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Fast analysis of nicotine related alkaloids in tobacco and cigarette smoke by megabore capillary gas chromatography

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Abstract

A novel fast megabore capillary gas chromatographic (MCGC) method for analysis of 7 nicotine related alkaloids in tobacco and cigarette smoke, including nicotine, nornicotine, myosmine, nicotyrine, anabasine, anatabine and 2,3-dipyridyl, was developed. The use of megabore capillary column GC methodology, equipped with flame ionization detector (FID), provided rapid, unambiguous nicotine related alkaloids analysis. One gram flue-cured tobacco (or Cambridge filter pad), 20 ml ether, and 5 ml 10% sodium hydroxide solution, added with *n*-heptadecane as the internal standard, were placed in a flask, and the flask was capped and placed in an ultrasonic bath for 15 min. A 1 μl volume was analyzed by capillary GC operating in split-injection mode on a mega bore Simplicity™-5 column. This simple procedure was compared with the previously reported packed column GC method and the Griffith still-colorimetric method. The application of the method for analysis of various flue-cured tobaccos and cigarette smoke was discussed.

Keywords: Tobacco; Cigarette smoke; Alkaloids; Nicotine; Nornicotine; Myosmine; Nicotyrine; Anabasine; Anatabine; 2,3-Dipyridyl

1. Introduction

Tobacco and cigarette smoke contain a number of structurally related alkaloids (Fig. 1). Alkaloids in tobacco (*Nicotiana tabacum* L.) have been widely recognized for their contributions to tobacco quality and usability [1,2]. The nature and underlying neurobiology associated with nicotine, the principal tobacco pyridine alkaloid, and its role in pleasurable sensations and continuation of tobacco use have been reviewed by Ashton et al. [3] and Martin [4]. Also, alkaloids and other chemical components associated with tobacco usage and attendant health risks were summarized by Davis [5].

The quantitative analysis of tobacco alkaloids has always been of great interest to tobacco scientists. In commercial tobacco, the major alkaloid is nicotine, accounting for about 95% of total alkaloid fraction [6]. Nornicotine and anatabine are the two most abundant minor alkaloids, present in roughly equal amounts, each accounting for about 2–3% [7]. Anabasine is generally present in concentrations of about 0.3% of the total alkaloids. Anabasine is present in numerous

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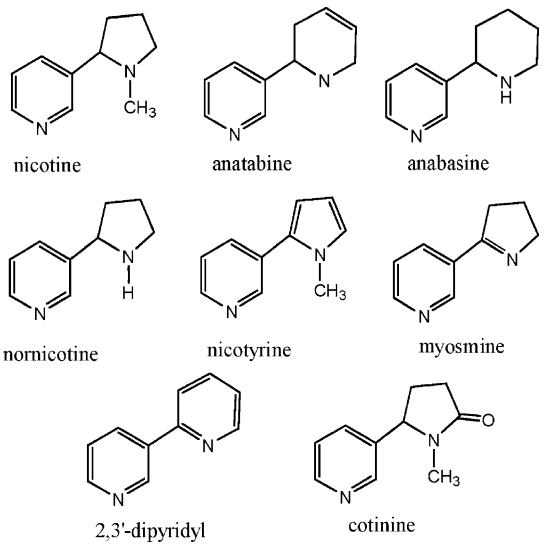

Fig. 1. Chemical structures of nicotine related alkaloids in tobacco and cigarette smoke.

Nicotiana as well as other related plant families [7]. In some, notably *Nicotiana glauca*, anabasine is the principal alkaloid. Tobacco and cigarette smoke contain smaller amounts of 2,3-bipyridyl, metanicotine, etc. [8].

Alkaloids contribute to the organoleptic properties of cigarette smoke, and alkaloid data have been used traditionally as an indicator of tobacco quality. Alkaloid determinations have shown that high levels of nornicotine in the cured tobacco produce an undesirable product. Recent work indicates that nicotine and nornicotine are precursors of the carcinogenic *N*-nitrosonornicotine, *N*-nitrosoanatabine, *N*-nitrosoanabasine, and other tobacco-specific nitrosamines (TSNA) that have also been identified in tobacco and cigarette smoke [9]. Thus, data on levels of total alkaloids and individual components are important parameters in evaluating tobacco products for potential biochemical activity or marketing quality.

