

Characterization of complex hydrocarbons in cigarette smoke condensate by gas chromatography–mass spectrometry and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry

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Abstract

Gas chromatography–mass spectrometry with electron ionization and positive-ion chemical ionization and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOF–MS) were applied for the characterization of the chemical composition of complex hydrocarbons in the non-polar neutral fraction of cigarette smoke condensates. Automated data processing by TOF–MS software combined with structured chromatograms and manual review of library hits were used to assign the components from GC × GC–TOF–MS analysis. The distributions of aliphatic hydrocarbons and aromatics were also investigated. Over 100 isoprenoid hydrocarbons were detected, including carotene degradation products, phytadiene isomers and carbocyclic diterpenoids. A total of 1800 hydrocarbons were tentatively identified, including aliphatic hydrocarbons, aromatics, and isoprenoid hydrocarbons. The identified hydrocarbons by GC × GC–TOF–MS were far more than those by GC–MS.

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1. Introduction

Tobacco smoke condensate contains a large number of hydrocarbon compounds. Dube and Green [1] estimated that there are 755 hydrocarbons in tobacco and tobacco smoke. They are transferred from the tobacco waxes, which contain alkanes, alkenes, alcohols, carboxylic acids, esters, aldehydes, ketones and alkaloids. The hydrocarbons transfer either structurally intact or fragmented into alkanes and alkenes. Many of these compounds may contribute significantly to the organoleptic and physiological properties of tobacco smoke. Alkanes, alkenes, aromatics and isoprenoid hydrocarbons are the major subgroups of tobacco smoke hydrocarbons.

A number of analytical methods have been developed to explore the hydrocarbon composition of the tobacco smoke, especially polynuclear aromatic hydrocarbons. These included gas chromatography [2,3], high-performance liquid chromatography (HPLC) [4–6] and hyphenated methods such as gas chromatography–mass spectrometry (GC–MS) [4,5–16].

Comprehensive two-dimensional gas chromatography (GC × GC) is a multidimensional separation technique [17,18], in which two columns with different kinds of selectivity are serially connected. The sample is firstly separated on a high-resolution first column. A modulator is installed between the columns to separate the first column elute into a very large number of adjacent small fractions. Each individual fraction is refocused, and subsequently, re-injected into the second GC column. The second separation is made to be fast enough (e.g. 1–10 s) to permit the continual

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introduction of subsequent, equally small fractions from the first column without mutual interference [19]. The main advantages [19–22] of GC \times GC over conventional gas chromatography are increased peak capacity, increased sensitivity and “structured” chromatograms [21] that facilitate the recognition of unknowns. Time-of-flight mass spectrometry (TOF-MS), which can acquire up to 500 mass spectra per second, is the only available MS technology to match GC \times GC. GC \times GC–TOF-MS has been successfully used to analyze petrochemicals [23], essential oils [24], pesticides in food extracts [25], flavor mixtures [26], airborne particulate matter [27] and cigarette smoking [28–30].

Our initial investigation found that the co-eluting components heavily existed in the smoke condensate analysis even when the powerful GC \times GC–TOF-MS technique was used. To acquire more useful information, a suitable pretreatment for reducing complexity of the samples was necessary. Therefore, several fractions of cigarette smoke condensates including acidic, basic and neutral fractions were prepared. In our previous papers [29,30], the chemical composition of the acidic and basic fractions in cigarette smoke condensates has been reported. The principal aim of this study was to characterize the chemical composition in the non-polar neutral fraction by using GC \times GC–TOF-MS.

2. Experimental

2.1. GC–electron ionization-MS and GC–positive-ion chemical ionization-MS

A Shimadzu GC–MS QP 2010 quadrupole mass spectrometer in electron ionization EI and positive-ion chemical ionization (PCI) mode using methane was used for the identification of hydrocarbons in non-polar neutral fraction. The data were collected in the mass range from m/z 33–450 with two spectra per second. A 50 m \times 0.2 mm i.d., 0.5 μ m DB-Petro column was used. The carrier gas was helium at a constant velocity of 35 cm/s. The temperature of the GC column was programmed at 2 °C/min from 50 °C to 260 °C, held for 25 min. A sample size of 3 μ l was used in the split mode with the split ratio of 60:1.

2.2. GC \times GC–TOF-MS apparatus and column sets

The GC \times GC–TOF-MS system consisted of an Agilent 6890 GC (Agilent Technologies, Wilmington, DE, USA), a time-of-flight mass spectrometer Pegasus III (Leco St. Joseph, MI, USA) and a cold-jet modulator KT-2001 Retrofit prototype (Zoex Lincoln, NE, USA). The cold-jet modulator consisted of two cold and two hot jets, with the nozzles providing the cold jets mounted orthogonally to the hot jets. The detailed description of the setup and its operation can be found in the literature [31]. Additional TOF-MS instrumentation parameters, the data conversion and processing methods have been described earlier [29,30].

A 50 m \times 0.2 mm, 0.5 μ m DB-Petro column was used as the first dimensional column and a 2.5 \times 0.1 mm, 0.1 μ m DB-17th stationary phase column as the second dimensional column. The columns were connected by means of a press-fit connector. Two columns were installed in the same oven. The carrier gas was helium at a constant pressure of 600 kPa. The linear velocity of the carrier gas was 24 cm/s for the first column, and 96 cm/s for the second column at 50 °C. A split injection was used with injector temperature at 290 °C. The temperature program used was 50 °C to 260 °C with a ramp rate of 2 °C/min, held for 60 min. Three microliters of sample was injected in the split mode with the split ratio of 30:1.

