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Quantitative analysis of tobacco alkaloids by gas chromatography*

Utilization of gas–liquid chromatography (GLC) to separate and quantify tobacco alkaloids has received much attention recently. Most methods described in the literature have been concerned with the difficulties of complete resolution of the tobacco alkaloids. QUIN[1] first reported the use of GLC to separate tobacco alkaloids and resolved nicotine, nornicotine-myosmine, and anabasine into three respective fractions. ALWORTH *et al.*[2,3] modified the technique of QUIN[1] and partially separated nicotine, nornicotine, anabasine and anatabine. Others[4–9] have reported the use of different liquid phases for separating various fractions of tobacco alkaloids. YASUMATSU[10] separated nicotine, nornicotine, anabasine and anatabine by using 25 % DC 550 as the liquid phase. WEEKS *et al.*[11] improved the method by using 10 % DC 550, modified the operating conditions and reported a minimum of 95 % resolution of nicotine, nornicotine, anabasine and anatabine.

LYERLY[12] described a direct vapor chromatographic determination of nicotine in smoke. Quantitative determinations were made rapidly and were equivalent to data obtained by spectrophotometry. JACIN *et al.*[13] determined the quantities of nicotine in tobacco and tobacco smoke using a 10 % neopentyl glycol adipate column. With many different tobacco samples they obtained a 3 % maximum deviation of the mean and a 100 ± 3 % recovery of nicotine added to various tobacco samples. YASUMATSU AND MURAYAMA[14] have reported quantitative determination of nicotine in tobacco samples by GLC with isoquinoline as an internal standard.

All quantitative procedures described in the literature are for nicotine and no quantitative procedures have been described for the other tobacco alkaloids. The objective of this investigation was to develop a quantitative GC procedure for the four most important tobacco alkaloids. The procedure described is based primarily on the separation of the tobacco alkaloids as previously reported by WEEKS *et al.*[11].

Procedure

The gas chromatograph was a Varian Model 1740 with flame ionization detectors. Columns were 3.05 m × 2 mm I.D. coiled glass packed with 10% DC 550 (methylphenyl silicone oil) on acid-washed, dimethyldichlorosilane-treated 60–80 mesh Chromosorb W. The oven temperature, injection port temperature and detector oven temperature were 185°, 250° and 300°, respectively. The carrier gas, nitrogen was metered at 30 ml/min at 60 p.s.i. Quinoline, nicotine, nornicotine and anabasine were obtained from K & K Laboratories. Anatabine was obtained from Dr. E. Glock, American Tobacco Company, Hopewell, Va. The alkaloids were dissolved in benzene and 0.2 to 20 μg (0.5 to 2.0 μl) either alone or in a mixture were injected. Peak areas were determined by peak height × peak width at half-height.

The extraction and isolation of alkaloids from tobacco samples was accomplished by a modification of the procedure of KELLER *et al.*[15]. 1 g of dried tobacco tissue ground to pass 40-mesh screen, 250 mg of barium hydroxide and 5 ml of a saturated

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aqueous solution of barium hydroxide were placed in an 125-ml erlenmeyer flask and mixed. 40 ml of benzene–chloroform (9:1, v/v) solution containing the appropriate amount of internal standard (quinoline) was added and the mixture was mechanically shaken for 30 min. 750 mg of Celite were then added and dispersed by shaking the flask. The organic phase was filtered through Whatman No. 1 filter paper. 10 ml of filtrate was reduced *in vacuo* at 50° to 0.2 to 0.5 ml and approximately 1 μl was injected into the gas chromatograph for determination of nornicotine, anabasine and anatabine. To determine nicotine, 10 ml of the filtrate was reduced to approximately 1 ml prior to injection of approximately 1 μl. Because of the great difference in quantities of the minor alkaloids (nornicotine, anabasine and anatabine) and nicotine present in most tobacco samples two extractions, each with an appropriate amount of internal standard, were required for a complete assay.

Exogenous tobacco alkaloids were added to tobacco tissue by dissolving the alkaloids in ethyl acetate and spraying the alkaloid solution on tobacco at a rate of 250 ml per 1000 g tobacco. The control samples were also sprayed with ethyl acetate at the same rate. Extractions were then performed as outlined above.

Results and discussion

WEEKS *et al.*[11] reported that 10% DC 550 on Chromosorb W was superior to other liquid phases tested for qualitative resolution of tobacco alkaloids. To maximize the use of this procedure for quantitative analysis of tobacco alkaloids one must minimize potential errors. To eliminate the need for injection of exact volumes and frequent determination of calibration curves, quantitation was accomplished by use of an internal standard. This method required knowledge of the relative detector response of all the components. The relative weight response (*RWR*) of the four principal tobacco alkaloids are presented in Table I. The greatest percentage standard deviation (7.2%) was observed with nornicotine and the smallest deviation (2.0%) was observed for nicotine. The *RWR* will change, sometimes significantly, with changes in operating conditions—carrier gas flow-rates, cleanliness of detector, and temperature. Therefore, *RWR* must be determined for each detector and each set of operating conditions. It is suggested that an authentic alkaloid sample with the internal standard be run at least daily to correct for such differences.

The detector responses for each alkaloid must be independent of the presence of other alkaloids in the sample. Different weight ratios of two alkaloids or one alkaloid

TABLE I

RELATIVE WEIGHT RESPONSES (*RWR*) OF TOBACCO ALKALOIDS IN A FLAME IONIZATION DETECTOR

All values are relative to quinoline and were determined over three month period. n = number of determinations.