Commonly used methods for determining tobacco alkaloids are steam distillation-spectrophotometric method of Griffith [10] (the Griffith still method) and the automated procedures of Harvery et al. [11] and Davis [12]. These methods yield excellent data for total alkaloids (calculated as nicotine), but additional steps are required to estimate the minor alkaloids.

Gas chromatography method for quantitative analysis of nicotine related alkaloids had employed unmodified or potassium hydroxide-modified liquid phases. Bush extracted the alkaloids with a mixture of saturated aqueous barium hydroxide–benzene–chloroform and analyzed them by GC on a DC-550 column [13]. Rosa reported a pyrolysis-GC method for the estimation of nicotine and nornicotine [14]. In this method, the alkaloids were directly volatilized from the tobacco sample on to the GC column in an injection-port pyrolyzer. The steps and time required for extraction in the first two GC methods and the inability to use an internal standard in the pyrolysis-GC method made those procedures undesirable for the screening of large numbers of samples in our quality, and health related studies.

Consequently, we developed a novel method involving a brief extraction step with fast analysis 7 nicotine related alkaloids by mega bore capillary GC, coupled with FID. The method was applied to both flue-cured tobacco and cigarette smoke sample; extraction and analysis time was sufficiently decreased to permit the screening of numerous samples.

2. Experimental

2.1. Apparatus

A Shimadzu GC-14B (Shimadzu, Japan) gas chromatograph equipped with a split/splitless injector, a temperature programmer and a flame ionization detector (FID) was used. In all cases, the chromatograms were recorded and calculated by using a Shimadzu CR-7A (Shimadzu, Japan) computing integrator.

A Borgwaldt RM 20/CS smoking machine (HB Co. Ltd., Germany) was used to collect cigarette smoke.

EB2000 Rotavapor (Beijing Analytical Instrument Co. Ltd., China), thermostated by water circulation and furnished with a vacuum pump SP3200 ultrasonic bath (Shanghai Ultrasonic Co. Ltd., China).

2.2. Reagents and materials

Analytical-reagent grade materials were used unless otherwise indicated. Water was glass-stilled.

Nicotine (Chromatographic purity, Aldrich) was distilled before used. Anabasine (Chromatographic purity, Tridom Chemical, Hauppauge, NY, USA)

and *n*-heptadecane (Chromatographic purity, Aldrich) were used as received. Ether was analytical grade.

Tobacco samples were flue-cured leaves from Xuchang, Henan provinces of PR China. Cigarette smoke condensate was collected on a Cambridge filter pad (five cigarettes per pad).

2.3. Extraction procedure

One gram of ground flue-cured tobacco (60–80 mesh) or Cambridge filter discs was weighed into a flask, 20 ml ether and 10 ml 5% sodium hydroxide were added, then 100 μl 10.0 μg/ml *n*-heptadecane (used as internal standard solution) was added. After ultrasonicated for 15 min, the two layers were separated, and the ether layer transferred to vial. One microliter of the sample was injected into GC and GC–MS to be analyzed.

2.4. Smoke collection

All cigarettes had a total 85 mm, with a 20 mm filter. They were conditioned selected on a weight and pressure drop basis. They were smoked according to the Coresta Standard Method No. 10 [15], Chopra et al. [16] and the Federal Trade Commission (FTC) [17], i.e. puff duration, 2 s; puff frequency, 1 puff/min; puff volume, 35 ml; butt length, 23 mm. The smoke was collected on a 92 mm diameter Cambridge filter disc. The alkaloids were extracted separately from the Cambridge filter disc. Because of the very low quantities of alkaloids to be dealt within cigarette smoke, five cigarettes had to be smoked to determine all those alkaloids that had been found in the tobacco. After each of the five cigarettes were smoked, the old Cambridge filter pad was replaced by a new Cambridge filter pad. The Cambridge filter pads were collected and extracted with the method that was described above.

2.5. Qualitative analysis by GC–MS

Autosystem TurboMass GC–MS (Perkin-Elmer, USA) was used to qualitatively analyze nicotine related alkaloids in tobacco and cigarette smoke. A HP-5 MS column (30 m length, 0.25 mm i.d., 0.25 μm d.f. cross-linked SE-54) was used in this investigation. Helium was used as carrier gas with head pressure 12.5 psi. The split ratio was 20:1. Temperature program was from 120 to 250 by 10 °C/min. The mass spectrum was operated at 170 °C in the electron impact mode (70 eV), scanning from *m/z* 33 to 500 in 0.3 s with the 0.2 s interval time of the scan. The voltage of the photoelectric multiplier tube (PMT) was 230 V above tuning.