2.3. Samples

A commercially available brand of cigarettes was used for this study. The particulate phase was collected from the mainstream smoke of 100 cigarettes on glass fiber pads under a set of internationally agreed standard smoking conditions [32]. The glass fiber pads, 400 ml water, 120 g sodium chloride and 40 ml of CH₂Cl₂ were placed in a sample flask of a simultaneous distillation and extraction apparatus (SDE). The solvent flask of SDE contained 50 ml of CH₂Cl₂. The two flasks were boiled for 2 h with efficiently cooling of the vapor by a condenser attached. The CH₂Cl₂ SDE extract was washed three times with 25 ml of 5% sodium hydroxide solution, and the aqueous layer was extracted twice with 10 ml of CH₂Cl₂. The CH₂Cl₂ extracts were mixed. Then, CH₂Cl₂ extract was washed three times with 25 ml of 5% hydrochloric acid solution, and the aqueous layer was extracted twice with 10 ml of CH₂Cl₂. The CH₂Cl₂ extracts were mixed, dried by sodium sulfate and filtered. The total neutral fraction was concentrated to approximately 2 ml. The total neutral fraction was separated by silica acid column [33], elution with 30 ml of 10% ether in hexane yielded a non-polar neutral fraction. Finally, the fraction extract was concentrated to approximately 1 ml.

3. Results and discussion

3.1. GC–EI-MS and GC–PCI-MS analysis

GC–MS with (EI) mode of operation is the common mode of confirmation for all the components. The total ion current (TIC) chromatogram from GC–EI-MS of the non-polar neutral fraction is shown in Fig. 1A. Over 300 compounds were detected, and many of them are aliphatic hydrocarbons. Some long-chain alkanes are observed at higher elution temperature.

For long-chain compounds, the intensity of the molecular ion peak usually is very low, a library search can produce erroneous result. Because of the importance of the molecular weight for aiding the identification of long chain components, PCI was used. Using the same column and GC

