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Simultaneous Quantitative Gas Chromatographic Determination of Atropine and Scopolamine

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Abstract □ Atropine and scopolamine as free bases were simultaneously quantitated by GLC. Atropine and its sulfate salt produced peaks as apoatropine and atropine base. Scopolamine base produced peaks of scopolamine and a product of lower molecular weight. Linear standard curves were obtained between 0.5 and 200 mcg. for each alkaloid. Pure compounds and plant extracts were compared with the USP XVII assay for total alkaloids of belladonna.

Keyphrases □ Atropine, scopolamine—simultaneous determination □ TLC—identity □ GLC—analysis

The work of Quin (1) on tobacco smoke and Lloyd (2) on the gas chromatography of high molecular weight alkaloids led Brochmann-Hanssen and Svendsen (3) to attempt separation of a large number of alkaloid mixtures from various plant sources, including atropine and scopolamine. No attempt was made to quantitate these alkaloids, but some of the difficulties to be anticipated in a quantitative GLC procedure were enumerated. The formation of apoatropine catalyzed by glass wool in the column was discussed. The use of GLC in the simultaneous determination of methapyrilene fumarate, ephedrine hydrochloride, and codeine phosphate in syrup was recently demonstrated by Wesselman and Koch (4). In an intensive study Rader and Aranda (5) extended the applicability of GLC quantitative procedures to various drug mixtures. Wesselman (6) assayed terpin hydrate and codeine elixir by GLC procedures.

This paper reports the quantitation of atropine and scopolamine simultaneously as pure compounds and from plant extracts.

EXPERIMENTAL

Equipment—A linear programmed temperature gas chromatograph[1] equipped with a flame-ionization detector was used. The detector signal was printed on a 1-mv. recorder[2] with a chart speed of 1.27 cm. (0.5 in.)/min. and 1-sec. full-scale response. The 2-μl. samples were injected with a 10-μl. syringe.[3] Continuous extraction apparatus was used for all extractions of plant powders according to the method of USP XVII (7). No attempt to estimate the combined precision of the extraction procedure with the GLC procedure was made.

Materials—The carrier gas was helium. Hydrogen and air were used in the flame-ionization detector. The stationary phase was diatomite aggregate,[4] DMCS 80/100 with methyl silicone gum rubber[5] liquid phase at a concentration of 2.5%. Dual borosilicate glass columns 182.88 cm. (6 ft.) × 0.19 cm. (0.075 in.) inside diameter were filled with prepared packing material[6] under reduced pressure with uniform vibration. Packing material was held in place in the column by the smallest pledgets of Pyrex glass wool practicable. Chromatographically pure chloroform was used throughout as the solvent for the free bases of atropine and scopolamine. Atropine sulfate was dissolved in reagent grade anhydrous methyl alcohol. The powders of *Atropa belladonna* and *Datura stramonium* used in this work were 60-mesh powders. Thin-layer chromatograms of each compound indicated one spot for each standard.[7]

Operating Conditions—The column temperature was programmed from 150–275° at the rate of 6°/min. At the end of each run the column was cooled for 10 min. and then equilibrated for 3 min. at 150° before injecting the next sample. The injection port temperature was maintained at 315°. The mean helium flow rate was 99.5 ± 0.5 ml./min. Air and hydrogen were maintained at 48 and 24

[1] Perkin-Elmer model 881.
[2] Sargent model SR (S-72180-20).
[3] Hamilton No. 701.
[4] Chromosorb G, acid-washed.
[5] SE30/S.
[6] Perkin-Elmer Co.
[7] Atropine and scopolamine used as standards were obtained from the Aldrich Chemical Co.

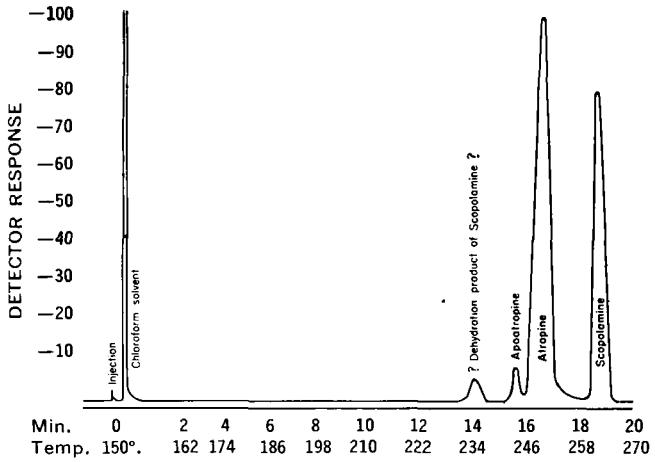

Figure 1—*Gas chromatogram of atropine and scopolamine as free bases in chloroform. Programmed 150 to 275° at 6°/min. Attenuation ×20.*

psig. inlet pressure, respectively. Attenuations of ×10, ×20, ×50, ×100, ×200, and ×500 were used to obtain good precision over a useful range of samples.

