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Gas chromatographic–mass spectrometric determination of polycyclic aromatic hydrocarbons formed during the pyrolysis of phenylalanine

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Abstract

The formation of polycyclic aromatic hydrocarbons (PAHs) during pyrolysis process of phenylalanine had been studied. Ten PAHs, including fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, and benzo[*a*]pyrene were analyzed by gas chromatography–mass spectrometry using selective ion monitoring mode. This technique offers the capability to analyze trace amounts of PAHs in phenylalanine pyrolyzates. The pyrolysis was carried out in a micro-furnace with quartz furnace liner. The injection was conducted with glass pelletizer syringe to avoid metal contamination. Qualitative results were obtained at 900 °C and quantitative analysis of 10 PAHs was done for 700 and 900 °C.

Keywords: Pyrolysis; Cigarette smoke; Polynuclear aromatic hydrocarbons; Benzo[*a*]pyrene; Phenylalanine

1. Introduction

Cigarette smoke analysis for specific compounds such as polycyclic aromatic hydrocarbons (PAHs) was the subject of numerous studies [1–4]. Rodgman [5] summarized the identification, tobacco precursors and control of levels of PAHs in cigarette mainstream smoke. The PAHs are generally found in trace amounts in the particulate matter of smoke in the presence of a very complex matrix. Chromatography and spectrometry techniques are commonly used for the analysis. PAHs are well known as a group of environmental carcinogens and are subject of intensive investigations [6,7]. Their most important source is the incomplete combustion of organic materials [8–10]. Ingestion and inhalation are the main pathways into the human body.

Stotesbury et al. [11,12] had predicted tobacco additives behaviour in a burning cigarette through pyrolysis and pyrolyzed cigarette ingredients labelled with stable isotopes. Forehand et al. [13] studied the formation of several PAHs in the pyrolysis of two phytosterols and of cellulose with the purpose of determining the origin of PAHs in cigarette smoke. Their work demonstrated that pyrolysis could be applied usefully to the study of smoke chemistry.

Phenylalanine is one of the major amino acid in tobacco. Free phenylalanine in high quality smoking grade blends is 0.24 mg/g in flue-cured and 0.50 mg/g in burley [14]. It is significant to investigate whether or not it will produce PAHs during pyrolysis. Upon pyrolysis, phenylalanine produced a complex mixture of various classes of compounds [15]. The determination of the possible trace PAHs in the mixture presents a challenging task to the analytical chemist, since it demands critical chromatographic and spectrometric conditions, especially to the analysis of benzo[*a*]pyrene which is one of the most potential carcinogenic PAH. To our knowledge, information on benzo[*a*]pyrene level in phenylalanine pyrolyzates has not been reported in the literature. In this study, by choosing the MS detector in the selective ion monitoring (SIM) mode, both sensitivity and selectivity were increased, and trace amounts of PAHs including benzo[*a*]pyrene in phenylalanine pyrolyzates were found and quantified.

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Table 1
The mass and concentrations of the target PAHs

Compound	Mass (g)	Concentrations of E.S.* (ng/μl)
Fluorene	0.01010	32.3
Phenanthrene	0.01030	33.0
Anthracene	0.01036	33.1
Fluoranthene	0.01146	36.7
Pyrene	0.00964	30.8
Benzo[*a*]anthracene	0.00440	14.1
Chrysene	0.00386	12.4
Benzo[*k*]fluoranthene	0.00306	9.8
Benzo[*e*]pyrene	0.00301	9.6
Benzo[*a*]pyrene	0.00741	23.7

* External standards.

2. Materials and methods

2.1. Chemicals and reagents

DL-ß-Phenylalanine (99% purity) was purchased from Kangda amino acid factory, Shanghai, China; Fluorene (98% purity), phenanthrene (99% purity), fluoranthene (99% purity), pyrene (98% purity) and benzo[e]pyrene (99% purity) were purchased from Aldrich; anthracene (99% purity), benzo[*a*]anthracene (99% purity), chrysene (98% purity), benzo[*k*]fluoranthene (99% purity) and benzo[*a*]pyrene (98% purity) were purchased from Acros. A solution of PAHs in toluene was prepared containing external standards. The mass and concentration values for PAHs are summarized in Table 1.

