Characterization of polychlorinated aromatic hydrocarbons by reversed-phase liquid chromatography with ultraviolet absorbance and mass spectrometric detection

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Abstract

Ten polychlorinated aromatic hydrocarbons (PCAHs), perchlorobenzene (**I**), perchlorobiphenyl (**II**), perchlorobenzo[*f*]inden-1-one (**III**), perchloronaphthalene (**IV**), perchloropenta[*def*]phenanthren-4-one (**V**), 6H-1,2,4,5,7,8,9,10,11-nonachloro-benzo[*cd*]pyrene-3-one (**VI**), perchloroacenaphthylene (**VII**), 6-(*p*-tolyl)-6H-1,2,3,4,5,7,8,9,10,11-decachlorobenzo[*cd*]pyrene (**VIII**), perchlorofluoranthene (**IX**) and 6-(*p*-tolyl)-6H-1,11-dihydroxy-2,3,4,5,7,8,9,10-octachlorobenzo[*cd*]pyrene (**X**) have been separated with reversed-phase liquid chromatography (RPLC) by using two gradient eluents (toluene/methanol and cyclo-hexane/methanol), and were detected by ultraviolet (UV) and mass spectrometry (MS). The separation and detection of half of these ten compounds, which are of extremely high molecular weights, is an improvement over the chromatographic analysis of PCAHs. When the procedure was applied to the analysis of PCAHs of the products from the combustion of chlorobenzene and carbon tetrachloride, **I**, **II**, **IV**, **VIII** and **X** could be detected.
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1. Introduction

Great concerns about polychlorinated aromatic hydrocarbons (PCAHs) are mainly due to improper utilization, disposal and destruction of them in the environments [1–4]. Growing data [5–10] reveal that toxic PCAHs are the final products of combustion, discharge reaction and pyrosynthesis of organochlo-rine compounds, which are widely used as solvents in syntheses and as starting materials in polymer and pesticide. PCAHs have been detected in chlorinated tap and surface water [11], in urban air and auto-mobile exhausts [12], in emissions from municipal waste incinerators [13], etc. These PCAHs are ca-pable of causing a number of adverse health effects even with very low exposures [14]. Some are calling for a complete ban on the use of chlorine [15].

PCAHs having less than 12 carbon atoms can be conveniently detected by using chromatographic

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methods, and the limits of detection of these compounds have been significantly lowered through developments in capillary gas chromatography (GC) with mass spectrometry (MS) and atomic emission [16]. For example, a number of key polychlorinated biphenyls have been detected by high-resolution GC with an electron capture detector; this requires the use of an achiral column after a fractionation step on a 2-(1-pyrenyl)ethyldimethylsilylated silica column [17]; Enantiomers of 19 stable chiral polychlorinated biphenyl atropisomers were separated on seven commercially-available chiral capillary GC columns containing modified cyclodextrins [18]. In our exploratory experiment [19], separation and identification of a library of fully chlorinated PCAHs using reversed-phase liquid chromatography (RPLC) with ultraviolet (UV) absorption spectrometric detection have been preliminarily described. The separation of one class of the PCAHs bearing more than 12 carbon atoms, however, have not been well documented, and the reason may be attributed to a lack of reference compounds. In this report, we describe an RPLC eluted by toluene/methanol (and also by cyclohexane/methanol) combined with UV absorption and atmospheric pressure chemical ionization (APCI) mass spectrometric detection (LC–UV–MS) for identification of five PCAHs having 13–26 carbon atoms, as well as five reference compounds that are fully chlorinated PCAHs previously reported [19]. These PCAHs, including perchlorobenzene C_6Cl_6 (**I**), perchlorobiphenyl $C_{12}Cl_{10}$ (**II**), perchlorobenzo[*f*]inden-1-one $C_{13}Cl_8O$ (**III**), perchloronaphthalene $C_{10}Cl_8$ (**IV**), perchloropenta[*def*]phenanthren-4-one $C_{15}Cl_8O$ (**V**), 6H-1,2,4,5,7,8,9,10,11-nonachlorobenzo[*cd*]pyrene-3-one $C_{19}Cl_9HO$ (**VI**), perchloroacenaphthylene $C_{12}Cl_8$ (**VII**), 6-(*p*-tolyl)-6H-1,2,3,4,5,7,8,9,10,11-decachlorobenzo[*cd*]pyrene $C_{26}Cl_{10}H_8$ (**VIII**), perchlorofluoranthene $C_{16}Cl_{10}$ (**IX**) and 6-(*p*-tolyl)-6H-1,11-dihydroxy-2,3,4,5,7,8,9,10-octachlorobenzo[*cd*]pyrene $C_{26}Cl_8H_{10}O_2$ (**X**), were produced from the reaction of sodium and carbon tetrachloride [20].