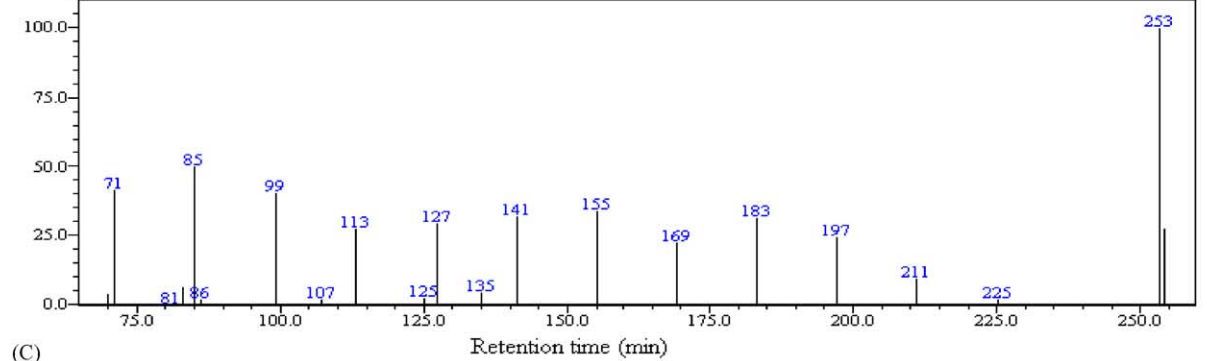
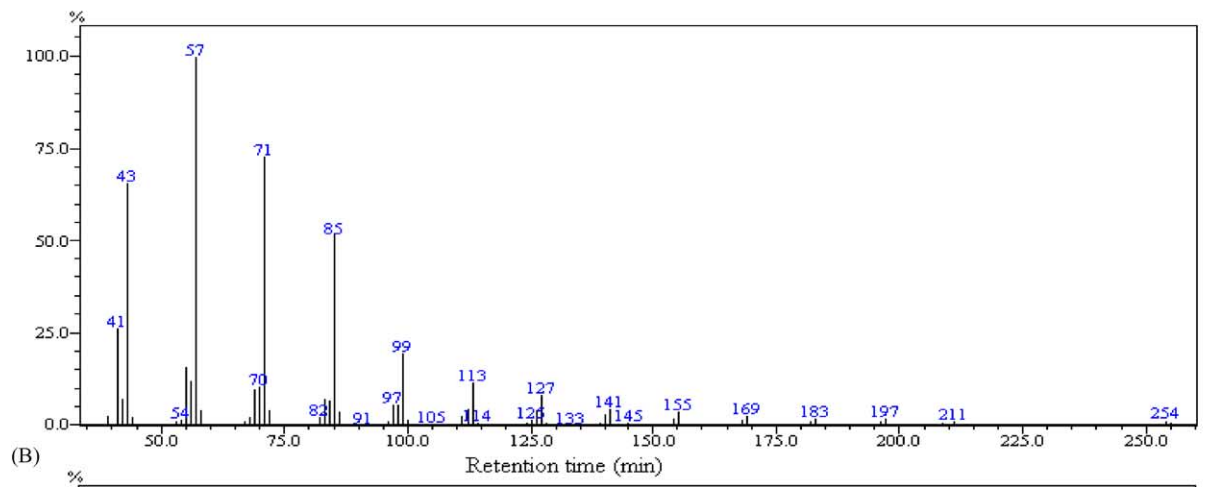
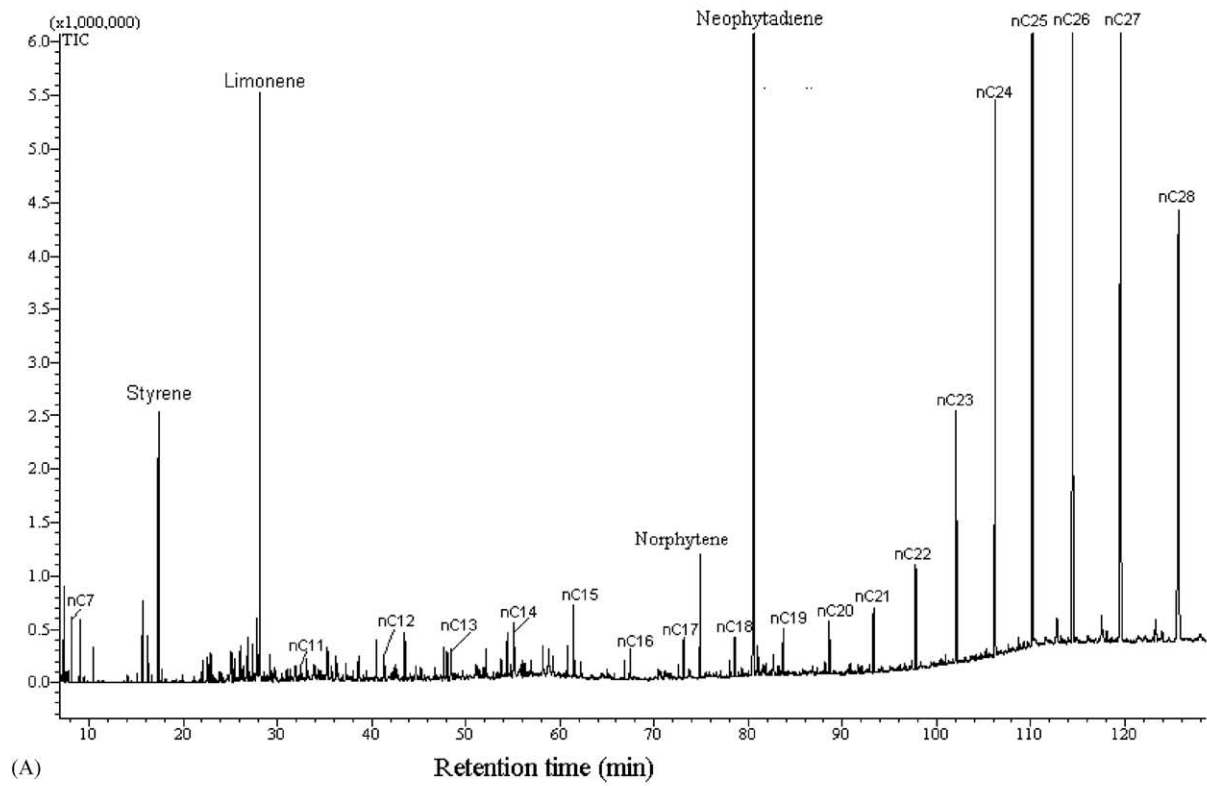


Fig. 1. (A) GC-EI-MS TIC chromatogram obtained from the non-polar hydrocarbon fraction of Virginia cigarette smoke condensate. (B) EI mode MS spectrum of *n*-octadecane. (C) PCI mode MS spectrum of *n*-octadecane.

Table 1
Normal alkanes and alkenes in non-polar hydrocarbon fraction found by GC–EI-MS and GC–PCI-MS

Retention time (min)	Compound	Pseudo-molecular ions of PCI	PCI ionization mode	Area (%)
26.4	<i>n</i> -Decane	141	[<i>M</i> – H]	0.16
33.1	1-Undecene	155	[<i>M</i> + H]	0.16
33.9	<i>n</i> -Undecane	155	[<i>M</i> – H]	0.10
40.5	1-Dodecene	169	[<i>M</i> + H]	0.38
41.3	<i>n</i> -Dodecane	169	[<i>M</i> – H]	0.23
47.6	1-Tridecene	183	[<i>M</i> + H]	0.31
48.4	<i>n</i> -Tridecane	183	[<i>M</i> – H]	0.35
54.4	1-Tetradecene	197	[<i>M</i> + H]	0.37
55.1	<i>n</i> -Tetradecane	197	[<i>M</i> – H]	0.71
60.8	1-Pentadecene	211	[<i>M</i> + H]	0.26
61.5	<i>n</i> -Pentadecane	211	[<i>M</i> – H]	0.71
66.9	1-Hexadecene	225	[<i>M</i> + H]	0.17
67.5	<i>n</i> -Hexadecane	225	[<i>M</i> – H]	0.29
72.6	1-Heptadecene	239	[<i>M</i> + H]	0.13
73.2	<i>n</i> -Heptadecane	239	[<i>M</i> – H]	0.41
78.0	1-Octadecene	253	[<i>M</i> + H]	0.18
78.6	<i>n</i> -Octadecane	253	[<i>M</i> – H]	0.35
83.2	1-Nonadecene	267	[<i>M</i> + H]	0.09
83.7	<i>n</i> -Nonadecane	267	[<i>M</i> – H]	0.43
88.2	1-Eicosene	281	[<i>M</i> + H]	0.11
88.6	<i>n</i> -Eicosane	281	[<i>M</i> – H]	0.48
92.9	1-Heneicosene	295	[<i>M</i> + H]	0.07
93.3	<i>n</i> -Heneicosane	295	[<i>M</i> – H]	0.61
97.4	1-Docosene	309	[<i>M</i> + H]	0.09
97.8	<i>n</i> -Docosane	309	[<i>M</i> – H]	1.00
101.7	1-Tricosene	323	[<i>M</i> + H]	0.05
102.1	<i>n</i> -Tricosane	323	[<i>M</i> – H]	2.49
106.2	<i>n</i> -Tetracosane	337	[<i>M</i> – H]	5.51
110.2	<i>n</i> -Pentacosane	351	[<i>M</i> –H]	8.53
114.5	<i>n</i> -Hexacosane	365	[<i>M</i> – H]	9.10
119.5	<i>n</i> -Heptacosane	379	[<i>M</i> – H]	10.23
125.7	<i>n</i> -Octacosane	393	[<i>M</i> – H]	9.09