The identifications of nicotine related alkaloids were carried out by comparing to the National Institute of Standards and Technology (NIST98, Gaithersburg, MD) mass spectral library as well as to the Wiley 6.0 (Wiley, New York, NY) mass spectral library. Qualitative analysis (mass spectral data) was verified by comparing the retention indices and mass spectra of identified nicotine related alkaloids with those of authentic reference substances.

2.6. Quantitative analysis by megabore capillary GC

Shimadzu GC-14B gas chromatography equipped with split/splitless injector and FID detector. Data processing was carried out on a CR-7A integrator. Analyses were carried out on a 15 m length, 0.53 mm i.d. megabore fused silica column, with 2 μm thickness cross-linked SE-54 liquid film (HP-5, Hewlett-Packard Co. Ltd., USA). The analysis conditions were as follows: model of sample introduction was hot needle injection; split ratio was 3:1; injector temperature was at 250 °C; detector temperature was at 250 °C; hydrogen was used as carrier gas with 20 ml/min. In preliminary tests, the oven temperature program was from 100 to 250 °C by 10 °C/min; in routine analysis the oven temperature was hold at 170 °C.

Quantitative data for nicotine related alkaloids were obtained by the internal standard method using heptadecane as internal standard or standards as reference substances, respectively, without considering calibration factors (i.e. $F = 1.00$ for all compounds).

3. Results and discussion

3.1. Effects of extract method

During analysis, in order to know if the neutral chemical constituents could infect nicotine related alkaloids analysis or not, the ether extract was extracted

three times with 5% sulfuric acid, the nicotine related alkaloids were transferred into aqueous layer, the neutral chemical constituents left in ether layer. The aqueous layer was added with ether and 30% sodium hydroxide to re-extracted nicotine related alkaloids into ether again. The neutral and alkaloids extracts were analyzed by GC, respectively. The result shown that analysis of nicotine related alkaloids was not affected by neutral chemical constituents extracted by ether in this investigation.

3.2. Optimization of sample introduction

In this investigation, the sample introduction model was hot needle injection. Partial solvent evaporation inside the syringe needle (optimized as "hot needle injection") produced thermospray: the sample liquid was nebulized upon leaving the needle [21]. The resulting fog was rapidly slowed and moved with the gas. Solute evaporation largely occurred from microparticles suspended in the gas phase. According to this theory, empty liners were the most suitable. Nevertheless, in this experiment, the injector liner was packed with 5 mm sodium carbonate (60–80 mesh). Packing small amount of sodium carbonate in GC injector liner had three advantages: (1) eliminating active absorption of alkaloids; (2) retarding contaminates, increase the lifetime of column; and (3) increasing heat capacity, allowing sample vaporizing more completely and quickly. This model of sample introduction of GC analysis would be beneficial to eliminate discriminative effects of early-eluted peaks, improve the accuracy of sample volumes and the quality of the results.

3.3. Megabore capillary GC analysis

Gas chromatography had been widely used to determine individual alkaloids in tobacco or cigarette smoke. According to the different GC column used, the previous investigations could be classified into three cases: (1) packed column with potassium hydroxide-modified PEG-20M stationery [18]; (2) 0.25 mm i.d. wall coated open tubular column (WCOT) glass or fused silica capillary column with polar stationery [19]; (3) 0.32 mm i.d. WCOT fused silica capillary column with nonpolar stationery (SE-54) [20]. Many investigators used nitrogen phosphor selective detector (NPD) to eliminate interference. The large differences in contents between nicotine and other nicotine related alkaloids required large capacity of the column. The structural similarities of the alkaloids required high resolving capability of the column. High polarity of nicotine related alkaloids required high inertness of the column. Packed column had large capacity, but its resolution and inertness were not desirable. 0.25–0.32 mm i.d. fused silica capillary columns had high resolving capability, but its capacities were not enough, and detection of nicotine related alkaloids other than nicotine did not meet the demount.

In this investigation, a megabore fused silica capillary column was used to analysis of nicotine related alkaloids in tobacco and cigarette smoke. The megabore capillary column combined the advantages of packed and conventional capillary columns; not only the large capacity, which was similar to that of packed column, but also the high resolution, which was like to the conventional capillary columns.