2.2. Pyrolysis operation

The sample was pyrolyzed using a SGE Pyrojector II. Phenylalanine might be introduced using the solid injector by septumless mode. The sample needed to be in ground state, so that it could be introduced into the pyrolyzer. The pyrolysis procedure used the glass pelletizer injection device to avoid metal contamination. Only a small amount of sample should be deposited in the tube. When the sample had been placed in the tube it should be gently compressed using two plungers. The sample should not be overcompressed, as this would make it difficult to expel the sample from the pelletizer into the furnace, and overcompression would also affect the dispersion and rapid heating of the sample in the furnace. Pressing either the up or down temperature set buttons displayed the set point temperature of the temperature control circuit. When the conditions in the pyrolyzing furnace and gas chromatograph were ready for injection, the sample was rapidly expelled into the hot quartz furnace liner. It was important that the sample was sent all the way into the middle of the hot furnace zone and caught in the quartz wool. A plug was provided to block the open end of the quartz lined pyrolysis zone while the pelletizer had been removed for cleaning or sample loading. Before the next sample was prepared the pelletizer tube should be clean and free of particulate material. Because the plunger was a very accurate fit in the glass barrel any remained material might jam the plunger and possibly crack the glass.

2.3. Reproducibility of the experiments

In order to test the reproducibility of the system, several experiments were performed by using a combination of a SGE Pyrojector II and a Hewlett-Packard gas chromatograph HP 6890 equipped with a split/splitless injector, and flame ionization detection (FID) system. The GC system was operated at a constant pressure of 15 psi (1 psi = 6894.76 Pa). Helium (99.999%) was used as carrier gas. The helium flowed through the column at a rate of 1.3 ml/min. The FID system was operated at 300 °C. The injector temperature was set at 250 °C. A 30 m × 0.25 mm J & W DB-1 fused silica capillary column with 0.25 μm film thickness was temperature programmed from 50 °C (held for 2 min) at a rate of 4 °C/min to 280 °C and held for 5 min. The split rate was 1:100. The pressure in the pyrolysis furnace was set to 20 psi. The septum purge was approximately 4.5 ml/min. Commercially available DL-ß-phenylalanine was used as received. The sample was weighed and pyrolyzed at 700 °C.

2.4. Pyrolysis–gas chromatography–mass spectrometry

Pyrolysis–GC–MS was carried out by using a SGE micro-furnace Pyrojector II interfaced directly to a Hewlett-Packard 6890 gas chromatograph equipped with a 5972 mass spectrometer detector. An HP-5MS (50 m × 0.20 mm i.d., 0.33 μm film thickness column) was used. The following analysis conditions were used: injector temperature, 300 °C; split rate, 1:50; temperature program, 50–280 °C (held for 30 min) at 5 °C/min; carrier gas, helium (flow rate, 1.0 ml/min); constant pressure, 30.00 psi; temperature of transfer line, 290 °C. The electron impact ionization (electron energy 70 eV) was used to generate the ions. The MS source temperature was 230 °C. The mass spectrometer was operated in two modes. One was the scan mode for qualitative analysis of the pyrolysis products, with a scan range from *m*/*z* 55 to 400; the other was the SIM mode for quantitative analysis of PAHs. The characteristic ions (*m*/*z*) monitored in the SIM mode for each PAH are shown

Table 2
Ions used for SIM mass spectra

PAH	Ion masses (*m*/*z*)
Fluorene	166
Phenanthrene	178
Anthracene	178
Fluoranthene	202
Pyrene	202
Benzo[*a*]anthracene	228
Chrysene	228
Benzo[*k*]fluoranthene	252
Benzo[*e*]pyrene	252
Benzo[*a*]pyrene	252

