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Gas-liquid Chromatographic Analysis of Some Tropane Alkaloids

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Abstract—The aim of this investigation was to develop a quantitative gas-liquid chromatographic method of analysis forhyoscyamine and scopolamine, and to apply this method to the analysis of preparations. The trimethylsilyl(TMS) derivatives of the alkaloids were found to be far superior to the nonsilylating compounds in chromatograms. Bis(trimethylsilyl) acetamide(BSA) was evaluated and found to be a good reagent for silylation of the alkaloids. The optimum derivatization conditions were heating the alkaloids in a closed tube at 70° for 30 min with a 150 molar excess of BSA to the alkaloids. Calibration curves for the two alkaloids were found to be linear over a sampleweight range of 1~9 μg of each alkaloid. The standard deviations were 1.1% for hyoscyamine and 1.5% for scopolamine. The minimum detectable amount using the hydrogen flame ionization edtector was determined to be 2×10^{-11} moles of each alkaloid injected.

Both hyoscyamine and scopolamine are tropane alkaloids found mostly in the Scopoliae Rhizoma in nature and are used as pharmaceuticals in the form of scopolia ex. or powdered scopolia ex. The ratio of the two alkaloids varies from 9 : 1 to 6 : 4 in various plants and these plants also differ in their pharmacological effect.

In the method of quantitative analysis of tropane alkaloids in herbs and scopolia ex., which is now specified in the Korean Pharmacopoeia, each alkaloid is not analyzed separately, but total alkaloids are analyzed at the same time by titration in aqueous media[1,2] as hyoscyamine. And generally adopted quantitative analysis of scopolia ex. contained in the ordinary complicated preparations is limited to analyzing only hyoscyamine colorimetrically[3] by spectrophotometer at 430 nm. However, as only hyoscyamine can be analyzed by this method, the total quantity of both alkaloids contained in the scoplia ex. especially abundant with scopolamine are often lessened.

For the qualitative and quantitative analyses of the tropane alkaloids, the main stream was as follow: 1) Specific color formation, 2) measuring its absorbance after separating alkaloids by TLC[4] 3) titration in aqueous media, and 4) UV absorption[5,6] or IR[7].

The method of qualitative identification of tropane alkaloids by gas-liquid chromatography[8~10] was reported by Hanssen, *et al.*[11] and Kazyak, *et al*[12]. But the method of quantitative analysis of tropane alkaloids by GLC has not been reported.

Paying attention to this point, the authors established a new method of quantitative and simultaneous analysis of both alkaloids by using trimethylsilylating reagent.

EXPERIMENTAL

Apparatus and reagents—A GC-4BM Gas Chromatograph(Shimadzu) equipped with dual hydrogen flame ionization detectors was used. The chromatographic columns used were 3% OV-17 on 80-100 mesh Shimalite. These column materials were packed in glass columns (borosilicate) which were 1 m long with an inside diameter of 3 mm. The instrumental conditions were fixed at 170°.

The detector was operated at 250°. The gas flow rate was as follows: N_2, 60 ml/min; H_2, 0.8 kg/cm^2; air, 1.2 kg/cm^2. The attenuation was 6.4×10^{-8} ampere full scale deflection (a.f.s.).

The reagents used are followings: HyoscyamineHBr and scopolamineHBr were purchased from E. Merck Co., and were chromatographically pure. Bis(trimethylsilyl) acetamide (BSA) was purchased from Sigma Chemical Co.

Analytical method—Stock solutions of hyoscyamine and scopolamine were prepared by dissolving 50mg of each alkaloid in 10 ml of pyridine.

Each 0.5 ml of stock solution was measured into 16×75 mm screw cap culture tube and 2.5 mg of caffeine as the internal standard was added. After the addition of 0.5 ml of BSA, the tube was tightly capped with a teflon lined cap. The sample was heated in the closed tube for 30 min at 70° in oil bath, then cooled to room temperature and the aliquots of 2 μl were injected into the gas chromatograph. The molar response of the TMS alkaloid relative to caffeine, RMR A./Caf., was calculated from the weighed area of each experimental chromatographic peak.