2. Experimental

2.1. Chemicals

All the solvents used in the experiments were commercially available. These solvents were ana-

lytical grade and were further distilled before LC experiments.

2.2. Standard compounds

All the standard compounds including **I**–**X** were prepared in the laboratory. Syntheses of compound **I**, **II**, **IV**, **VII** and **IX** have been described in [19]. Compounds **III**, **V**, **VI**, **VIII** and **X** can be prepared as follows: 3.0 g metal sodium was sealed in a stainless-steel autoclave, which was filled with 25 ml carbon tetrachloride. The autoclave was maintained at 300 °C for 40 h and then allowed to cool to room temperature. The crude products was sublimated at 160 °C to isolate compound **I**, followed by separated and purified on a neutral Al_2O_3 column using cyclohexane/toluene as eluent. The compound **III** was identified by MS, UV, IR and ^{1}H NMR, and the compound **V**, **VI**, **VIII** and **X** were characterized by single crystal X-ray diffraction, MS, and IR. Details of preparation and identification of these reference compounds can be found in [20].

2.3. LC–UV–MS analysis

LC–UV–MS analysis was performed on a TSP model P2000 pump coupled with a TSP UV3000 detector and a Finnigan LCQ MS detector equipped with atmospheric pressure chemical ionization (APCI) source. The UV and MS detecting ranges were 200–400 nm and 150–1000 *m/z*, respectively. The APCI vaporization temperature was 350 °C, and the N_2 flow rates for sheath gas and aux gas were 65 and 15 ml/min, respectively. RPLC was performed on a 250 mm × 4.6 mm Hypersil RP-ODS2 column. The eluents of methanol/toluene and methanol/cyclohexane were used in gradient elution mode. The flow rate was 1.0 ml/min and the injection volume was 20 μl.