Table 3
Compounds released from pyrolysis of phenylalanine at 900 °C

No.	t_r (min)	Compounds	M_r	Formula
1	4.12	2-Propenenitrile	53	C_3H_3N
2	4.27	1,3-Cyclopentadiene	66	C_5H_6
3	5.56	Benzene	78	C_6H_6
4	7.85	Toluene	92	C_7H_8
5	10.48	Ethylbenzene	106	C_8H_{10}
6	10.71	Benzene, 1,3-dimethyl-	106	C_8H_{10}
7	11.45	1,3,5,7-Cyclooctatetraene	104	C_8H_8
8	11.53	Benzene, 1,2-dimenthyl-	106	C_8H_{10}
9	13.22	Benzene, 2-propenyl-	118	C_9H_{10}
10	14.27	Aniline	93	C_6H_7N
11	14.44	α-Methylstyrene	118	C_9H_{10}
12	14.58	Benzonitrile	103	C_7H_5N
13	14.89	Benzene, 1-ethenyl-3-methyl-	118	C_9H_{10}
14	15.01	Benzene, 1-ethenyl-4-methyl-	118	C_9H_{10}
15	15.96	Benzene, 1-propenyl-, (E)-	118	C_9H_{10}
16	16.77	Indene	116	C_9H_8
17	17.48	Benzonitrile, 4-methyl-	117	C_8H_7N
18	18.20	Benzonitrile, 3-methyl-	117	C_8H_7N
19	18.67	Benzonitrile, 2-methyl-	117	C_8H_7N
20	18.96	Benzene, 1,3-diethenyl-	130	$C_{10}H_{10}$
21	19.79	Benzyl nitrile	117	C_8H_7N
22	20.32	2-Methylindene	130	$C_{10}H_{10}$
23	20.63	1H-Indene, 1-methylene-	128	$C_{10}H_8$
24	21.27	Benzeneacetonitrile, α-methylene-	129	C_9H_7N
25	21.43	2-Propenenitrile, 3-phenyl-, (E)-	129	C_9H_7N
26	21.61	Naphthalene	128	$C_{10}H_8$
27	23.29	Quinoline	129	C_9H_7N
28	23.99	Isoquinoline	129	C_9H_7N
29	24.88	Indole	117	C_8H_7N
30	25.11	Naphthalene, 2-methyl-	142	$C_{11}H_{10}$
31	25.67	Naphthalene, 1-methyl-	142	$C_{11}H_{10}$
32	27.57	Naphthalene, 2-ethenyl-	154	$C_{12}H_{10}$
33	28.10	1,1′-Biphenyl, 2-methyl-	168	$C_{13}H_{12}$
34	29.07	Diphenylmethane	168	$C_{13}H_{12}$
35	29.85	Acenaphthylene	152	$C_{12}H_8$
36	29.92	Pyridine, 3-phenyl-	155	$C_{11}H_9N$
37	30.53	1,1′-Biphenyl, 4-methyl-	168	$C_{13}H_{12}$
38	30.71	2,2′-Dimethylbiphenyl	182	$C_{14}H_{14}$
39	30.80	1,1’-Biphenyl, 3-methyl-	168	$C_{13}H_{12}$
40	31.04	2-Naphthalenecarbonitrile	153	$C_{11}H_7N$
41	31.36	Ethylene, 1,1′-diphenyl-	180	$C_{14}H_{12}$
42	31.61	1,1′-Biphenyl, 2,3′-dimethyl-	182	$C_{14}H_{14}$
43	31.72	1-Naphthalenecarbonitrile	153	$C_{11}H_7N$
44	31.82	Benzene, 1-methyl-2- (phenylmethyl)-	182	$C_{14}H_{14}$
45	31.96	Benzene, 1-methyl-4- (phenylmethyl)-	182	$C_{14}H_{14}$
46	32.09	1-Aminonaphthalene	143	$C_{10}H_9N$
47	33.28	3,3′-Dimethylbiphenyl	182	$C_{14}H_{14}$
48	33.49	Fluorene	166	$C_{13}H_{10}$
49	33.59	4,4′-Dimethylbiphenyl	182	$C_{14}H_{14}$
50	33.83	9H-Fluorene, 2-methyl-	180	$C_{14}H_{12}$
51	34.45	1.1′-Biphenyl, 4-ethenyl-	180	$C_{14}H_{12}$
52	36.39	(E)-Stilbene	180	$C_{14}H_{12}$
53	36.58	9H-Fluorene, 1-methyl-	180	$C_{14}H_{12}$
54	36.87	Acridine	179	$C_{13}H_9N$
55	37.18	9H-Fluorene, 9-methylene-	178	$C_{14}H_{10}$
56	37.28	1H-Indene, 1-phenyl-	192	$C_{15}H_{12}$
57	37.95	1H-Indene, 2-phenyl-	192	$C_{15}H_{12}$
58	38.57	Phenanthrene	178	$C_{14}H_{10}$
59	38.78	Anthracene	178	$C_{14}H_{10}$
60	38.95	Phenanthrene, 9,10-dihydro-1-methyl-	194	$C_{15}H_{14}$
61	39.04	Anthracene, 1-methyl-	192	$C_{15}H_{12}$
62	39.17	Phenanthrene, 9,10-dihydro-2-methyl-	194	$C_{15}H_{14}$
63	40.10	1-Phenylnaphthalene	204	$C_{16}H_{12}$
64	40.24	1H-Indene, 3-phenyl-	192	$C_{15}H_{12}$
65	40.97	Iminostilbene	193	$C_{14}H_{11}N$
66	41.09	Anthracene, 2-methyl-	192	$C_{15}H_{12}$
67	41.24	Phenanthrene, 2-methyl-	194	$C_{15}H_{14}$
68	41.71	Phenanthrene, 1-methyl-	194	$C_{15}H_{14}$
69	41.83	Phenanthrene, 4-methyl-	194	$C_{15}H_{14}$
70	42.65	2-Phenylnaphthalene	204	$C_{16}H_{12}$
71	44.20	1H-Indole, 2-phenyl-	193	$C_{14}H_{11}N$
72	44.84	Fluoranthene	202	$C_{16}H_{10}$
73	45.34	Pyrene	202	$C_{16}H_{10}$
74	45.59	9-Anthracenecarbonitrile	203	$C_{15}H_9N$
75	46.05	1,4-Diphenylbenzene	230	$C_{18}H_{14}$
76	48.30	11H-Benzo[*b*]fluorene	216	$C_{17}H_{12}$
77	52.80	1-Methylchrysene	242	$C_{19}H_{14}$
78	53.86	Benz[*a*]anthracene	228	$C_{18}H_{12}$
79	54.22	Chrysene	228	$C_{18}H_{12}$
80	54.76	Anthracene, 9-phenyl-	254	$C_{20}H_{14}$
81	55.27	1,2′-Binaphthyl	254	$C_{20}H_{14}$
82	58.66	2,2′-Binaphthyl	254	$C_{20}H_{14}$
83	65.61	Perylene	252	$C_{20}H_{12}$
84	67.12	Benz[*e*]acephenanthrylene	252	$C_{20}H_{12}$