operational conditions as the EI mode, the fraction was analyzed under PCI condition. *n*-Octadecane was used as an example to show the combination of two modes of MS for identification of alkane. EI mode MS spectrum and PCI mode MS spectrum of *n*-octadecane are shown in Fig. 1B and 1C, respectively. From Fig. 1B it can be deduced it is a linear-chain alkane, and from Fig. 1C the molecular mass (253 + 1) is obtained. Therefore, the peak identified is *n*-octadecane. The components were finally identified through comparison of mass spectra from both EI and CI mode. Normal and iso-aliphatic hydrocarbons can be easily distinguished through mass fragments produced by EI. In addition, based on pseudo-molecular ion peak [*M* – H] of saturated alkanes and [*M* + H] for others of PCI mode, the aliphatic hydrocarbons were finally identified. It was found that the major alkanes in the fraction are normal C₁₀–C₂₈ alkanes, and the major alkenes are the normal C₁₁–C₂₃ alkenes. It can be predicted that when the column temperature is increased, the hydrocarbons with higher carbon number will be eluted.

The qualitative results and relative area percentage of normal alkanes and alkenes are listed in Table 1. The concentration of long-chain normal alkanes is obviously higher,

Table 2
The distribution of components in non-polar hydrocarbons by 1D-GC–MS

Group	Component number	Area (%)
Alkanes	32	50.7
Alkenes	16	19.7
Aromatics	38	6.4
Terpene hydrocarbons	20	7.8
Other hydrocarbons	9	2.1
Unknown	200	13.3

and normal heptacosane is the most abundant alkane. The distribution of components in the non-polar neutral fraction is listed in Table 2. A total of 115 components have been tentatively identified including 32 alkanes, 16 alkenes, 38 aromatics and 20 terpenes. The relative area percentage of alkanes and alkenes is over 70%. Neophytadiene (16.8%) is the most abundant alkene, and D-limonene (4.8%) is the most abundant monoterpene. Although unidentified compounds represent 13.3% of the total area, the number of unknowns are up to two-third of the total number of the peaks. The determination of these trace components is not possible for the limitation of both sensitivity and resolution of one-dimensional (1D) GC–MS.

3.2. GC × GC analysis

The two-dimensional (2D) GC × GC flame ionization detection (FID) chromatogram for the non-polar neutral fraction of cigarette smoke condensates is shown in Fig. 2. A boiling point elution order on the first dimension was produced by the non-polar first column, and a chemical functional group separation was produced in the second dimension by a moderately polar column. The typical base peak widths are 10–30 s in the first dimension, and 0.15–0.4 s in the second dimension. Compared with the results of 1D-GC, much more compounds are visualized on the 2D plane and most of them are well separated. A total of 3400 compounds were found by the GC × GC software. The distribution of hydrocarbons on 2D plane is similar to that of diesel oil [23]. A band of alkanes is at the bottom of plane, then (going up in the second dimension) alkenes, cycloalkanes, cycloalkenes, monocyclic-aromatics, dicyclic-aromatics and tricyclic-aromatics along the y-axis. The components with greatest second dimension retention are fluoranthenes and pyrenes. The tobacco-specific monoterpenes, sesquiterpenes and diterpenes can also be observed on the 2D plot. This is the most obvious difference of hydrocarbon compositions between cigarette smoke and petroleum fraction.