RESULTS AND DISCUSSION

Silylation of alkaloids using BSA and its optimal requirement for molar excess—When the BSA method of derivatization was compared to the TMSC (trimethyl silyl chloride) method and non-silylating method, BSA was found to have the advantages of speed, simplicity of the peaks on the GLC and higher yield of the derivatives (Fig. 1). Therefore, it was shown to be a superior method of derivatization.

The different amount of BSA added were corresponded to 30, 65, 100, 150 or 210 times of molar excess, respectively. The data presented in Fig. 2 show that a minimum of a 100 molar excess of BSA was necessary to obtain a maximum yield of the TMS derivative of alkaloid.

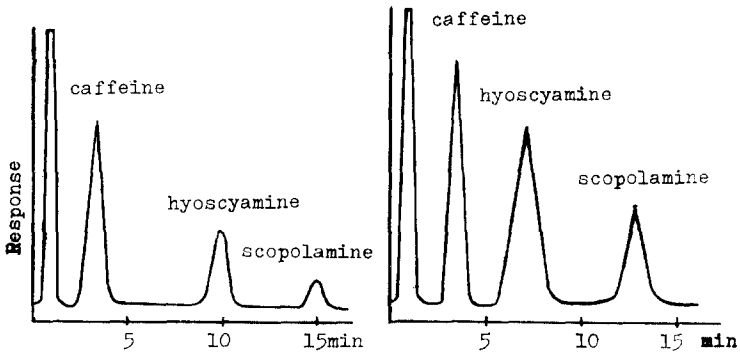

Fig. 1—Comparative gas-liquid chromatogram of non silylated (left) and silylated(right) alkaloids.

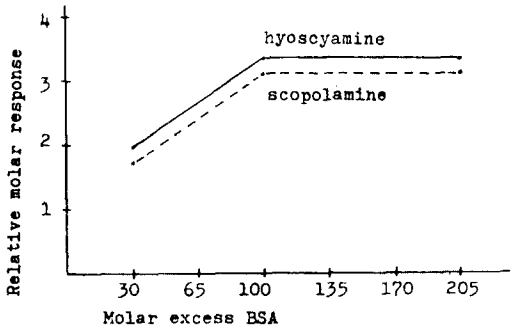

Fig. 2—Effect of BSA concentration on silylation.

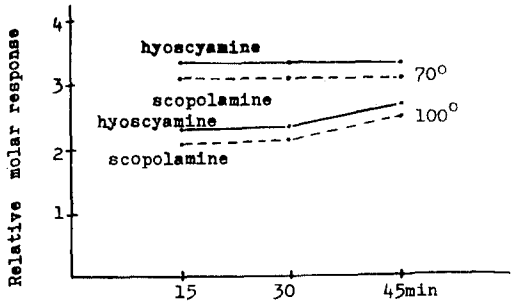

Fig. 3—Effect of silylation time and temperature on RMR.

Time and temperature required for maximum derivatization—The relative molar response, RMR A./Caf., for each alkaloid was plotted as a function of reaction time. It was shown in Fig. 3. The optimum silylation conditions for hyoscyamine and scopolamine, carried out by heating 15, 30 and 45 min at the temperature of 70° and 100°, were found to be 70° in a close tube for 30 min with a over 100 molar excess of BSA.

Calibration curves of hyoscyamine and scopolamine—Calibration curves were prepared to demonstrate the quantitative aspects and reproducibility of the linear range of response for the formation of the TMS alkaloid derivatives with BSA. The curves were found to be linear over a sample weight range of 1 to 9μg of each alkaloid, as shown in Fig. 4.

The minimum detectable amount using the hydrogen flame ionization detector was determined to be 2×10^{-11} moles of each alkaloid injected.

Reproducibility of silylation and stability of silylated derivatives using BSA—Optimum silylation conditions for the two alkaloids using BSA were chosen from the previous experiments at 70° for 30 min. These reaction conditions were used to check the reproducibility of silylation and the stability of the TMS alkaloids with time. The retention time and RMR are given in Table I, including standard deviation for the alkaloids.