3. Results and discussions

3.1. LC isolations and retentions of the PCAHs

Fig. 1 shows the LC–UV and LC–MS chromatograms of the ten reference PCAHs eluted by toluene/methanol (Fig. 1a and b) and cyclohexane/methanol (Fig. 1c and d) eluents. The Fig. 1a and b suggest that
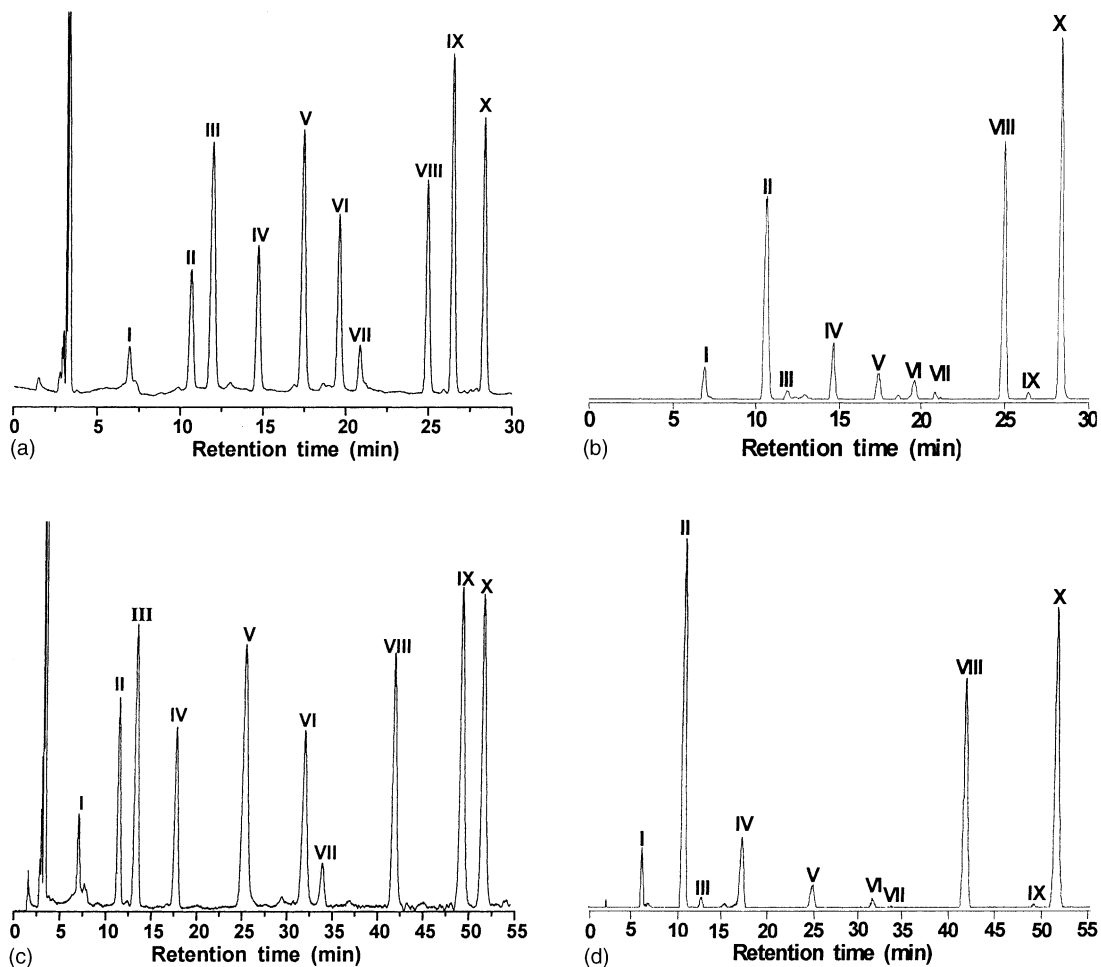
Fig. 1. LC–UV (recorded in 300 nm) and LC–MS (total ions current chromatogram) of the 10 reference PCAHs: (a) LC–UV eluted by toluene/methanol; (b) LC–MS eluted by toluene/methanol; (c) LC–UV eluted by cyclohexane/methanol; (d) LC–MS eluted by cyclohexane/methanol. Concentrations of C_6Cl_6 (**I**), $C_{12}Cl_{10}$ (**II**), $C_{13}Cl_8O$ (**III**), $C_{10}Cl_8$ (**IV**), $C_{15}Cl_8O$ (**V**), $C_{19}Cl_9OH$ (**VI**), $C_{12}Cl_8$ (**VII**), $C_{26}Cl_{10}H_8$ (**VIII**), $C_{16}Cl_{10}$ (**IX**) and $C_{26}Cl_8H_{10}O_2$ (**X**) are 7.58, 13.7, 0.31, 3.59, 0.34, 0.31, 0.42, 0.15, 0.10 and 0.61 mg/l, respectively. Detailed chromatographic parameters are listed in Table 1.

the PCAHs can be effectively eluted and separated by a linear increase of toluene from 0 to 30% (against a linear decrease of methanol from 100 to 70%) within 30 min. The disadvantage arisen from the strong absorption of toluene in the range of 200–280 nm, however, hindered the experiment from recording UV spectra within this region. Therefore, cyclohexane/methanol was used as another eluent considering their low absorption in the UV wavelength region. When the percent of cyclohexane was higher than 15% in the eluents of cyclohexane/methanol, the 10

PCAHs were not well separated due to less retention, especially for the compounds **I**–**VII**. Reducing the concentration of cyclohexane in the cyclohexane/methanol mixture would be favorable for the PCAHs isolation but resulting in long retention time. Two groups of peaks (i.e. the peaks of **II** and **III**, and the peaks of **IX** and **X**) could not be separated even in lower concentration of cyclohexane (e.g. 10%). As shown in Fig. 1c and d, however, base-line separation of the 10 PCAHs was fulfilled using a gradient cyclohexane/methanol eluent, which includes a linear