Based on GC–MS, a total of 115 hydrocarbons were tentatively identified. About 200 peaks were unknown. Most of these unknowns were trace or overlapped components. It was difficult to identify co-eluting peaks by GC–MS system. Using GC × GC–TOF-MS, about 4000 compounds were found, which is more than GC × GC–FID because of the deconvolution of TOF-MS software. Much more trace components were detected, and many overlapped and

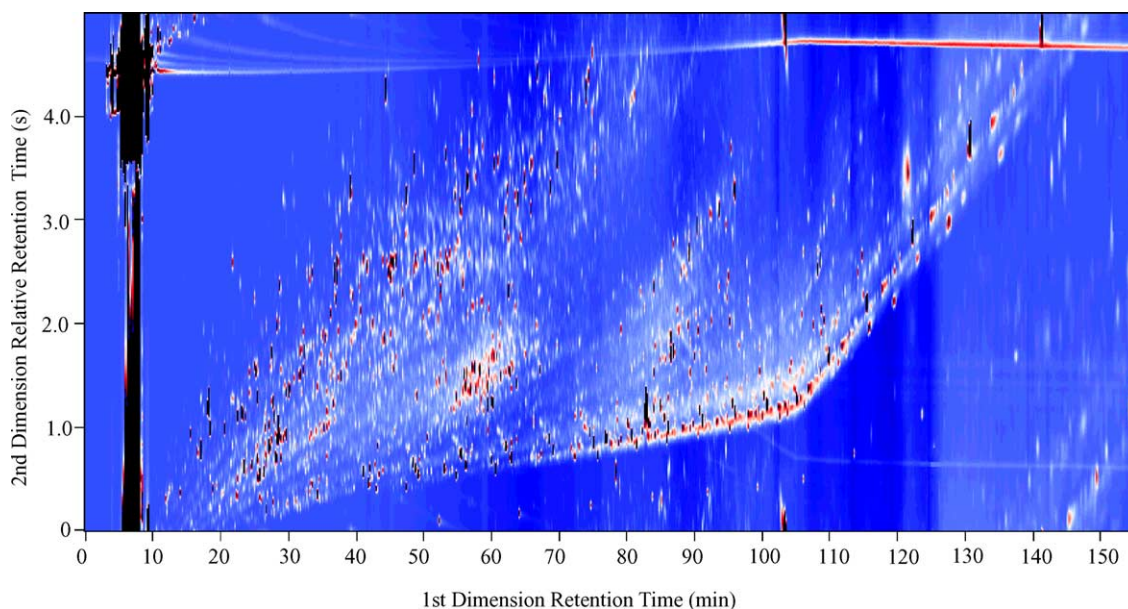


Fig. 2. GC \times GC-FID contour plot obtained from non-polar hydrocarbon fraction of Virginia tobacco cigarette smoke condensate. The column system and conditions are described in the text.

embedded peaks in GC-MS were well resolved. The number of tentatively identified hydrocarbons was over 1800.

3.3. Identification of hydrocarbons in the cigarette smoke condensate

Automated data processing by TOF-MS software combined with the structured chromatogram and manual review was used to identify the components in our earlier study [30]. Welthagen et al. [27] suggested search criteria and rules for the classification procedure. Using a similar method, the

components of hydrocarbon in the non-polar neutral fraction can be tentatively identified.

Using automated peak location and identification routines (built into the TOF-MS software) based on spectral deconvolution, several thousands of peaks were found in the non-polar neutral fraction. The general formulas of hydrocarbons were from C_nH_{2n+2} to C_nH_{2n-22} . However, further review of peak identification is often necessary for reliable characterization due to erroneous library searches. “Structured chromatograms” [21] have been used to identify the group-type separation of petroleum fractions [23]. It can also

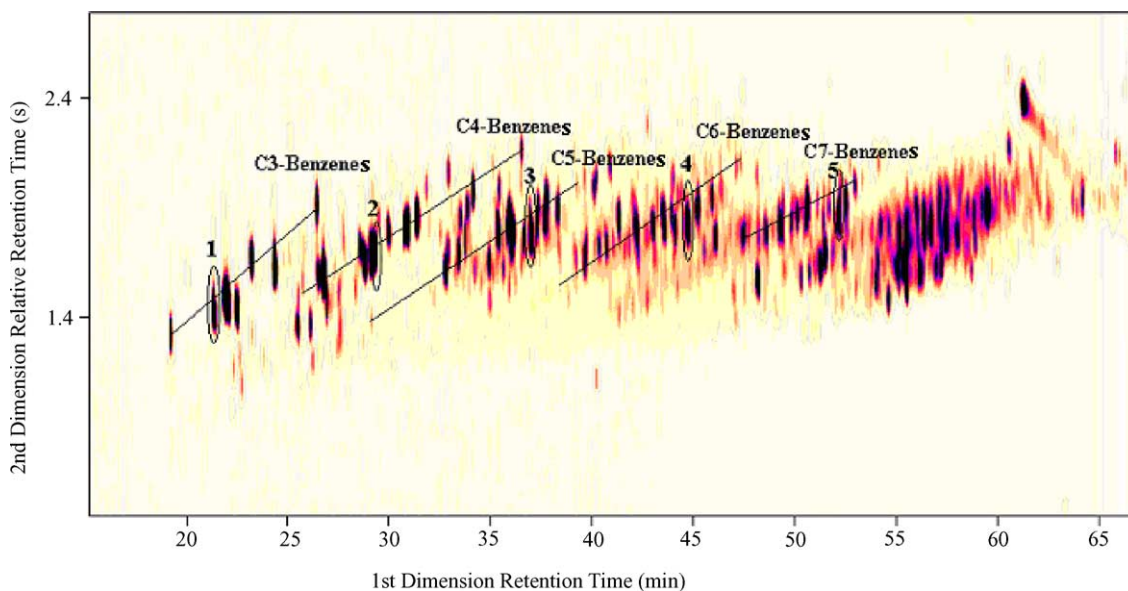


Fig. 3. Contour plot of three-carbon-substituted to seven-carbon-substituted benzenes based on summed ions with the masses of 120, 134, 148, 162 and 176, which are unique for C_3 – C_7 alkyl-substituted benzenes.

