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Gas chromatographic separation of tobacco alkaloids*

Many attempts have been made to separate tobacco alkaloids by gas chromatography. Methods described in the literature have resulted in incomplete resolution of some of the minor alkaloid components, extremely long retention times, poor base line, or a combination of these factors. QUIN[1] reported the separation of tobacco alkaloids using 25 % polyethylene, polypropylene or polybutylene glycol as the liquid phase. Relative retention times indicate that nicotine, nornicotine–myosmine, and anabasine were separated into three respective peaks. ALWORTH *et al.*[2,3] modified this technique by using 10 % polybutylene glycol on KOH-treated firebrick at 169° with a carrier gas flow of 300 ml/min. Relative retention times of 1.00, 1.64, 2.08 and 2.56 were obtained for nicotine, nornicotine, anabasine, and anatabine, respectively. Recently ANASTASOV *et al.*[4]. separated nicotine, nornicotine–myosmine, and anabasine into three respective peaks with 20 % Apiezon L on Chromosorb W with retention times of 30 to 70 min.

KOBASHI AND WATANABE[5] used 2 m columns packed with 24 % polyethylene glycol, a carrier gas flow of 80 ml /min, and a column temperature of 180° to separate myosmine from nornicotine; however, retention times were 100 min and resolution was not complete. MASSINGILL AND HODGKINS[6] separated nicotine and anabasine with 1 % liquid phase of SE-52, JXR or XE-60 using a programmed temperature of 100–300° at 12°/min on 1.83 m columns with flow rates of 60–70 ml/min. MCNIVEN *et al.*[7] separated nicotine from cotinine with a 14.5 % SE-30 liquid phase, a column temperature of 200° and a carrier gas flow of 10 to 20 ml/min. YASUMATSU[8] separated nicotine, nornicotine, anabasine and anatabine, with respective relative retention times of 1.00, 1.57, 1.97 and 2.36, using a 2.25 m column of 25 % DC 550, a column temperature of 200°, and a carrier flow adjusted to 60 ml/min. Actual retention times ranged from 15 min 30 sec for nicotine to 36 min 40 sec for anatabine.

Capillary columns have also been tested for separation of tobacco alkaloids. MASSINGILL AND HODGKINS[6] used 30.5 m columns coated with QF-1 or Apiezon L and a 61 m column treated with SE-30 to separate nicotine and anabasine. Compared with nicotine, anabasine had relative retention times of 2.04, 3.03 and 1.25, respectively. More recently, HARKE AND DREWS[9] attempted separation of tobacco alkaloids on a 50 m capillary column coated with UCON LB 550 (polypropylene glycol) and obtained good resolution of nicotine, nornicotine and anabasine but were unable to separate nornicotine from myosmine.

This investigation compared the analyses of nicotine and four of the minor tobacco alkaloids on three gas chromatographic column substrates for retention times, effective plate value, and resolution.

Procedure

The gas chromatograph was a Barber-Coleman Model 5000 equipped with a dual

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flame ionization detector. The columns were 2.44 m coiled glass with 3.5 mm I.D. Unless otherwise stated the column temperature was 170°, the injector temperature was 225° and the detector temperature was 370°. Helium was the carrier gas at a flow of 30–40 ml/min at 50 p.s.i. Samples of nicotine, nornicotine and anabasine were obtained from K&K Laboratories. Myosmine was obtained from Dr. T. C. Tso, United States Department of Agriculture, Beltsville, Md., and anatabine was obtained from Dr. E. GLOCK, American Tobacco Company, Hopewell, Va. The alkaloids were dissolved in methyl acetate and 0.5 to 3.0 μl were injected with a 10 μl syringe. The attenuation of the gas chromatograph was 32 or 64 $\times$ 10^{-9} A input current for full-scale output voltage.

The columns were packed with acid-washed and dimethyldichlorosilane-treated 60–80 mesh Chromosorb W (Applied Science) coated with 5 % SE-30 (methyl silicone rubber), 10 % Versamid 900 (an ethylenediaminelinoleic acid polyamide resin) or 10 % DC 550 (methylphenyl silicone oil). The liquid phase SE-30 was selected because it is recommended as the most suitable liquid phase for alkaloid separations. The liquid phase DC 550 was selected because it is similar to SE-30 but has 1/6 of the methyl groups replaced by phenyl groups. Versamid 900 was selected because it is recommended for aromatic and nitrogen compounds. The results presented in this report are representative of our experience with these liquid phases and were obtained under comparable conditions, which allows evaluation of relative merits. Achievement of optimum temperature, flow rate of carrier gas, and quantity of liquid phase was attempted for each column.

Results

The gas chromatographic separations obtained with the five tobacco alkaloids with the three liquid phases are presented in Tables I and II. All relative retention values (r) are given with respect to nicotine and the effective plate value (N) was calculated according to ETTRE[10]. The order of elution was nicotine, nornicotine, anabasine and anatabine with all column substrates. However, when myosmine was injected with the other alkaloids it was eluted before nornicotine on the Versamid 900 column

TABLE I

RELATIVE RETENTION TIME (r), EFFECTIVE PLATE VALUES (N) AND RESOLUTION (R) OF TOBACCO ALKALOIDS

The 2.44 m columns were packed with 10 % DC 550 or 5 % SE-30 on Chromosorb W. Column temperatures were 170° and 190°, and carrier flow was 40 and 30 ml/min, respectively.

