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Note

Rapid determination of certain alkaloids, other than nicotine, in tobacco

D. THORBURN BURNS*
Loughborough University of Technology, Loughborough, Leics. (Great Britain)
and
E. J. COLLIN
John Player and Sons, Imperial Tobacco Ltd., Nottingham (Great Britain)
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Quantitative analysis of alkaloids other than nicotine in tobacco is difficult due to their chemical similarity to, and the preponderance of, nicotine in most types of tobacco. Classical separations, now of little interest, have been achieved in selected areas with some degree of quantification[1–3]. The separation of nicotine alkaloids has been achieved by gas chromatography (GC) and in a few examples quantification was attempted[4–12]. In all cases, lengthy sample preparation schemes were specified.

The objective of the present study was to develop a rapid quality control procedure, with the minimum number of manual manipulations. The extraction of the alkaloids from tobacco and various separation and quantification systems were examined with this object in view.

A gas–liquid chromatographic (GLC) procedure for the determination of nicotine had previously been developed[13]. An extension of this is the basis of the optimised procedure described herein for the determination (expressed as nornicotine) of nornicotine, anabasine/myosmine, and nicotyrine in tobacco. Soxhlet extraction (2 h) of tobacco with methanol, after addition of quinaldine internal standard, is followed by GLC analysis (20 min) using a 6% Carbowax 20M + 1% KOH on 80–100 mesh Diatomite M column.

EXPERIMENTAL

Apparatus and materials

A Perkin-Elmer Model F11 (Mark 2) gas chromatograph with single flame ionisation detector (Perkin-Elmer, Beaconsfield, Bucks., Great Britain) was used with the injector block at 275° and the oven held isothermally at 180°. GLC was performed using a single glass column, 4 m × 3 mm I.D. packed with 6% Carbowax 20M, 1% KOH on 80–100 mesh Diatomite M, with oxygen-free nitrogen as carrier gas. The retention times and peak areas were obtained using a Hewlett-Packard Model 3370B electronic integrator (Hewlett-Packard, Wokingham, Great Britain).

* Present address: Department of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, Great Britain.

Basic procedure

The extraction of various samples of tobacco was performed by weighing 2-g samples of each tobacco into thimbles (Whatman cellulose 18 × 55 mm) of a compatible Soxhlet system, extracting each with methanol (A.R.) for 2 h over a steam-bath. After cooling, the extracts were made up to 50 ml with methanol (A.R.) and 1 ml of a 0.05% methanol solution of quinaldine (2-methylquinoline) was added as internal standard.

Five nornicotine calibration standards were prepared and used to cover the range 0–0.005% (w/v) nornicotine in methanol (equivalent to 0–0.125% nornicotine in tobacco), the internal standard being added as before. Calculation of % alkaloid (as nornicotine) was from the calibration graph, % nornicotine and ratio of the peak areas for nornicotine and quinaldine, prepared using the data obtained for the standard solutions.

Relative retentions have been calculated as the ratio of the time from injection to alkaloid peak to the time from injection to nicotine peak.

RESULTS AND DISCUSSION

The identification of peaks was effected by using a combination of fully characterised reference alkaloids available and published retention data for a Carbowax 20M column[14]. Comparisons were also made with tobacco (Japanese flue-cured) extract (Table I). A further peak at relative retention 2.90 was ascribed to a solvent impurity since it persisted with several injections of solvent at the same level.

TABLE I

RELATIVE RETENTIONS OF NICOTINE ALKALOIDS

Alkaloid	*Reference alkaloid measured relative retention*	*Published relative retention*[14]	*Tobacco extract relative retention*
Nicotine	1.00	1.00	1.00
Nornicotine	2.39	2.37	2.39
Myosmine	2.66	2.58	2.66
Anabasine	2.66	2.66	2.66
Isonicotine	—	3.18	—
Nicotyrine	—	3.74	3.80
Anatabine	—	—	4.10
Cotinine	—	16.30	—

Since both sets of data based on pure compounds are in reasonable agreement, application of the published data was considered valid for alkaloids for which no standard was available.