Standard Curve—Although dehydration occurred, quantitation of both alkaloids was accomplished (Fig. 1). A linear function for the quantity of alkaloid *versus* peak area for the two alkaloids and their respective dehydration products was obtained (Table I). Approximation of the equations for the two standard curves were obtained by regression analysis (8). Pilot studies indicated that atropine sulfate and hydrobromide could be quantitated but the scopolamine salts (especially the hydrobromide) underwent extensive decomposition. The error of quantitating apoatropine as atropine (0.018%) was less than the estimated precision (3.07%). The apoatropine peak was identified by addition of apoatropine base and hydrochloride to atropine in the base and sulfate forms. Data obtained and used for the standard curve were determined each day over an 8-day period. Blind unknowns containing weighed amounts of atropine and scopolamine in chloroform were used to substantiate the reliability of the standard curve.

Precision and Accuracy Study—Weighed samples containing the two alkaloids in chloroform were assayed 3 times daily for 5 successive days by both the GLC and USP methods to determine the relative precision and accuracy.

Quantitation of the Alkaloids of Plant Powders—Approximately 10 g. of the 60-mesh dried powders of *A. belladonna* and *D. stramonium* were extracted independently according the USP XVII (7) method of extraction and isolation of the total alkaloids of belladonna leaf. Two samplings of each extract (2 μl. each) were quantitated on the gas chromatograph and an estimate of precision was made. Two samples of 1 ml. each from the same extracts were quantitated by the USP assay for total alkaloids and the estimated precision of the assay on the same samples was determined.

RESULTS AND DISCUSSION

Initial experimentation indicated that the sulfate and hydrobromide salts of atropine dissolved in anhydrous methanol could be quantitated in the range of 0.5 to 200 mcg. Salts and free bases of atropine and apoatropine produced peaks with comparable retention times (apoatropine 10 min. 36 sec. ± 5 sec. and atropine 12 min. 6 sec. ± 13 sec.) between 150–300° programmed at 10°/min. The peaks always came off at 242° for apoatropine and 255° for atropine. It appears that a dehydration of scopolamine occurred when chromatographed as the free base. Scopolamine applied as the hydrobromide salt gave a chromatogram with seven separate peaks possibly indicating that decomposition and rearrangement had occurred. Because of the abnormalities that may occur when the alkaloids are present as certain salts, further studies emphasized the use of alkaloids as free bases.

Use of the base forms of the alkaloids in chloroform programmed at 6°/min. between 150–275° was quantitative in the range of 0.5–210.4 mcg. for atropine and 0.5–187.5 mcg. for scopolamine. Dehydration still occurred but if the two respective products for each alkaloid were quantitated together as atropine and scopolamine, the error was insignificant compared with the precision of successive runs. Linearity was obtained at all levels tested. Blind unknowns validated the accuracy of the standard curve (Table II).

A precision and accuracy study (Table III) was made on both high and low concentrations of both alkaloids in mixtures. The precision of the high concentrations of the two alkaloids were comparable (3.07 and 3.65%) while the determinations of scopolamine were more precise than atropine at low concentrations (5.01 and 1.77%). Five replications of three samples blocked by successive days were used to take into account daily fluctuations of the method as described by Snedecor in his chapter on Two-Way Classifications (8). The USP method was more precise than the GLC method in higher alkaloid concentration (53.52 mg./ml. total alkaloid expressed as atropine) but the accuracy was not as good (−11.08%). This discrepancy was attributed to the heating of the sample and back titrating to the methyl red end point rather than to pH 7.0. Both the accuracy and precision of the USP method at low ranges (2.095 mg./ml. total alkaloid expressed as atropine) were poor (−30.39%). The USP method appeared to be precise in the intended assay range. For research work including samples with extremes of alkaloid content the GLC method appears to have better accuracy and precision.

Samples of two solanaceous plants were examined by the GLC and USP methods for precision in successive samplings of plant extracts (Table IV). The mean estimated precision for atropine and scopolamine in the GLC assay compared favorably with the mean estimated precision for total alkaloids in the USP assay. Estimated precision using GLC for atropine and scopolamine was 2.650 and 2.50%, respectively, whereas that of the total alkaloid USP assay was 2.245%. In addition, it should be noted, however, that the USP assay required milligram amounts of alkaloid in order to obtain high precision. The GLC assay was quantitative on both milligram and microgram levels from 53 mg. (Table II) to as low as 2 mcg. (Table III) and had the added advantage of determining the absolute amounts of atropine and scopolamine present in the sample simultaneously.

SUMMARY

Atropine and scopolamine were quantitated from mixtures of pure compounds and plant extracts. A linear relationship between peak area and concentration of alkaloid was obtained for both alkaloids.