in Table 2. During standard solution analysis, 1 μl was injected using auto-injector with a solvent delay of 8.00 min. The phenylalanine sample (0.00022 g) was pyrolyzed at 900 °C while GC–MS was applied in the scan mode, and 0.00021 g of sample at 700 °C, and 0.00020 g at 900 °C while GC–MS was applied in the SIM mode.

3. Results and discussion

3.1. Reproducibility of the system

The experiment reflected good reproducibility of the retention times and peak intensities between the consecutive runs. The mean run-to-run variability was estimated using the relative standard deviations (R.S.D.) by averaging the relative analyte peak area calculated from three runs with area normalization method. For the main compounds, the R.S.D. were within good limits. 30 peaks were chosen between 15 and 64 min of the retention time, the minimum R.S.D. was 1%, the maximum R.S.D was 24%, and the average R.S.D. was 7.8%.

Table 4
The quantity of PAHs from pyrolysis of phenylalanine (ng/mg)

Compounds	Retention time (min)	700 °C	900 °C
Fluorene	33.48	2.9×10^2	6.8×10^3
Phenanthrene	38.53	2.0×10^3	2.7×10^4
Anthracene	38.77	4.7×10^2	4.4×10^3
Fluoranthene	44.83	11	7.6×10^2
Pyrene	46.02	16	3.4×10^2
Benzo[*a*]anthracene	53.86	8.8	2.9×10^2
Chrysene	54.21	15	5.8×10^2
Benzo[*k*]fluoranthene	65.94	5	4.5×10^2
Benzo[*e*]pyrene	69.55	9	1.3×10^2
Benzo[*a*]pyrene	70.36	31	2.0×10^2