Table 3
Number of alkanes and alkenes/cycloalkanes in the non-polar hydrocarbon fraction of a cigarette smoke condensate

Alkanes	Number	Alkenes/cycloalkanes	Component number
C ₈ H ₁₈	1		
C ₉ H ₂₀	4	C ₉ H ₁₈	5
C ₁₀ H ₂₂	14	C ₁₀ H ₂₀	11
C ₁₁ H ₂₄	22	C ₁₁ H ₂₂	27
C ₁₂ H ₂₆	21	C ₁₂ H ₂₄	15
C ₁₃ H ₂₈	20	C ₁₃ H ₂₆	20
C ₁₄ H ₃₀	19	C ₁₄ H ₂₈	24
C ₁₅ H ₃₂	22	C ₁₅ H ₃₀	17
C ₁₆ H ₃₄	18	C ₁₆ H ₃₂	29
C ₁₇ H ₃₆	23	C ₁₇ H ₃₄	26
C ₁₈ H ₃₈	19	C ₁₈ H ₃₆	27
C ₁₉ H ₄₀	19	C ₁₉ H ₃₈	17
C ₂₀ H ₄₂	19	C ₂₀ H ₄₀	26
C ₂₁ H ₄₄	16	C ₂₁ H ₄₂	23
C ₂₂ H ₄₆	16	C ₂₂ H ₄₄	15
C ₂₃ H ₄₈	13	C ₂₃ H ₄₆	19
C ₂₄ H ₅₀	23	C ₂₄ H ₄₈	12
C ₂₅ H ₅₂	20	C ₂₅ H ₅₀	20
C ₂₆ H ₅₄	28	C ₂₆ H ₅₂	21
C ₂₇ H ₅₆	35	C ₂₇ H ₅₄	9
C ₂₈ H ₅₈	19	C ₂₈ H ₅₆	4
Total	391		367

Table 4
Number of aromatics found in the non-polar hydrocarbon fraction

Formula	Group	Compound number
Alkyl-substituted benzenes		
C ₇ H ₈	Toluene	1
C ₈ H ₁₀	Benzene, C ₂	3
C ₉ H ₁₂	Benzene, C ₃	7
C ₁₀ H ₁₄	Benzene, C ₄	18
C ₁₁ H ₁₆	Benzene, C ₅	24
C ₁₂ H ₁₈	Benzene, C ₆	24
C ₁₃ H ₂₀	Benzene, C ₇	7
C ₁₄ H ₂₂	Benzene, C ₈	1
C ₁₅ H ₂₄	Benzene, C ₉	2
C ₁₆ H ₂₆	Benzene, C ₁₀	4
C ₁₇ H ₂₈	Benzene, C ₁₁	8
C ₁₈ H ₃₀	Benzene, C ₁₂	3
C ₁₉ H ₃₂	Benzene, C ₁₃	3
C ₂₀ H ₃₄	Benzene, C ₁₄	1
Alkenylbenzenes/naphthoaromatics		
C ₈ H ₈	Styrene	1
C ₉ H ₁₀	Indane/benzene, C ₃	7
C ₁₀ H ₁₂	Tetrahydronaphthalene/methylindane/benzene, C ₄ -alkenylated	24
C ₁₁ H ₁₄	Tetrahydronaphthalene, C ₁ /indane, C ₂ /benzene, C ₅ -alkenylated	31
C ₁₂ H ₁₆	Tetrahydronaphthalene, C ₂ /indane, C ₃ /benzene, C ₆ -alkenylated	56
C ₁₃ H ₁₈	Tetrahydronaphthalene, C ₃ /indane, C ₄ /benzene, C ₇ -alkenylated	56
C ₁₄ H ₂₀	Tetrahydronaphthalene, C ₄ /indane, C ₅	10
C ₁₅ H ₂₂	Tetrahydronaphthalene, C ₅ /indane, C ₆ /benzene, C ₉ -alkenylated	6
C ₁₆ H ₂₄	Tetrahydronaphthalene, C ₆ /indane, C ₇ /benzene, C ₁₀ -alkenylated	11
Polycyclic aromatics		
C _n H _{2n-12}	Naphthalenes	84
C _n H _{2n-14}	Biphenyls/acenaphthenes/alkenyl naphthalenes	82
C _n H _{2n-16}	Fluorenes/dihydroanthracenes/dihydrophenanthrenes	74
C _n H _{2n-18}	Anthracenes/phenanthrenes	31
C _n H _{2n-20}	Alkenylanthracenes/alkenylphenanthrenes	9
C _n H _{2n-22}	Fluoranthenes/pyrenes	10

be used to validate or correct automated peak identification of GC × GC–TOF–MS. As many different classes exist in the complex hydrocarbons mixture, the border of different groups of compounds is not clearly observed in the TIC contour plot of GC × GC–TOF–MS. But the extracted ion chromatograms (EIC) can be used to separately show the different groups in the samples.