Qualitative analysis was verified by comparing mass spectra of nicotine related alkaloids with those of authentic reference substances in National Institute of Standards and Technology (NIST98, Gaithersburg, MD) mass spectral library as well as to the Wiley 6.0 (Wiley, New York, NY) mass spectral library (Fig. 2 and Table 1). As shown in Fig. 3, nicotine and other alkaloids achieved baseline separation, nicotine related alkaloids were clearly detected, nicotine peak showed no "overload" (tailing). Oven temperatures held in 170, the analysis of 7 nicotine related alka-

Table 1
Nicotine related alkaloids analyzed by megabore capillary GC and GC–MS

Number	Nicotine related alkaloids	Retention time (min) of megabore GC	Identified method
1	Nicotine	2.10	MS, S
2	Nornicotine	2.67	MS
3	Mysomine	3.08	MS
4	Nicotyrine	3.17	MS
5	Anabasine	3.37	MS, S
6	Anatabine	3.72	MS
7	2,3-Dipyridyl	3.91	MS
IS	*n*-Heptadecane	4.06	MS, S

S, identified by authentic standards; MS, identified by comparing mass spectra data to NIST or Wiley mass spectral library.

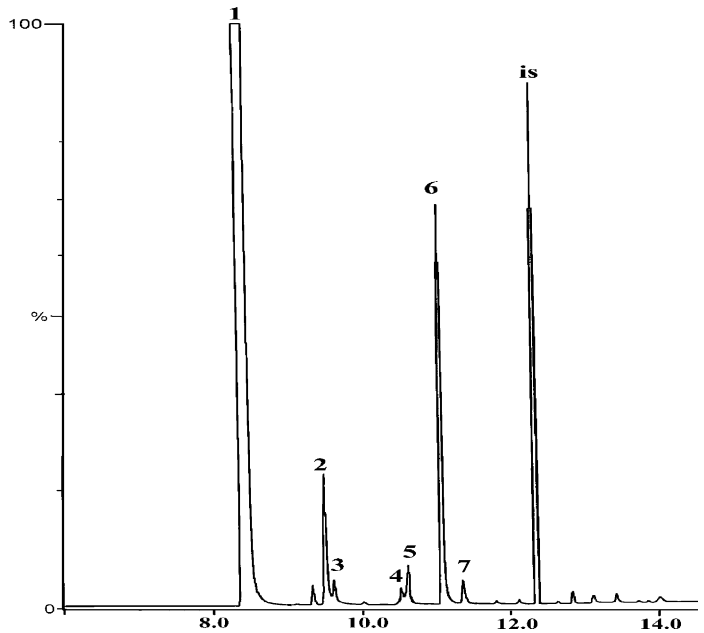

Fig. 2. GC–MS total ion chromatogram (TIC) of nicotine related alkaloids in tobacco added with *n*-heptadecane as internal standard (IS). For identified peaks number see Table 1.

loids only needed 4.5 min. So this method can be used as fast routine screening.

3.4. Method check-up

As shown in Table 2, we found that a fast megabore capillary GC analysis yielded results identical with those obtained by the more laborious methods of Burns and Collin [18]. The total alkaloid levels obtained by the widely used Griffth still method [10]. Good agreement between the two methods was obtained over a wide range of alkaloids (Table 2). The recovery of nicotine was 98.9%, the dynamic liner range of nicotine was from 0.7 to 14.1%, and the R.S.D. of this method was between 1.5 and 10.3%.

This method was found to be effective for simultaneous determination of 7 nicotine related alkaloids. The 7 nicotine related alkaloids examined in this study were monitored by the proposed method in five tobacco samples and one cigarette smoke sample. The results were shown in Table 3. The determination limit was 0.01 μg/mg for 7 nicotine related alkaloids by the proposed method. The results showed that the different type of tobacco contain different amount of nicotine related alkaloids and their distributions. Therefore, this method could be extended for the screening of nicotine related alkaloids and their distributions in tobacco and cigarette smoke.

Table 2
Comparison of data from megabore capillary GC with other methods

Tobacco sample[a]	Total alkaloids content (%)		
	Megabore capillary GC	Packed GC	Griffth still method
B1F	2.30	2.45	2.45
C3F	1.93	2.04	2.05
X2F	1.67	1.71	1.74
Cigarette smoke[b]	1.26	1.27	–[c]

[a] Grown and cured at Xuchang, Henan, China, 2000.
[b] The unit is mg per cigarette.
[c] Trace amount.

