled downward movement of the die at a rate proportionately less than the downward displacement of the upper punch. The resulting compression effect is the same as if the die were fixed and the lower punch moved upward at a controlled rate equal to, greater than, or less than the downward movement of the upper punch. The system selected also simplifies the measurement of compaction and ejection forces using the Instron machine[1].

DESIGN DETAILS

The schematic diagram of the simulator (Fig. 1) shows the apparatus assembled with 33-mm. diameter plane-faced compression tooling (A, B, and C) from a rotary tablet machine[2]. The upper and

[1] Model TTDM, Instron Corp., Canton, Mass.
[2] Stokes DS3, F. J. Stokes Corp., Philadelphia, Pa.