3.3.1. Alkanes, alkenes and cycloalkanes

It is clearly observed from Fig. 2 that significant amounts of trace branched-chain alkanes and alkenes existed. The distributions of the components with the same formulas are given in Table 3. Alkenes and cycloalkanes have similar fragment ions and 2D distribution pattern, so the formula C_nH_{2n} is used to describe both alkenes and cycloalkanes. A total of 391 alkanes and 367 C_nH_{2n} are found in the sample. Many more alkanes and alkenes/cycloalkanes were detected and identified using GC × GC–TOF–MS method than GC–MS.

3.3.2. Aromatic hydrocarbons

The isolation and identification of alkylbenzenes is important because these compounds are linked to phys-

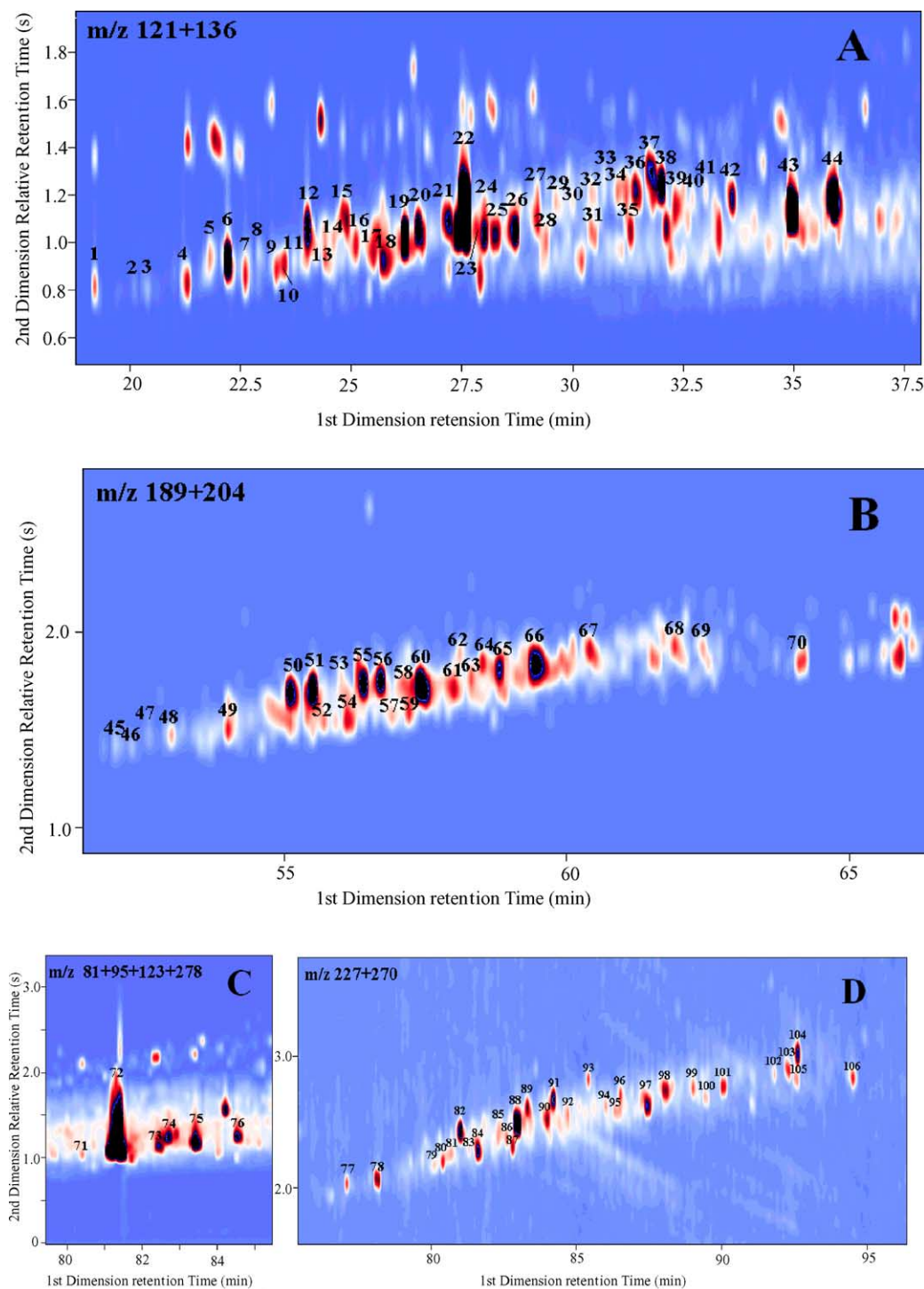


Fig. 4. GC \times GC–TOF–MS extracted ion chromatogram of terpenes. (A) Monoterpenes, 1–44: monoterpenes with molecular formula $C_{10}H_{16}$ (B) sesquiterpenes, 45–70: sesquiterpenes with molecular formula $C_{15}H_{24}$ (C) acyclic diterpenes, 71–76: phytadiene isomers with molecular formula $C_{20}H_{38}$ and (D) 77–106: carbocyclic diterpenes with molecular formula $C_{20}H_{30}$.

biologic toxicity. By selecting appropriate unique masses 120, 134, 148, 162 and 176, C_3 – C_7 alkyl-substituted benzenes could separately be shown in Fig. 3. Circles 1–5 mark the homologous series of linear alkyl benzene from *n*-propylbenzene to *n*-heptylbenzene. It should be noted

that some specific non-aromatics in cigarette smoke condensate are intermingled in the alkylbenzenes band, but they were easily identified by their mass spectra. The number of alkylbenzenes found in non-polar neutral fraction is listed in Table 4. A total of 106 alkylbenzenes are tenta-

tively identified. The distribution of alkylbenzenes is from toluene to 14-carbon-atom alkyl-substituted benzenes. The most abundant alkylbenzenes are five-carbon-atom and six-carbon-atom alkyl-substituted benzenes. All of the linear alkyl benzenes from toluene to *n*-tetradecylbenzenes were seen in the sample, but branched-chain octylbenzene isomers were not detected.