Table 1
Structures and chromatographically analytic parameters of the reference PCAHs
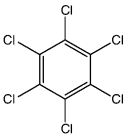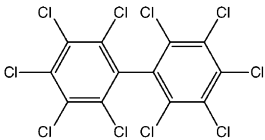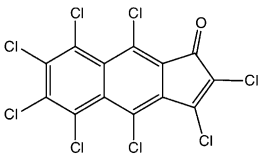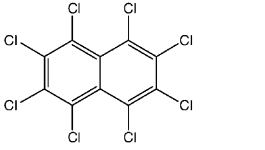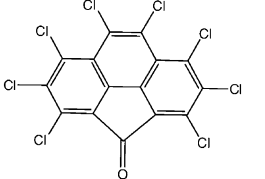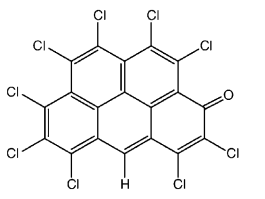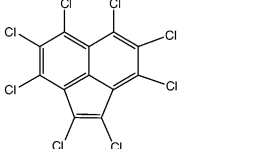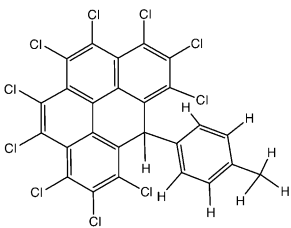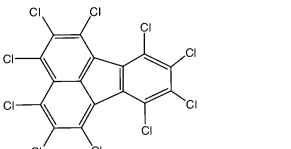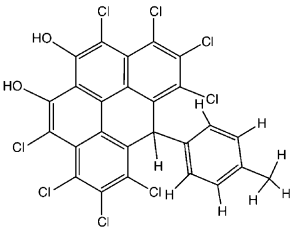
Compounds	Formulas	Structures	k'[a]	LC–UV		LC–MS				
				Detected in 300 nm		Total ions current determination		Selected ions monitoring		
				R[b]	DL[c] (ng/ml)	R	DL (ng/ml)	Mass (*m/z*)	R	DL (ng/ml)
I	C_6Cl_6		3.6	0.9937	0.6	0.9982	1.1	263–269	0.9987	1.0
II	$C_{12}Cl_{10}$		6.1	0.9948	1.1	0.9995	0.7	475–485	0.9956	0.6
III	$C_{13}Cl_8O$		6.9	0.9927	0.2	0.9990	0.4	452–462	0.9958	0.6
IV	$C_{10}Cl_8$		8.8	0.9989	1.0	0.9914	1.3	381–389	0.9975	2.0
V	$C_{15}Cl_8O$		10.6	0.9988	0.05	0.9981	0.01	475–487	0.9946	0.01
VI	$C_{19}Cl_9HO$		12.0	0.9990	0.4	0.9944	0.01	560–570	0.9858	0.04
VII	$C_{12}Cl_8$		12.8	0.9953	0.01	0.9983	0.01	423–433	0.9972	0.01

Table 1 (*Continued*)

Compounds	Formulas	Structures	k'^a	LC–UV		LC–MS				
				Detected in 300 nm		Total ions current determination		Selected ions monitoring		
				R^b	DL^c (ng/ml)	R	DL (ng/ml)	Mass (m/z)	R	DL (ng/ml)
VIII	$C_{26}Cl_{10}H_8$		15.5	0.9987	0.1	0.9968	0.06	670–680	0.9963	0.1
IX	$C_{16}Cl_{10}$		16.5	0.9981	0.05	0.9992	0.3	542–552	0.9971	0.2
X	$C_{26}Cl_8H_{10}O_2$		17.8	0.9906	0.6	0.9974	0.4	634–644	0.9991	0.1

[a] The t_0 was measured to be 1.51 min.
[b] R is the linear correlation coefficient; calibration curves for the PCAHs were constructed by plotting the detected peak area of each compound as a function of its concentration (within the range of 0.01–0.1 mg/ml).
[c] DL is the detection limit; defined as the concentration which produced an detected signal triplicate the peak-to-peak noise.

increase of cyclohexane from 0 to 5% in 15 min and from 5 to 15% in next 30 min and holding for 10 min. In comparison with the toluene/methanol eluent, cyclohexane/methanol allow to record fully UV spectra of the PCAHs but consume more time (55 min) in a LC run. In practice, we prefer to choose toluene/methanol as eluent for shortening analytic time. Table 1 shows the chromatographic parameters under the elution of toluene/methanol and structures of the standard PCAHs, as well as the LC–UV and LC–MS analytical performance data such as linear correlation coefficient and limitation of detection.

The reference PCAHs in the present experiment can be classified into two groups of chlorinated compounds: fully chlorinated aromatic hydrocarbons (i.e.

