Gas Chromatographic Analysis of Cigarette Smoke by Trimethylsilylation Method*

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1. INTRODUCTION

Recently, tobacco smoke components have been investigated extensively because of the organoleptic or biological interest. The total number of the smoke components summarized by *Stedman* (1) in 1968 reached about 1200. Since then more effort (2–9) has been made to elucidate the compositions of tobacco smoke. Now it seems that more than 2500 compounds are known as tobacco smoke components. Gas chromatography has been widely used to investigate the tobacco smoke containing such a large number of components. *Grob* (10, 11) analyzed the semi-volatiles and vapor phase components by glass capillary column gas chromatography. Tobacco smoke, however, includes not only volatile compounds but also non-volatile acids, phenols and polyhydroxy neutral compounds. *Guerin* (12, 13) employed the trimethylsilylation method to investigate gas chromatographic component profiling of cigarette smoke. Using this method, non-volatile compounds can easily be converted into their volatile trimethylsilyl (TMS) derivatives. So we developed the trimethylsilylation method and quantitatively determined the major components in the total particulate matter (TPM) of the cigarette smoke.

2. EXPERIMENTAL

Samples: The sample used in this study was a cigarette made from flue-cured (Bright yellow) tobacco lamina (70 mm in length, 25 mm in circumference and 1.00 ± 0.02 g in weight).

Preparation of the Sample for Gas Chromatography: Four lamina cigarettes were smoked under the standard smoking conditions (35 ml/puff, 2 s/puff, 1 puff/min and 30 mm in butt length) and TPM of the smoke was

collected on a glass fiber filter pad (37 mm ϕ). The filter pad was put into a glass vial immediately and 300 μ l of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was added together with 0.3 mg of L-arabitol as internal standard. The vial was allowed to stand at 80 °C for 1 hour and then 1 μ l of the solution was injected into the gas chromatograph.

Gas Chromatography: The gas chromatographic separation was made on a Shimadzu Model Mini-1 gas chromatograph equipped with a glass capillary column (0.25 mm inside diameter $\times$ 60 m) coated with OV-101, and a flame ionization detector (FID). The column oven temperature was programmed from 50 °C to 200 °C at a rate of 2 °/min. The injection port was kept at 220 °C. Helium was used as carrier gas with a flow rate of 1 ml/min through the column. Split ratio was 1/50.

Identification of Smoke Components: Peaks were tentatively identified by comparison of gas chromatographic retention times with those of authentic compounds. Gas chromatography / mass spectrometry (GC-MS) was carried out on a Nippon Electric Varian Model TE-600 mass spectrometer.

Quantitative Determination of Major Components in TPM: Calibration curves were prepared on the basis of the relation between concentration and peak height of authentic compounds and that of L-arabitol. Levels of the major components in TPM were calculated from the calibration curves.

3. RESULTS AND DISCUSSION

A typical gas chromatogram of TPM of lamina cigarette smoke is shown in Fig. 1, while those compounds identified from the gas chromatogram are listed in Table 1. As shown in Table 1, various classes of com-

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Figure 1. Gas chromatogram of TPM of lamina cigarette smoke (trimethylsilyl derivatives).

Column: 0.25 mm inside diameter $\times$ 60 m glass capillary column coated with OV-101.
 Column temperature: 50 $^{\circ}\text{C}$ $\rightarrow$ 2 $^{\circ}/\text{min}$ $\rightarrow$ 200 $^{\circ}\text{C}$.
 Carrier gas: Helium (1.0 ml/min).

pounds, viz.: phenols, organic acids, nitrogenous compounds, lactones, hydrocarbons, polyalcohols, sugars and miscellaneous compounds, could be analysed simultaneously and the total number of the compounds identified was approximately 70. Levels of major components in Fig. 1 are listed in Table 2. In addition to nicotine, the major components were phenol, catechol, hydroquinone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one [I], 4-vinylcatechol, levoglucosan [II], quinic acid γ -lactone [III] and neophytadiene.