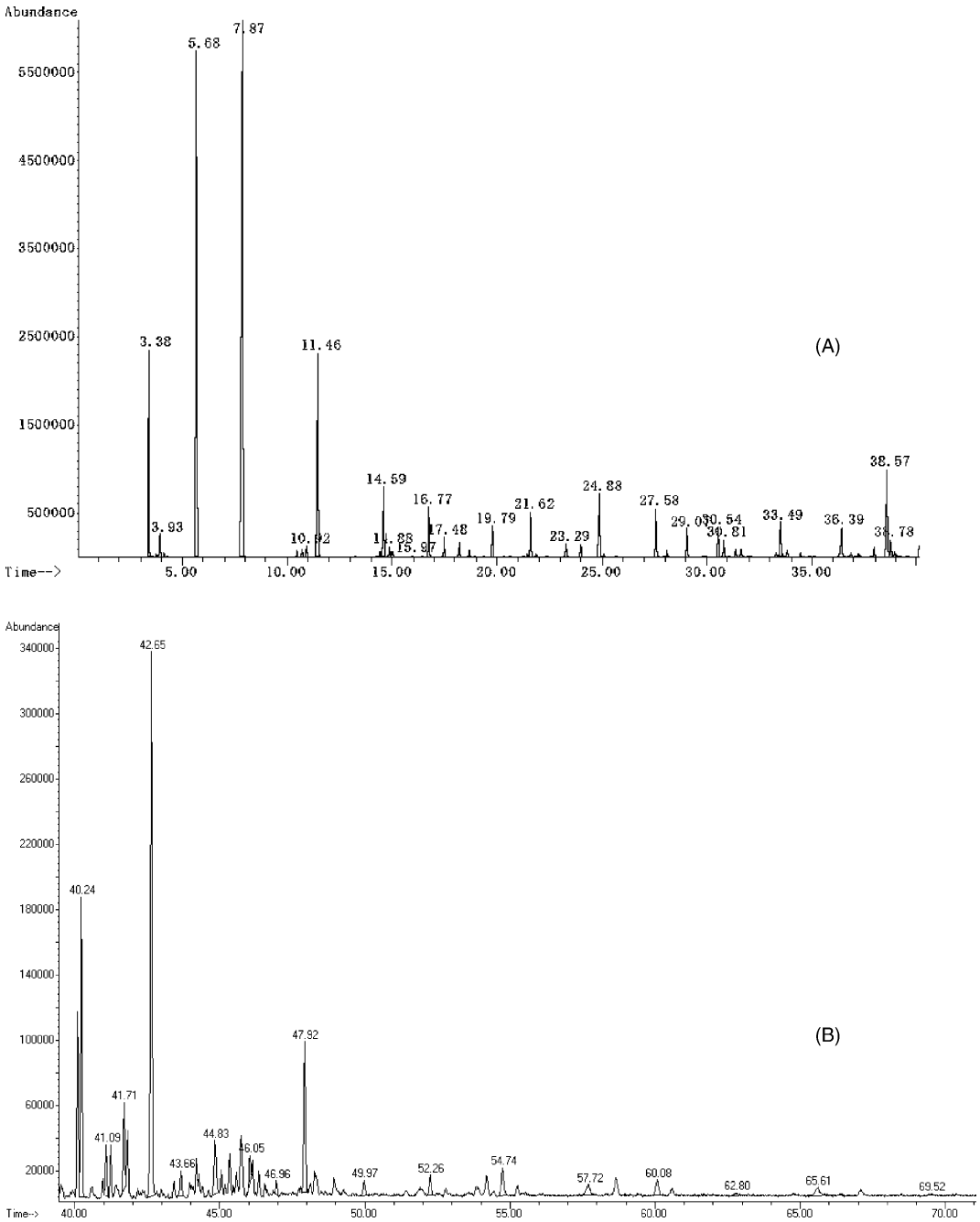

Fig. 1. TIC chromatograms of pyrolysis of phenylalanine at 900 °C. (A) Retention time: 0–40 min; (B) retention time: 40–71 min.

3.2. SIM quantitation

The total ion chromatogram (TIC) of phenylalanine pyrolyzed at 900 °C is shown in Fig. 1, and the GC–MS identification results are shown in Table 3. The mass spectra identifications were carried out by comparing to the NIST (US National Institute of Standards and Technology) as well as to the Wiley (New York, USA) mass spectral libraries. As seen in Table 3, phenylalanine pyrolysis produced various PAHs, but some of the important PAHs in pyrolyzates such as benzo[*e*]pyrene and benzo[*a*]pyrene could not be detected in scan mode, due to their very low levels.