I, **II**, **IV**, **VII** and **IX**) and polychlorinated quinones (i.e. **III**, **V** and **VI**). As for each group of the PCAHs, retention time is found to rise with increasing number of their fused rings. The same correlation was also found in their polycyclic aromatic hydrocarbons parents. Retention time of later group, however, is shorter than that of the former one. For instance, the retention times of **VII** and **IX** are much longer than those of **III** and **V**, respectively. The evidence may attribute to higher hydrophilic when chlorine is replaced by oxygen in the polycyclic aromatic system.

Compound **VI**, **VIII** and **X** have the same carbon skeleton but different substituted group/atom. The eluting sequence of them in turn is **VI**, **VIII** and **X**, implying different substituted atoms/groups in the

carbon frame rims of PCAHs, in particular the large volume group such as the tolyl, will significantly affect on their retention.

3.2. LC–MS and LC–UV analysis

The relative abundance (i.e. peak area) in LC–MS chromatogram (see Fig. 1b and d) is greatly different from that in LC–UV chromatogram (see Fig. 1a and c). The LC–UV seems to be suitable for quantitative analysis due to the balanceable chromatographic peaks. As indicated in Table 1, the limitations of determinations and the linearity of the PCAHs are slightly different between LC–UV and LC–MS analyses. The later analytic procedure, however, provides MS spectra that

are helpful for characterizing the compositions of the PCAHs, especially for the newly observed compounds (vide infra). In addition, the selected ions chromatography origin from the LC–MS has higher selectivity for identification of the target PCAHs from complex mixture (vide infra).

As for each component with the same concentration, the pertinent LC–UV peaks (areas) eluted by either toluene/methanol or cyclohexane/methanol are similar, while the LC–MS peaks may be imbalance in the two eluents. For instance, the peak of **II** is stronger in Fig. 1d as compared with that in Fig. 1b, implying the APCI ionization efficiency of **II** in cyclohexane/methanol environment is better than that in toluene/methanol eluent.
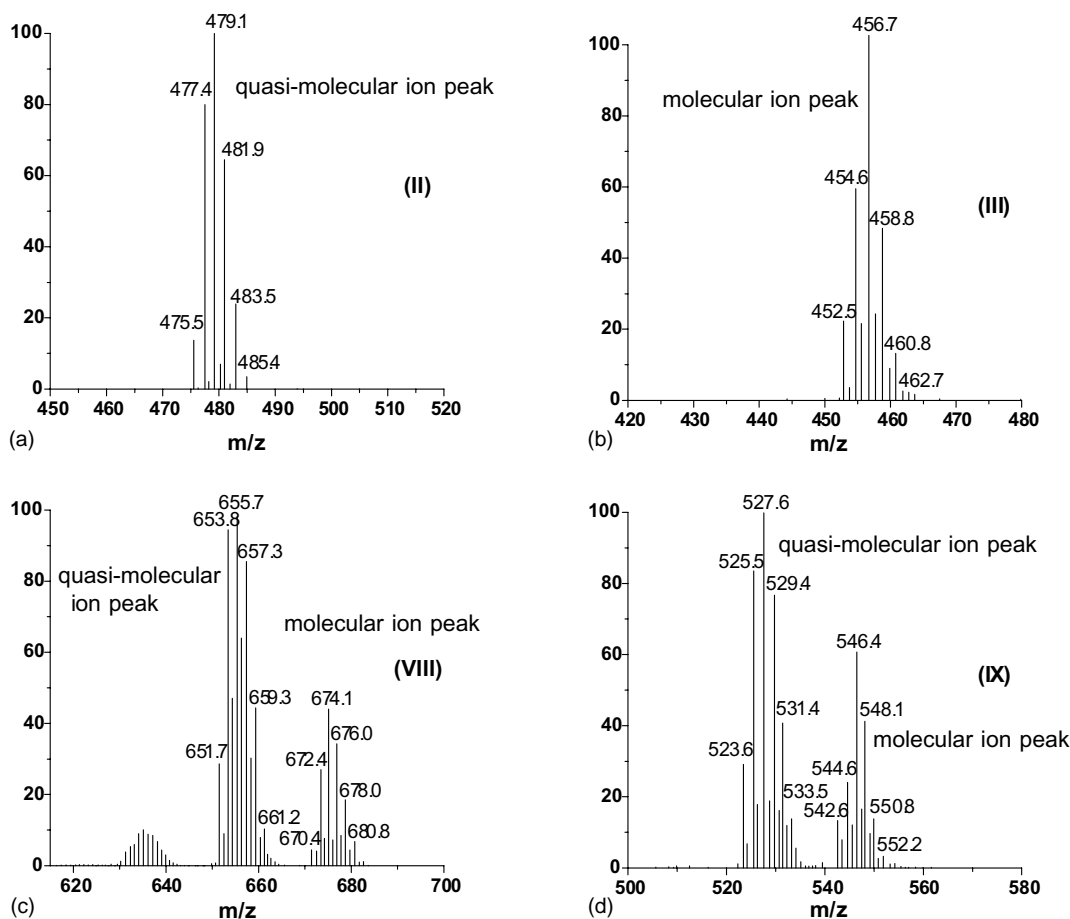