Alkenylbenzenes and naphthoaromatics such as alkylindanes and alkyltetrahydro-naphthalenes have also been tentatively identified in the non-polar neutral fraction (Table 4). A total of 202 alkenylbenzenes and naphthoaromatics were also tentatively identified. The isomers of C₁₂H₁₆ and C₁₃H₁₈ are the major species in this group. No eight-carbon-atom alkenyl-substituted benzenes are found in the fraction.

A total of 98 indenes are also tentatively identified, including 7 methylindenes, 19 C₂ alkyl-substituted indenes, 25 C₃ alkyl-substituted indenes, 26 C₄ alkyl-substituted indenes, 10 C₅ alkyl-substituted indenes and 10 C₆ alkyl-substituted indenes. Similarly, alkylindanes, the isomers of C₃ and C₄ alkyl-substituted indanes are the richest in this subgroup. A total of 290 polycyclic aromatic hydrocarbons (PAHs) are tentatively identified as shown in Table 4. They are two- and three-ringed aromatics, including naphthalenes, alkenylnaphthalenes, biphenyls, acenaphthenes, fluorenes, dihydroanthracenes, dihydrophenanthrenes, anthracenes, alkenylantracenes, phenanthrenes, alkenylphenanthrenes, fluoranthenes and pyrenes. No hydrocarbons containing four or more condensed rings are detected because of the limitation of detection sensitivity. For the determination of these trace PAHs in cigarette smoke, condensates from more cigarettes or higher sensitive detection is necessary.

3.3.3. Isoprenoid hydrocarbons

Isoprene is the basic unit of the terpenes. Terpenes in tobacco may arise by pyrolysis of solanesol and chlorophyll or carotene degradation. The monocyclic cembranoids and bicyclic labdanoids are part of the surface cuticular waxes where they occur along with the sucrose tetraesters, wax hydrocarbons, esters and alcohols. Over 100 terpene hydrocarbons are identified in the non-polar neutral fraction. The GC × GC–TOF–MS extracted ion chromatograms of monoterpenes, sesquiterpenes and diterpenes are shown in Fig. 4A–D. Selecting unique ions 121 and 136 for monoterpenes compounds, a total of 44 compounds are tentatively identified as shown in Fig. 4A (compounds 1–44), including camphene, α-fenchene, α-terpinene, D-limonene, α-terpinolene, Z-β-ocimene, E-β-ocimene and γ-terpinene. Most of the detected monoterpenes are carotene degradation products. Selecting unique ions 189 and 204 for sesquiterpenes, 26 compounds were identified as shown in Fig. 4B (compounds 45–70), including β-elemene, γ-elemene, α-farnesene, thujopsene and δ-gurjunene. Phytane, cembrenes and labanene are the major diterpenes identified in the non-polar neutral fraction. Fig. 4C (compounds 71–76) shows the extracted ion chromatogram

of phytadiene isomers, including neophytadiene and five other phytadiene isomers which have a single set of conjugated double bonds in different terminal and internal positions. Excluding aliphatic diterpene, a total of 30 cyclic diterpene with formula C₂₀H₃₀ are found in the fraction as shown in Fig. 4D (compounds 77–106). The probable structures are labdanenes based on their mass spectra. They may be produced by dehydration of labdanoids. A total of 14 megastigatriene isomers and six membrane isomers were also detected in the non-polar neutral fraction. The identification of individual terpene isomers was not within the scope of this study owing to the lack of both standard compounds and additional structure information.

In the previous papers [29,30], the acidic and basic fractions of cigarette smoke condensates were investigated using GC × GC–TOF–MS. The number of detected peaks was about 1000 for acidic fraction, and 800 for basic fraction. A total of 139 organic acids, over 150 phenols and 377 nitrogen-containing compounds were tentatively identified in the acidic fraction and the basic fraction, respectively. The composition of non-polar neutral fraction is much more complicated than that of the acidic fraction and basic fraction, about 4000 peaks were detected, and over 1800 hydrocarbons were identified in this work.

4. Conclusions

GC × GC in combination with TOF–MS detection has been demonstrated to be a useful tool for the characterization of complex hydrocarbon mixtures in cigarette smoke condensates. For the analysis of the non-polar neutral fraction of cigarette smoke condensates, about 4000 components have been detected. It is not possible with conventional analytical technologies. The GC × GC–TOF–MS identification results can be validated or corrected using extracted ion chromatograms of isomers and homologous series. Over 1800 hydrocarbon components were tentatively identified, including aliphatic hydrocarbons, aromatics, and isoprenoid hydrocarbons. Over 100 important isoprenoid hydrocarbons were detected. The investigation of cigarette smoke hydrocarbons is beneficial to the further understanding of the burning mechanism. Although the developed GC × GC–TOF–MS method has been demonstrated to be very useful for the characterization of hydrocarbons in cigarette smoke, additional structure information is still necessary for the further structural characterization of numerous isomers.

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