Typical electron impact ionization (EI) mass spectra of PAHs showed little fragmentation. This lack of fragmentation in the spectrum was a disadvantage in confirming ions for the identification of PAHs, but it was an advantage regarding the sensitivity when using SIM detection. All measurements for 10 PAHs were done using their molecular ions. The identification of compounds of PAHs was accomplished by comparing the retention time of the products

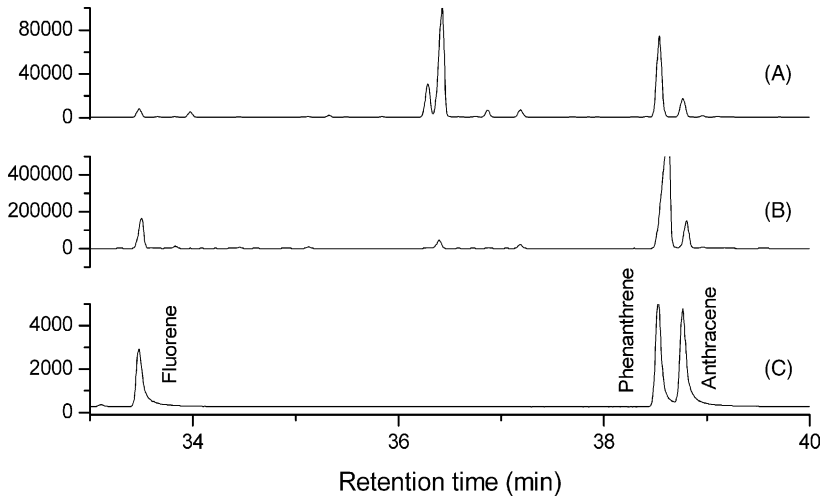

Fig. 2. Selected ion chromatograms, $m/z = 166$ (fluorene) and 178 (phenanthrene and anthracene). (A) At 700 °C; (B) at 900 °C; (C) standard.

to the retention time of PAH external standards run under the same conditions and the corresponding mass spectra. The SIM ion group data were converted into extracted ion chromatograms for each particular compound (ion), and the areas under each peak were determined for further quantitation. The detection limits for these 10 PAHs using the GC–MS–SIM mode were 1–10 ppb.

Since the ratio of mass fragments to parent ion was characteristic for each compound, using this approach allowed us to assign the retention time for each PAH under investigation unequivocally. This was particularly important for the isomers phenanthrene and anthracene. After this assignment, only the parent ions were used in the subsequent data analysis. The capability of verifying mass spectral patterns as well as retention times made the GC–MS technique a very reliable method for PAH analysis.

3.3. Analysis of phenylalanine pyrolysate

In the first puff, the tobacco in a cigarette started at ambient temperature and was rapidly heated to about 900 °C within 2 s [16]. All subsequent puffs started with an established coal smoldering at a temperature of about 700 °C [17]. So in this paper, pyrolysis temperature was chosen at 700 and 900 °C in a non-oxidizing atmosphere of helium gas. In Figs. 2–4, the mass traces for the native PAHs acquired during pyrolysis of phenylalanine were compared to the corresponding standard chromatograms, starting with fluorene (three-ring, shown in Fig. 2) and ending with the five-ring PAHs shown in Fig. 4. These chromatograms demonstrated the effectiveness of using the glass pelletizer injection. Virtually, no contaminations and proper quantification of the target compounds were obtained.

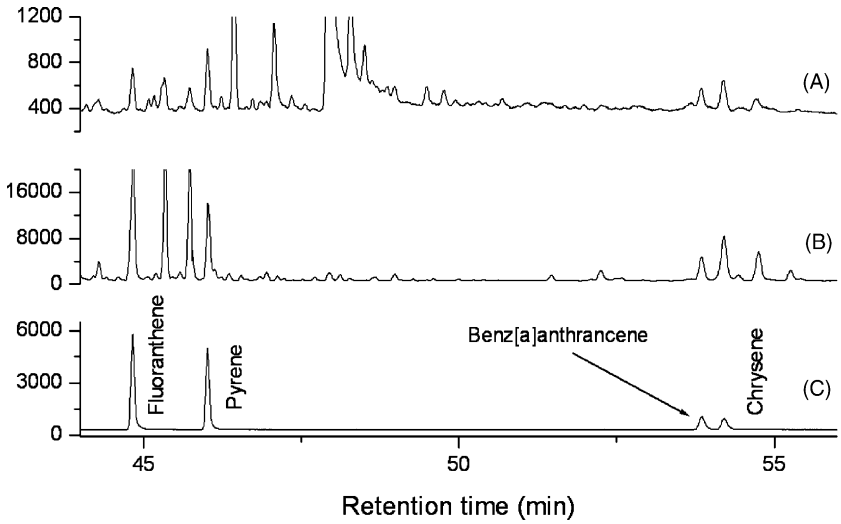

Fig. 3. Selected ion chromatograms, m/z 202 (fluoranthene and pyrene) and 228 (benzo[*a*]anthracene and chrysene). (A) At 700 °C; (B) at 900 °C; (C) standard.