Alkaloid	*n*	*Average RWR*	*S.E.*
Nicotine	8	0.81	0.017
Nornicotine	8	0.31	0.022
Anabasine	8	0.55	0.014
Anatabine	4	0.58	0.021

and the internal standard were compared for their response. Each alkaloid was tested with the internal standard, and the alkaloids, with the exception of anatabine, were tested in all possible combinations. Fig. 1 is a summary of these comparisons. Linear responses and zero intercepts were observed for all comparisons. These results would not be expected if component interaction occurred or irregular component loss occurred; thus, the detector response for each alkaloid was independent of the other alkaloids in the sample.

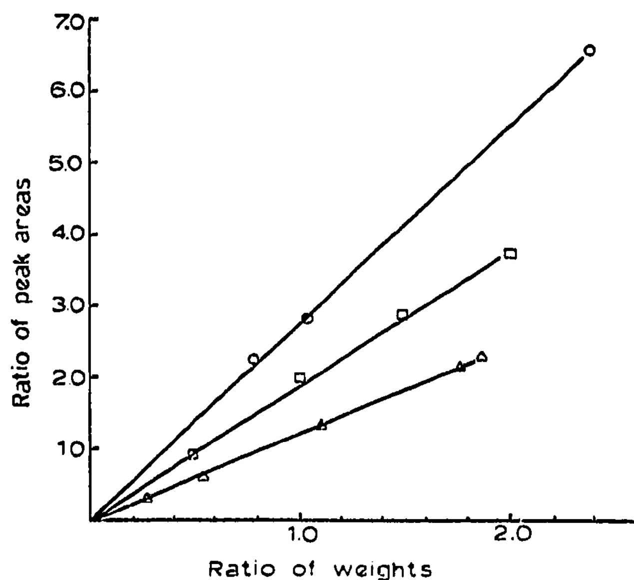

Fig. 1. Relative weight and peak area response for anabasine/nornicotine (○), nicotine/anabasine (□), and quinoline/nicotine (△).

Quinoline was selected as the internal standard because no interaction occurred with the alkaloids and its suitable retention time. With the exception of nornicotine the RWR was greater than 0.5 and the quantities of quinoline used could approximate the concentration of the alkaloids in tobacco tissue. If the RWR for quinoline is assumed as one relative to the other alkaloid components, the concentration of any alkaloid component is given by

$$C_A = \frac{C_Q}{RWR_A} \times \frac{A_A}{A_Q}$$

where C_A = the unknown concentration of the alkaloid component, C_Q = concentration of quinoline, RWR_A = relative detector response of the alkaloid component, A_A = the peak area of the alkaloid component, and A_Q = the peak area of quinoline.

The procedure was tested on several biological samples. A tobacco sample was spiked with two levels of alkaloids and the alkaloid content determined (Table II). The total alkaloids determined by the GLC method are a summation of the individual alkaloids, whereas the total alkaloids determined by steam distillation were expressed as nicotine equivalents. The GLC summations were 3.0 to 6.6 % greater than total alkaloids determined by steam distillation. The largest standard deviation for the

TABLE II

QUANTITATIVE COMPARISON OF ALKALOID ANALYSIS USING GAS CHROMATOGRAPHY AND STEAM DISTILLATION

Sample	*Gas chromatography*					*Steam distillation*
	Nicotine (*mg/g*)	*Nornicotine* (*µg/g*)	*Anabasine* (*µg/g*)	*Anatabine* (*µg/g*)	*Summation* (*mg*)	*Total* (*mg*)[a]
Tobacco	20.4 ± 0.2	642 ± 32	109 ± 5	541 ± 8	21.69	20.25
Tobacco + low-level exogenous alkaloids	30.6 ± 0.9	709 ± 20	304 ± 7	768 ± 16	32.38	31.40
Tobacco + high-level exogenous alkaloids	40.0 ± 0.8	1120 ± 35	549 ± 11	954 ± 18	42.62	40.80

[a] Expressed as nicotine equivalents.

TABLE III

PRECISION OF QUANTITATIVE ALKALOID ANALYSIS ON TOBACCO SAMPLES OF DIFFERENT ALKALOID COMPOSITION

Sample	*Nicotine* (*mg/g*)	*Nornicotine* (*µg/g*)	*Anabasine* (*µg/g*)	*Anatabine* (*µg/g*)
1	20.14 ± 0.19	393 ± 12.0	65 ± 3.0	408 ± 7.0
2	9.79 ± 0.26	$(47.6 \pm 1.53) \cdot 10^3$	trace	$(2.39 \pm 0.13) \cdot 10^3$
3	$(264 \pm 4.48) \cdot 10^{-3}$	1268 ± 45.6	not detected	35.3 ± 1.40

individual alkaloids was observed for nornicotine (3.6% average) and the smallest for anatabine (1.8%). These results indicate that the GLC method accurately and precisely determines both endogenous and exogenous tobacco alkaloids from plant tissue.

The technique was also tested on tobacco samples with greatly different quantities and ratios of the individual alkaloids (Table III). The standard deviation of the mean was less than 6% for all determinations. These findings indicate good precision of the method for samples with a wide range of alkaloid content. The accuracy of the method for these samples was difficult to ascertain because of the lack of valid comparisons with present methods on such diverse samples as Nos. 2 and 3.

The GLC procedure has been used successfully in our laboratory for analysis of many tobacco and tobacco smoke samples. The magnitude of difference between nicotine and the other alkaloids in most samples necessitates two extractions per sample if the amount of internal standard is going to be approximately equal to the amount of the alkaloids. This shortcoming can be eliminated or minimized if an electronic integrator is used in conjunction with the gas chromatograph. For deter-

mination of alkaloids in tobacco smoke, the sample must be chromatographed prior to addition of quinoline because small amounts of endogenous quinoline are present.

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