Table I—Summary of the Regression Analyses on the Standard Curves of Atropine and Scopolamine by GLC

	Atropine (Free Base)	Scopolamine (Free Base)
Equation of standard curve	$\hat{X} = 1.8600 + 0.1784\ Y_{x\ 200}$[a]	$\hat{X} = -0.1200 + 0.2472\ Y_{x\ 200}$[a]
95% Confidence limits of slope[b]	0.1752 and 0.1816	0.2427 and 0.2517
Minimum *SE* of $\bar{X}$[c]	at 75 mcg.	at 40 mcg.
	372 to 412 integrator counts	145 to 165 integrator counts
Minimum *SE* of $\hat{X}$[d]	at 75 mcg.	at 40 mcg.
	362 to 422 integrator counts	100 to 160 integrator counts

[a] Alkaloid value ($\hat{X}$) = 1.8600 mcg. + 0.1784 × no. of integrator counts adjusted to attenuation × 200. [b] Confidence limits of Slope b. In 95% of the cases the slope obtained from such a standard curve would be between the limits of the values given (8). [c] *SE* $\bar{X}$ = standard error of predicting mean value at a given point. [d] *SE* $\hat{X}$ = standard error of prediction of an individual value.

Table II—Simultaneous Determination of Unknown Amounts of Atropine and Scopolamine (Pure, Free Bases)[a]

Unknown Number	Atropine, mg./ml. Actual	Assayed	Accuracy Absolute	Accuracy Relative, %	Relative Precision, %	Scopolamine, mg./ml. Actual	Assayed	Accuracy Absolute	Accuracy Relative, %	Relative Precision, %
1	2.560	2.300	−0.26	9.00	3.03	13.45	12.99	−0.46	3.54	0.177
2	13.17	14.31	+1.140	8.35	1.68	4.538	3.56	−0.98	27.5	3.95
3	6.169	4.210	−1.952	28.2	4.75	14.100	12.68	−1.42	11.19	2.86
4	41.21	40.83	−0.379	1.01	0.795	8.383	9.44	+1.057	12.50	1.80
5	16.95	15.55	−1.395	8.21	1.88	15.871	15.44	−0.431	2.78	0.786
6	20.51	19.90	−0.610	2.97	1.66	17.92	17.87	−0.05	0.280	0.391
7	0.00	0.00	—	—	—	40.10	42.40	+2.297	5.41	0.708
8	1.582	1.740	−0.158	9.99	2.87	27.80	28.55	+0.753	2.63	0.609
9	53.28	54.25	+0.966	1.78	1.80	5.187	5.395	+0.208	3.85	2.78
10	32.53	35.54	+3.013	9.26	0.562	0.00	0.00	—	—	—

[a] Blind gravimetric unknowns of atropine + scopolamine (free bases) in chloroform.

Table III—Precision and Accuracy Study Alkaloid Determinations

	GLC Method				USP XVII Method	
	Atropine		Scopolamine		Total Alkaloids	
	High Concn.	Low Concn.	High Concn.	Low Concn.	High Concn.	Low Concn.
No. Detn. (N)	15	15	15	15	15	15
Mean of Detn. ($\bar{X}$)	38.71 mcg.	1.99 mcg.	22.44 mcg.	1.13 mcg.	53.52 mg.	2.095 mg.
SD	±1.19	±0.10	±0.818	±0.02	±0.00159	±0.6887
RSD	±3.07 %	±5.01%	±3.65%	±1.77%	±0.030%	±20.95%
Mean error	+0.42 mcg.	+0.07 mcg.	−0.52 mcg.	−0.02 mcg.	−6.67 mg.	−0.9147 mg.
Relative error	+1.19%	+3.66%	−2.26%	−1.74%	−11.08%	−30.39%

Table IV—Simultaneous Determination of the Alkaloids of Belladonna and Stramonium Powders Extracted by the USP Method[a]

Extract	GLC Method Wt. of Powd. Ext., g.	Atropine, % of Powd. Wt.	Estimated Precision, %[b]	Scopolamine, % of Powd. Wt.	Estimated Precision, %[b]	USP Method Total Alkaloids, % of Powd. Wt.	Estimated Precision, %[b]
Belladonna extracts							
1	10.36285	0.246	1.157	0.096	4.170	0.142	2.178
2	10.34959	0.240	6.513	0.075	4.640	0.318	1.119
3	10.15134	0.061	2.632	0.017	4.384	0.034	4.766
4	10.32442	0.237	2.602	0.072	0.000 (no diff.)	0.381	2.139
5	9.93384	0.211	2.595	0.077	3.111	0.244	1.615
Stramonium extracts							
1	10.33883	0.349	1.678	0.053	3.774	0.321	3.197
2	9.75524	0.276	4.343	0.055	0.000 (no diff.)	0.434	0.7571
3	9.74233	0.314	1.941	0.054	1.471	0.344	3.051
4	10.36721	0.291	1.615	0.052	0.000 (no diff.)	0.361	2.861
5	9.91891	0.317	1.420	0.060	3.535	0.350	0 7657

[a] Underlined digits indicate an estimated value. [b] Range $X \times 100\%$.

Atropine and scopolamine both produced two peaks. The maximum ranges of precision (atropine 3.07% and scopolamine 3.65%) were obtained at intermediate optimum concentrations.

The GLC method was found applicable to analyses of plant material with mean precisions comparable to the precision possible in determining pure compounds.

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