As regards the precursors of the components thus found in TPM, nicotine and diterpene neophytadiene (14) would be transferred directly to the main stream smoke from tobacco by distillation in the smoking process.

Catechol (6, 15), 4-methyl- (6, 15), 4-ethyl- (6, 15) and 4-vinylcatechol (6, 16) would be formed from chlorogenic acid (15) and/or 3,4-dihydroxycinnamic acid (17) (caffeic acid) by pyrolysis.

Cellulose pyrolysates have been comprehensively investigated. Levoglucosan (18), catechol (19), hydroquinone (19), glycolic acid (20), 5-hydroxymethyl-2-furaldehyde (18) have been reported as major pyrolytic products of cellulose.

Quinic acid γ -lactone (21) would be formed from quinic acid and/or chlorogenic acid (15) during combustion. Sugar-amino acid reactions seem to play a significant role in the formation of the flavor and aroma of tobacco smoke. As the reaction products (22), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 4-hydroxy-2,5-dimethyl-3(2H)-furanone [IV] were recognized in TPM. The other sugar degradation products might also contribute to the organoleptic properties of tobacco smoke. Lactic acid, glyceric acid, threonolactone [V], erythroneolactone, 3-deoxyxylonic acid γ -lactone and glucometasaccharinic acid γ -lactone [VI] could be prepared from

sugar by alkaline degradation (23). Thus, the composition of smoke constituents derived from various precursors can be clarified by the trimethylsilylation method. It would be one of the easy and useful methods of estimating the quality of tobacco smoke.

Figure 2.

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

4-Hydroxy-2,5-dimethyl-3(2H)-furanone

Glucometasaccharinic acid γ -lactone

Table 1. Constituents Identified in the total particulate matter (TPM) of cigarette smoke.

Peak No.	Compound (trimethylsilyl derivative)	Evidence
1	Formic acid	GC ^a
2	Acetic acid	GC
4	Propionic acid	GC
5	Methacrylic acid	GC
7	n-Butyric acid, acetoin	GC
8	Crotonic acid	GC
9	Furfuryl alcohol	GC-MS ^b
10	Ethylene glycol	GC-MS
12	Dipentene (limonene)	GC-MS
13	i-Caproic acid	GC
15	Phenol	GC-MS
16	Lactic acid	GC-MS
17	5-Methylfurfuryl alcohol	GC
18	Glycolic acid	GC-MS
19	Levulinic acid	GC-MS
20	2-Furoic acid, 3-hydroxypyridine, o-cresol	GC-MS
21	m-Cresol, α-hydroxy-n-butyric acid	GC-MS
22	p-Cresol, benzyl alcohol, β-hydroxypropionic acid	GC-MS
23	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	GC-MS
24	2-Hydroxy-3-methyl-2-cyclopentene-1-one (cyclopentene)	GC-MS
25	o-Ethylphenol	GC-MS
26	o-Methoxyphenol (guaiacol)	GC-MS
27	2,4-Xylenol	GC-MS
28	2-Phenyl ethanol, 5-methyl-2-furoic acid	GC-MS
29	Benzoic acid	GC-MS
30	2,6-Xylenol	GC-MS
32	4-Vinylphenol	GC-MS
33	m-Methoxyphenol	GC
34	Phenylacetic acid	GC-MS
35	5-Hydroxymethyl-2-furaldehyde	GC-MS
36	Glycerol	GC-MS
37	o-Toluic acid	GC
38	Succinic acid	GC
39	Catechol	GC-MS
40	Nicotine	GC-MS
42	Glyceric acid	GC-MS
43	2,3,4-Trihydroxy-n-butyric acid γ-lactone ^c (threonolactone)	GC-MS
44	Resorcinol	GC-MS
45	4-Methylcatechol	GC-MS
46	3-Methylcatechol	GC-MS
47	Hydroquinone	GC-MS
48	4-Vinylguaiacol, erythronolactone ^c	GC-MS
49	2,3-Dihydroxy-2-cyclopentene-1-one (reductic acid)	GC-MS
50	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	MS
51	4-Ethylcatechol	GC-MS
52	5-Hydroxymaltol	MS