The stability of the TMS alkaloids was maintained for 18 hrs on running for 24 hrs at

Table I—Retention time and relative molar response of the TMS derivatives

Compound	Retention time(min).	RMR A./Caf.	S.D.
Hyoscyamine	7.5	3.32	1.1
Scopolamine	12.5	3.06	1.5
Caffeine	3.5	1.00	—

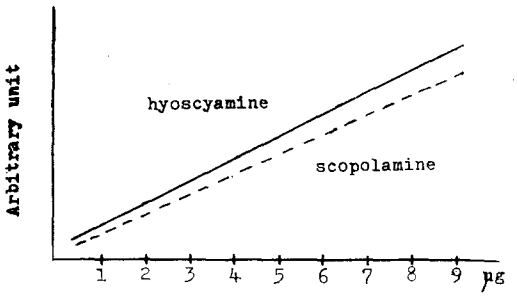

Fig. 4—Calibration curve.

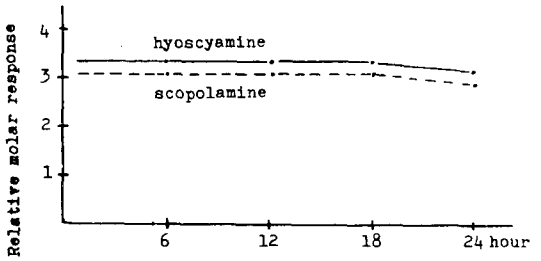

Fig. 5—Stability of the TMS alkaloid.

Table II—Results of analysis of preparations

Sample	Total sample(g)	Hyoscyamine(mg)	Scopolamine(mg)
Scopoliae rhizoma	10.000	69.50	4.83
Preparation A	20.001	1.16	0.10
Preparation B	20.001	0.36	0.03
Preparation C	20.000	0.09	—
Preparation D	20.005	0.18	0.02

the same chromatographic conditions, as shown in Fig. 5.

Analysis of preparations—The application of the developed method for tropane alkaloids to the analysis of preparations selling in the market was accomplished. In this five preparations the results obtained are as Table II. The extraction method of tropane alkaloid from he preparations was based upon the method guided by the Korean Pharmacopoeia.

CONCLUSIONS

The purpose of this investigation was to develop a quantitative gas liquid chromatographic method of analysis for the components of tropane alkaloids as hyoscyamine and scopolamine, and further to apply this method to the analysis of preparations selling in the market. Gas-liquid chromatography has the advantages of speed, accuracy and simplicity over the other methods of analysis.

The optimum derivatization conditions were heating the alkaloids in a closed tube at 70° for 30 min with a 150 molar excess of BSA to total alkaloids. Calibration curves for the two alkaloids were found to be linear over a sample weight range of 1~9 μg of alkaloid. The standard deviations are 1.1% for hyoscyamine and 1.5% for scopolamine.

The relative molar responses of the two alkaloids to caffeine as the internal standard were determined.

The GLC method for tropane alkaloid analysis gave the quantitative and reproducible results.

REFERENCES

1. Ministry of Health and Social Affairs, *Korean Pharmacopoeia*, 2nd Ed., **1967**, p-254.
2. R. Higuchi and E. Brochmann-Hanssen, *Pharmaceutical Analysis*, New York, London, **1961**, p-370.
3. F. Durick, *Am. Assoc. Sci. Ed.*, **39**, 680 (1950).
4. W.C. Evans and M.W. Partridge, *J. Pharm. Pharmacol.*, **4**, 769 (1952).
5. E. Berman and H.N. Wright, *Arch. Ind. Hyg. and Occupational Med.*, **8**, 518 (1953).
6. W.F. Elvidge, *Quart. J. Pharm. Pharmacol.*, **13**, 219 (1940).
7. R.S. Browning, *Anal. Chem.*, **27**, 7 (1955).
8. D. Lakings and C. Gehrke, *Chromatography*, **62**, 347 (1971).
9. *Ibid.*, **38**, 473 (1968).
10. *Ibid.*, **61**, 45 (1971).
11. E. Brochann-Hanssen and A. Baerheim Svendsen, *J. Pharm. Sci.*, **51**, 1095 (1962).
12. L. Kazyak and C. Edward, *Anal. Chem.*, **35**, 1448 (1963).