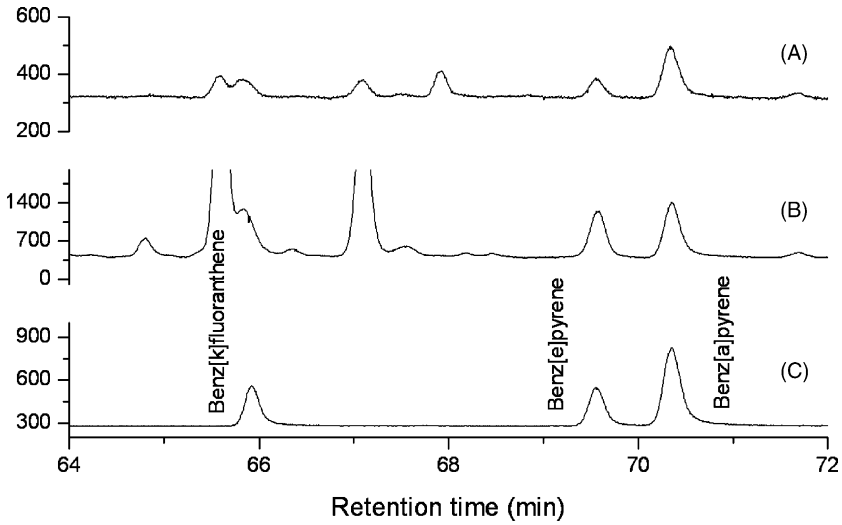

Fig. 4. Selected ion chromatograms, *m*/*z* 252 (benzo[*k*]fluoranthene, benzo[*e*]pyrene and benzo[*a*]pyrene). (A) At 700 °C; (B) at 900 °C; (C) standard.

We compared the PAH yield of an unknown sample with a known control standard solution. The quantitation was done based on the ratio of peak areas of the analytes and corresponding external standards. The $AREA_{E.S.}$ (The peak area of external standard) for each compound was obtained by averaging the integrated standard matter peak area calculated from three runs. The bounds of R.S.D.s were within very good limits, and seven out of the ten R.S.D.s were less than 6.7%. Some compounds at very low levels had higher R.S.D.s, but they were less than 17.6%. Extracted ion chromatograms were obtained for the characteristic ions for each of the compounds used for quantitative determination. The quantity of PAHs from pyrolysis of phenylalanine were calculated using the following formula:

Relative analyte level (ng/mg) = $[(AREA_{analyte}/AREA_{E.S}) \times Concentration_{E.S}(ng/\mu l) \times \mu l]$/[mass of pyrolysis sample(mg)].

The results of quantitative analysis are shown in Table 4.

The results indicated that the high-molecular-mass PAHs (4–5 rings) were found in much smaller quantity compared to those containing only three rings. This corresponds well to the information on PAH concentrations in the mainstream smoke of the Kentucky reference cigarette IR4F [1]. Phenanthrene was the dominating compound, followed by fluorene and anthracene. Benzo[*a*]anthracene, chrysene, benzo[*k*]fluoranthene and benzo[*a*]pyrene, the tumorigenic agents in tobacco smoke [18], were identified in the pyrolyzates at 700 and 900 °C. The yields of PAHs were considerably higher from the pyrolysis at 900 °C than that from the pyrolysis at 700 °C.

In the present work, we have demonstrated that it is important to reduce the temperature of the center of the burning zone on intent to reduce the level of PAH compounds in tobacco smoke, since the PAH yields mostly increased with temperature.

4. Conclusion

A method has been developed for the determination of up to 10 significant PAHs in the phenylalanine pyrolyzates. This method could effectively explore the origins of PAHs in cigarette smoke. Although the work presented only deals with phenylalanine, the method is also suitable for other types of components in cigarette.

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