Peak No.	Compound (trimethylsilyl derivative)	Evidence
53	3-Deoxyxylonic acid γ-lactone ^c	GC-MS
54	4-Vinylcatechol	GC-MS
55	Malic acid	GC-MS
56	5-Hydroxymethyl-2-furoic acid	GC-MS
59	m-Hydroxybenzoic acid	GC-MS
63	Unknown (cellulose pyrolysate)	
64	Unknown (anhydro- or deoxyfructose, mol. wt. 162)	
67	Unknown (lactone? mol. wt. 162)	
69	1,6-Anhydroglucopyranose (levoglucosan)	GC-MS
70	1,6-Anhydroglucofuranose ^c	MS
73	Arabitol (internal standard)	
74	3-Deoxyhexonic acid γ-lactone ^c (glucometasaccharinic acid γ-lactone)	GC-MS
75	Quinic acid γ-lactone	GC-MS
77	3-Methylene-7,11,15-trimethyl-1-hexadecene (neophytadiene)	GC-MS
78	Fructose	GC-MS
79	α-Glucose	GC-MS
80	Coniferyl alcohol	GC-MS
81	Palmitic acid	GC-MS
82	β-Glucose	GC-MS
83	Inositol ^c	GC-MS

a: Gas chromatographic retention time.

b: Gas chromatography / mass spectrometry.

c: Compound found for the first time in tobacco smoke.

Table 2. Levels of major components in TPM of lamina and midrib cigarette smoke.

Peak No.	Compound	Lamina (μg/cigarette)	Midrib (μg/cigarette)
10	Ethylene glycol	24	6
15	Phenol	94	38
16	Lactic acid	87	65
18	Glycolic acid	91	40
36	Glycerol	123	41
39	Catechol	135	43
40	Nicotine	3840	650
43	Threonolactone	40	187
47	Hydroquinone	152	52
50	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	120	41
54	4-Vinylcatechol	80	12
69	Levoglucosan	133	63
75	Quinic acid γ-lactone	178	96

SUMMARY

A gas chromatographic method was developed to quantitatively determine the major components in the total particulate matter (TPM) of cigarette smoke. Tobacco smoke condensate was collected on a glass fiber filter and

the components were converted to their trimethylsilyl derivatives and then subjected to glass capillary column gas chromatography. By this method, volatile and non-volatile tobacco smoke components, including unstable phenolic substances and water-soluble polyhydroxy compounds, were determined simultaneously.

ZUSAMMENFASSUNG

Es wurde ein gaschromatographisches Verfahren für die quantitative Bestimmung der hauptsächlichsten Inhaltsstoffe der Gesamtpartikelfase (TPM) des Cigarettenrauches entwickelt. Nach Niederschlagung des Kondensats auf einem Glasfaserfilter wurden die Bestandteile in ihre Trimethylsilylderivate umgewandelt und gaschromatographisch unter Einsatz von Glaskapillarsäulen untersucht. Mit dieser Methode wurden die flüchtigen und nichtflüchtigen Inhaltsstoffe des Tabakrauches einschließlich der instabilen phenolischen Substanzen und der wasserlöslichen Polyhydroxyverbindungen gleichzeitig bestimmt.

RÉSUMÉ

Une méthode a été mise au point pour la détermination quantitative des principaux constituants du condensat de fumée (TPM) de cigarette, par chromatographie en phase gazeuse. Du condensat de fumée du tabac a été récolté sur un filtre de fibre de verre et les composants ont été convertis en leurs dérivés triméthylsilyl et soumis ensuite à la chromatographie en phase gazeuse sur colonne capillaire de verre. Par cette méthode, les composants volatils et non volatils de la fumée du tabac, y compris des substances phénoliques instables et les composés polyhydroxyliques solubles dans l'eau, ont pu être déterminés simultanément.

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