Fig. 2. APCI mass spectra of **II**, **III**, **VIII** and **IX**.

Among all the APCI–MS analytical conditions, vaporization temperature is the most critical factor for achieving reliable MS pattern. Higher vaporization temperature could ionize the PCAHs more effectively, but molecular ion peaks of some compounds disappear due to serious fragmentation in the higher temperature condition. In the present experiment the APCI vaporization temperature was fixed at 350 °C.

As shown in Fig. 2, four examples of the APCI–MS patterns of **II**, **III**, **VIII** and **IX**, the PCAHs tend to produce ion peaks with masses 19 less than their molecular masses, which are named as quasi-molecular ion peaks [21]. In the cases of **I**, **II** and **IV**, only quasi-molecular ion peaks are observed. These quasi-molecular ion peaks are speculated to result from losing a chlorine atom and capturing an oxygen anion during the APCI process, which may reflect the stability of the corresponding PCAHs.

Fig. 3 shows the UV spectra of five newly investigated PCAHs acquired in LC–UV analysis when using cyclohexane/methanol as eluent. The absorptive wavelengths of these PCAH compounds locate in three region of 200–215, 260–280, and 320–330 nm. The UV absorptive peaks are observed to shift red with the increase of conjugated rings. Compounds **VI**, **VIII** and **X** have the same carbon skeleton (benzo[*cd*]pyrene) and similar UV absorption region, but the relative abundance of these UV peaks are different because of the dissimilar substituted group on the rim of the carbon skeleton.

3.3. The application of the proposed chromatographic procedures

In addition to the reference PCAHs, soot product from the combustion of organochlorine compounds (chlorobenzene C_6ClH_5 and carbon tetrachloride CCl_4) was analyzed by the proposed LC–UV–MS chromatographic procedure. The crude sample was prepared as follows: 60 ml C_6H_5Cl was burnt in the air under the pressure of 1 atm, and CCl_4 was injected into the flame in a rate of ca. 0.3 ml/min. After combusting for 3 h, about 3.5 g soot was collected for extraction with 100 ml toluene. Twenty microlitres toluene-soluble soot extract was injected for LC analysis after filtered through a 0.45 μm pore diameter filter. As shown in Fig. 4a, the LC–UV chromatogram of the soot extract eluted by toluene/methanol, a
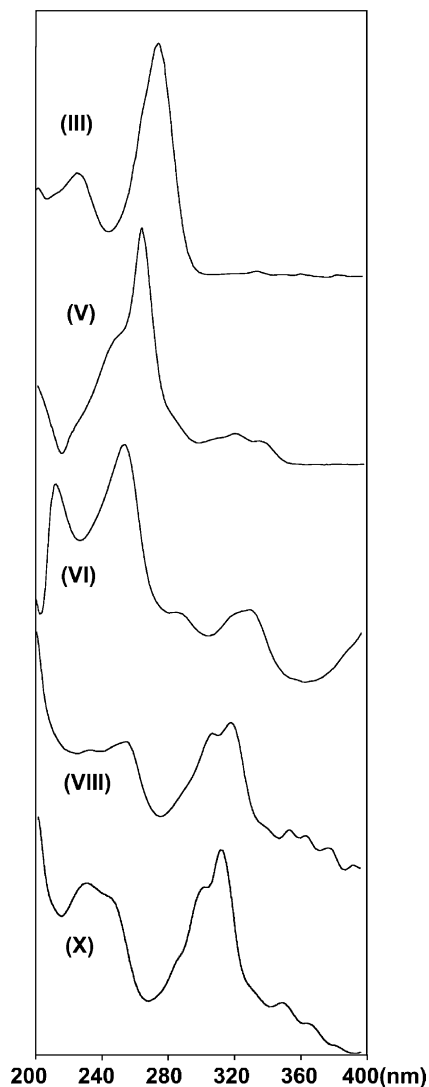