No separation between myosmine and anabasine was found possible on this otherwise satisfactory column. It was therefore necessary to quantify and designate this composite peak as anabasine/myosmine. A typical chromatogram is shown in Fig. 1. A tentative identification of anatabine has been made on the basis of almost linear regression of data herein and those of Bush[12], who included anatabine in a

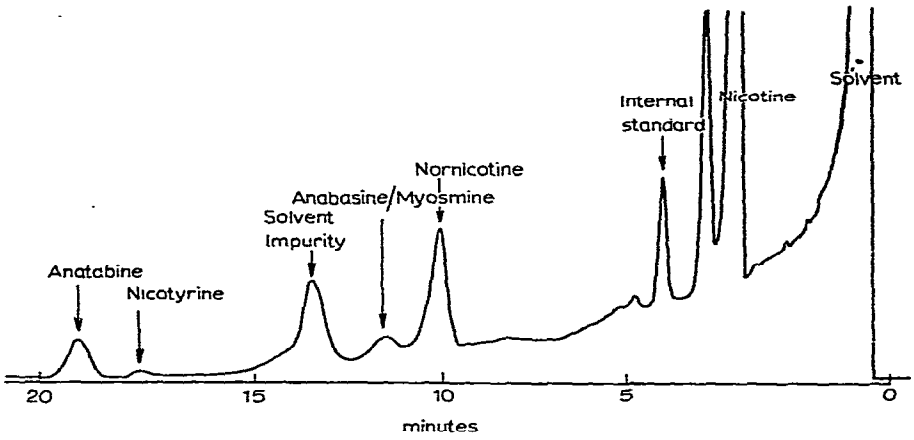

Fig. 1. Chromatogram of typical tobacco extract.

model study using a DC-550 column. With this system, myosmine is resolvable from anabasine but then interferes with nornicotine.

The Soxhlet extraction procedure was confirmed to be satisfactory by the absence of total nicotine alkaloids, determined by a colorimetric procedure[15], in a further aqueous extraction of the previous (methanol) Soxhlet extraction residue. Extraction of greater than 99% was obtained using a wide range of tobacco types.

In order to confirm that no degradation of alkaloids had occurred in the extraction and analysis stages, the recovery of nornicotine and anabasine added to tobacco was investigated. Addition of known quantities of these alkaloids to tobacco stem samples of very low inherent alkaloid content was performed by adding a solution of alkaloids in methanol and after air drying, the analytical procedure was carried out as before; each alkaloid being quantified in these experiments with respect to its own calibration standards. The results are shown in Table II. The recovery values shown are considered satisfactory for the purpose of quality control.

TABLE II

RECOVERY OF NORNICOTINE AND ANABASINE ADDED TO TOBACCO

Tobacco	*Recovery (%)*	
	Nornicotine	*Anabasine*
Stem + 0.025% alkaloid in tobacco	89.0	91.0
Stem + 0.050% alkaloid in tobacco	96.5	83.5
Stem + 0.075% alkaloid in tobacco	92.0	100.3
Stem + 0.100% alkaloid in tobacco	92.3	96.3
Stem + 0.125% alkaloid in tobacco	103.0	102.8
Mean weighted recovery	96.1	97.2

The accuracy of the method was examined by comparing the difference between estimates from total nicotine alkaloids determined colorimetrically and a nicotine-specific GLC procedure[13] with the summation of the alkaloids as measured previously (Table III).

A good correlation was obtained. Perfect correlation would not be expected due to

TABLE III

ACCURACY OF THE ALKALOIDS PROCEDURE

Tobacco type	*Total nicotine alkaloids (%) as nicotine (colorimetric)*	*Total nicotine alkaloids minus nicotine (%)*	*Sum of alkaloids other than nicotine (%)*
Italian cigarette	1.15	0.13	0.10
Japanese flue-cured	5.02	0.21	0.42
Stem	0.29	0.01	0.01
Tobacco sheet	0.52	0.00	0.01

relative responses of alkaloids other than nicotine being likely to be non-unity in the total nicotine alkaloids procedure.

The reproducibility of the procedure was assessed by performing complete analyses on samples from the same tobacco batch on four separate occasions. The mean % alkaloid (as nornicotine) (S.D. in parentheses) was for Japanese flue-cured tobacco: nornicotine, 0.20 (0.03); anabasine/myosmine, 0.12 (0.002); nicotyrine, 0.003 (0.001); anatabine, 0.33 (0.02); for Italian cigarette tobacco: 0.09 (0.01); 0.01 (0.002); 0.001 (—); 0.05 (0.01).

In addition, the repeatability of the GLC stage was evaluated by performing six replicate injections of the same extract of Italian cigarette tobacco. The mean % alkaloid (as nornicotine) (S.D. in parentheses) was: nornicotine, 0.09 (0.003); anabasine/myosmine, 0.02 (0.001); nicotyrine, 0.001 (—); anatabine, 0.04 (0.003).

CONCLUSIONS

It is considered that the results obtained with the procedure described herein show that an advance has been made in the analysis of alkaloids other than nicotine in tobacco. The method described has a simple extraction stage and is sufficiently precise and suitable for a laboratory engaged in routine testing and quality control in the tobacco industry.

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