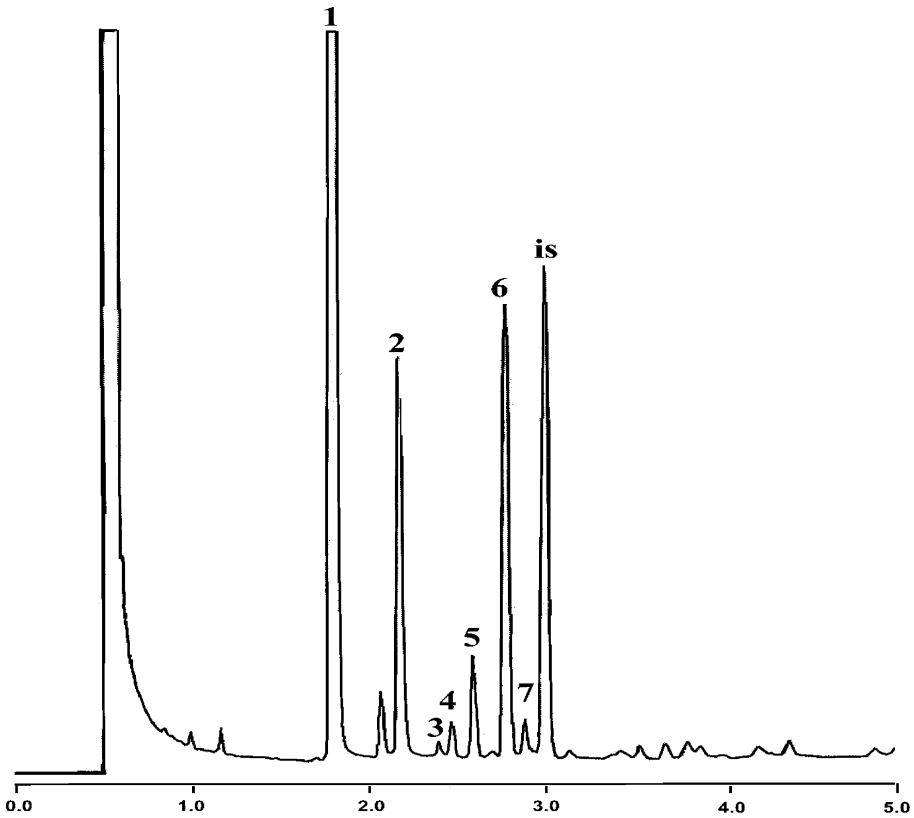

Fig. 3. Gas chromatogram of nicotine related alkaloids in tobacco added with *n*-heptadecane as internal standard (IS). For identified peaks number see Table 1.

Table 3
The content of alkaloids and its distribution in tobacco and cigarette smoke

Sample[a]	Total alkaloids(%)[b]	Distribution						
		Nicotine	Nornicotine	Mysomine	Nicotyrine	Anabasin	Anatabine	2,3-Dipyridyl
C1F	4.27	17.1	71.6	2.4	1.1	2.6	4.0	1.2
C2F	4.13	91.7	3.2	0.1	0.9	1.0	3.0	0.3
B1F	4.28	93.7	1.5	0.1	0.3	0.6	3.0	0.9
B2F	5.82	94.3	2.6	0.1	0.2	0.6	2.4	0.7
C3F	5.41	94.7	1.5	0.1	0.1	0.6	2.7	0.4
Cigarette[c]	1.23	98.3	0.1	–	–	–	–	–

[a] Grown and cured at Xuchang, Henan, China, 2000.
[b] Based on dry weight.
[c] The unit is mg per cigarette.

4. Conclusions

In this investigation, the 0.53 mm i.d. megabore fused silica capillary column was used to analyze 7 nicotine related alkaloids. A fast, simple and reliable method, based on a brief extraction and megabore capillary GG analytical techniques was developed, for determining total nicotine related alkaloids and its

distributions. This fast procedure permits a trained technician, using one automated megabore capillary GC system, to analyze about 100 samples per day. This permits the analyses of sufficient samples for the method to be useful in the evaluation of numerous samples for quality and consumer acceptability. Also, this method should have wide applicability to the analysis of alkaloids in other plant extracts or organic mixtures.

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