Alkaloid	*DC 550*			*SE-30*		
	r	N	R	r	N	R
Nicotine	1.00	2532		1.00	1390	
			5.12			1.57
Nornicotine	1.55	2320		1.21	1366	
			1.24			
Myosmine	1.87	—		—	—	1.20
			0.64			
Anabasine	2.03	2511		1.41	1302	
			2.18			
Anatabine	2.50	2000		—	—	

TABLE II

RELATIVE RETENTION TIME (r), EFFECTIVE PLATE VALUES (N) AND RESOLUTION (R) OF TOBACCO ALKALOIDS

The 2.44 m columns were packed with 10% Versamid 900 on Chromosorb W. Column temperature was 170° and carrier gas flow 40 ml/min.

Alkaloid	*Versamid 900*		
	r	N	R
Nicotine	1.00	1258	
			9.0
Myosmine	1.97	1290	
			0.52
Nornicotine	2.15	1330	
			1.78
Anabasine	2.60	1642	
			3.28
Anatabine	3.28	1580	

(Table II), whereas on the DC 550 column myosmine was eluted after nornicotine (Table I). The tobacco alkaloids had the shortest retention time on SE-30 (nicotine 2 min 3 sec). DC 550 and Versamid 900 liquid phases gave longer retention times but were similar to each other except for nicotine. Nicotine was eluted considerably faster on Versamid 900 (6 min 29 sec) than on DC 550 (9 min 4 sec), and consequently the relative retention times were greater for Versamid 900. Relative retention times were 1.00, 1.55, 1.87, 2.03 and 2.50 for nicotine, nornicotine, myosmine, anabasine, and anatabine, respectively, on DC 550, and the corresponding (r) values on Versamid 900 were 1.00, 2.15, 1.97, 2.60 and 3.28.

Decreasing the amount of liquid phase resulted in shorter retention times and increasing the liquid phase above 10% resulted in broadening of peaks, and in both instances poorer separations were achieved. Unsatisfactory separations were achieved with carrier flow above 30 ml/min with the SE-30 column. The optimum flow for the DC 550 and Versamid 900 columns was 40 ml/min. The temperature optima were 170° to 180° for the DC 550 and Versamid 900 columns and 190° for the SE-30 column. Decreased temperature increased elution time but resulted in poor separation and was expressed by a 3 to 8-fold decrease in N values. At our optimum conditions, Versamid 900 and SE-30 had similar N values (1300). At lower temperatures SE-30 had N values of 500 or less, whereas with Versamid 900 the N values were in the range of 1000 to 1800 as temperature ranged from 170° to 190° and carrier flow ranged from 30 to 60 ml/min. DC 550 had the highest N values (2000–3200) within the 170–190° temperature range and 30–60 ml/min range for carrier flow. Because only a minimal quantity of myosmine was available and because of the very low attenuation used (8×10^{-12} A input current for full-scale output voltage) the N value for myosmine on DC 550 was difficult to calculate but appeared lower than those obtained for the other alkaloids on the same column.

Resolution (R) or completeness of separation between two components was calculated by the equation

$$R = \frac{2(t_{r2} - t_{r1})}{W_2 + W_1}$$

where t_{r2} is the retention time, from injection, of the second eluting component and t_{r1} is the corresponding retention time for the first eluting component. The peak widths, W_1 and W_2, are determined from the intersection of the baseline by the tangents to the inflection points of the peaks. If $R = 1.5$, the two peaks are 99 % resolved and if $R = 1.0$ the two peaks are 95 % resolved.

Under the conditions used in these investigations the DC 550 and Versamid 900 columns were superior to the SE-30 column. On both the DC 550 and Versamid 900 columns nicotine and anatabine were completely resolved from the nearest component. The middle three alkaloids in the elution pattern were more difficult to separate. Nornicotine and myosmine were 95 % resolved on the DC 550 column but myosmine was not appreciably separated from anabasine. On the Versamid 900 column myosmine was eluted prior to nornicotine and the two peaks were poorly resolved but nornicotine and anabasine were 99 % resolved. With both the DC 550 and Versamid 900 columns nicotine, nornicotine, anabasine, and anatabine were 99 % resolved from each other. In tobacco tissue myosmine is usually present in smaller amounts than the other four alkaloids examined and hence causes little interference. But, because of the small amounts, it has been difficult to quantify in the presence of nornicotine and anabasine. Separation of all five alkaloids on the SE-30 column was not attempted because of the rapid elution pattern obtained for nicotine, nornicotine and anabasine and the poor separations of tobacco alkaloids on SE-30 reported in the literature[6]. Only 95 % resolution was obtained with the three major tobacco alkaloids on the SE-30 column, but because of peak symmetry and rapid elution this column may be useful for partially purified samples. The DC 550 and Versamid 900 columns were similar with respect to overall analysis but the retention time and resolution between nornicotine and anabasine were superior on DC 550 and this column is being used routinely for the analysis of tobacco samples in our laboratory.

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