Fig. 3. UV spectra of five reference PCAHs (**III**, **V**, **VI**, **VIII** and **X**).

variety of peaks with blurred boundary are detected. The products seem to be too complex to be characterized on basis of the LC–UV analysis only. Five PCAHs (i.e. **I**, **II**, **IV**, **VIII** and **X**), however, could be unambiguously identified by combining the LC–UV with LC–MS (principally with the selected ions chromatograms as shown in Fig. 4b). Similar chromatograms could be observed when the soot extract was analyzed using cyclohexane/methanol eluent. In
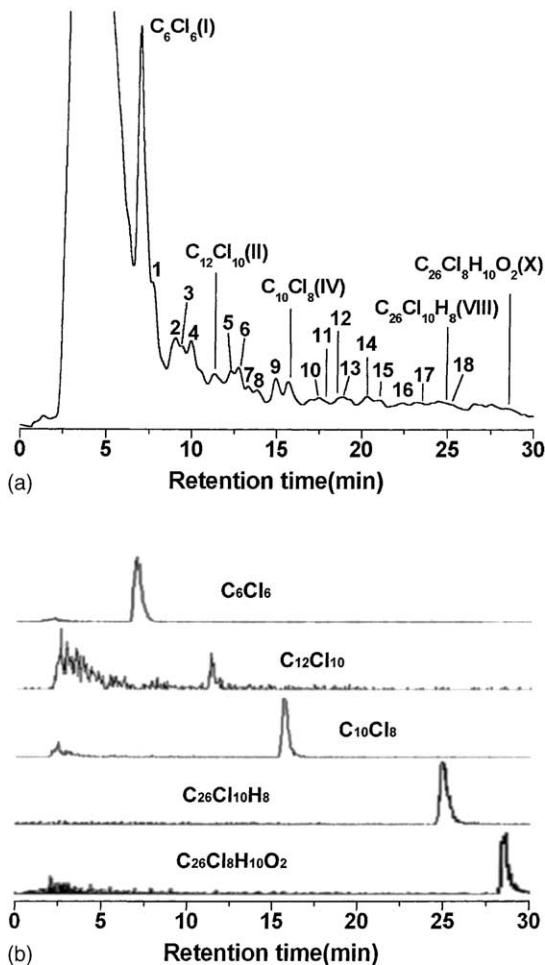
Fig. 4. LC chromatograms of the crude products from the combustion of C_6ClH_5 and CCl_4: (a) LC–UV chromatogram eluted by toluene/methanol and recorded in 300 nm; (b) selected-ion chromatograms of **I**, **II**, **IV**, **VIII** and **X** recorded in corresponding m/z ranges. Peaks are labeled by numbers, and their molecular formula are suggested from their isotope distributions in mass spectra as the following: 1, $C_{13}Cl_5H_5$; 2, $C_{12}Cl_9H$; 3, $C_{13}Cl_6H_4$; 4, $C_{12}Cl_4H_6$; 5, $C_{12}Cl_8H_2$; 6, $C_{10}Cl_6H_2$; 7, $C_{10}Cl_7H$; 8, C_9Cl_7H; 9, $C_{10}Cl_7H$; 10, C_9Cl_8; 11, C_9Cl_8; 12, $C_{14}Cl_8H_2$; 13, C_9Cl_7H; 14, $C_{16}Cl_7H_3$; 15, $C_{17}Cl_8H_3$; 16, $C_{23}Cl_{10}H_6$; 17, $C_{24}Cl_{10}H_8$; 18, $C_{26}Cl_{12}O$.

PCAHs would be helpful for the study of environmental contamination regarding organochlorine compounds combustion. PCAHs with characterized structure (particularly those with more than 13 carbon atoms), however, have rarely been reported in previous chemical literatures. To further chromatographic investigation of theses PCAHs, their structures remain to be investigated using some powerful methods such as single-crystal X-ray diffraction or nuclear magnetic resonance.

Acknowledgements

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addition to the identified five PCAHs, a number of other PCAHs with 9–26 carbon atoms were detected by the LC–UV–MS analysis in the soot, and among them 18 molecular formulas characterized by LC–MS are listed in the caption of Fig. 4. It is obvious that chromatographic identification of the newly observed

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