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Use of immobilized copper ion chromatography and on-line mass spectrometry with atmospheric pressure chemical ionization for the profiling of complex mixtures of polycyclic aromatic compounds☆

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Abstract

Immobilized copper ion chromatography was combined with either diode array detection or atmospheric pressure chemical ionization mass spectrometry for the profiling of complex mixtures of polycyclic aromatic compounds (PACs). A heated pneumatic nebulizer interface was used to convey the eluent from the column into the ionization region of the mass spectrometer. Several modifications were made to the heated pneumatic nebulizer interface originally designed for liquid chromatography–mass spectrometry so that the mass spectrometer could be safely operated with large quantities of organic solvent passing into the ionization region of the instrument. A copper-coated Zorbax 300 SCX column was used in conjunction with a hexane, chloroform and acetonitrile gradient to accomplish fractionation of three extracts containing PACs. The column was able to separate portions of the extracts into a number of fractions, each of which contained homologous series of different PAC classes. Using this technique, it is possible to gain considerable insight into the types and relative amounts of minor components present in the PAC mixtures.

1. Introduction

In the analysis of complex mixtures of polycyclic aromatic compounds (PACs), a continuing problem is that of identification of many of the heterocyclic compounds present in the mixture. Generally these compounds are present at levels of 1% or less of their polycyclic aromatic hydrocarbon (PAH) analogues; PAHs are almost always the dominant PACs in the mixture. The principal reason for the determination of the PAC content of any given sample is the carcinogenic and mutagenic nature of many of these compounds [1]. For this reason substantial effort has been expended in the development of methods for their reliable determination in a variety of matrices [1]. Almost all such methods depend

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on some form of chromatography in the final analytical step to separate the mixture into its various components. In previous work, we have developed the thesis that such analyses are most reliably performed by using a mass spectrometer as the chromatographic detector [2,3]. Despite the level of effort, there has been relatively little work on the development of methods for the determination of compounds other than the dominant PAHs.

Interest in methods for the separation of the heterocyclic PACs, particularly those containing sulfur or nitrogen, from their hydrocarbon analogues has been spurred by the fact that many of these compounds are potent carcinogens [4]. Thus, assessment of potential exposure hazards requires methods of analysis for these compounds in environmental samples. In general, these methods involve some step, where the compounds of interest are separated and concentrated from the PAHs. The reasons for this selective concentration step are twofold. First, unambiguous compound identification in any chromatographic–mass spectrometric analysis depends upon the chromatographic technique having sufficient resolving power to deliver the individual analytes to the mass spectrometer at a slow enough rate to permit the acquisition of mass spectra of sufficient quality for positive identification. Second, the overall column loading must be kept low enough to prevent peak shape distortion and thus losses in resolving power, which could lead to co-elution of compounds and thus unreliable identification, or to poor peak area integration and thus unreliable quantification. In other words, a compound class-selective preconcentration step provides a wider margin for compromise in the choice of chromatographic conditions for the final analysis.

A variety of techniques have been developed which permit the analyst to isolate one particular group of heterocyclic PACs, usually the sulfur compounds, from the other components of the mixture. Thus a separation on a column of silica impregnated with palladium chloride has been developed for the isolation of sulfur heterocycles from a PAC mixture [5], and this technique has been applied to the characterization of several coal- and petroleum-derived products [6]. Other techniques have been developed using silver nitrate-impregnated silica [7–9], or other stationary phases containing mercury [10,11], to achieve the same end. We have recently developed a technique, based on the use of copper immobilized on silica, which permits the separation of complex mixtures of PACs from environmental samples into a number of less complex fractions, containing members of different compound classes [12]. In that work we were able to obtain a satisfactory separation of compounds containing nitrogen and oxygen from other classes of PACs. The sulfur heterocycles were not separated from the PAH fraction, so that the technique may be considered to be complementary to those methods focused on isolating the sulfur heterocycles from all other components. The copper–silica fractionation [12] permitted us to separate the carbazoles from other nitrogen species, allowing unambiguous chromatographic–mass spectrometric identification of many minor components in the mixture. Despite their success in separating some components of the mixture from others, such techniques mostly share a common weakness; viz., they all rely on lengthy open-column chromatographic steps to isolate the fraction(s) of interest before the analytical step, and all such manipulations must be performed before the analyst can determine whether or not the target compounds sought are actually present in the sample.

In the present work we describe the development of an on-line chromatographic–mass spectrometric technique for the rapid profiling of PAC mixtures. The technique applies the concept of immobilized metal ion chromatography to the problem [13]. A similar technique, using a carboxylic cationic exchange resin loaded with copper for the isolation of sulfides from petroleum-derived products, has already been described in the literature [7]. We have drawn on that work and developed a stationary phase in which the copper is immobilized on a strong cation exchange resin, for use as part of an on-line HPLC–MS profiling technique.

2. Experimental

2.1. Column preparation and conditioning

The column used for these experiments was a 25 cm × 4.6 mm Zorbax 300 SCX with 6 μm particle size (Chromatographic Specialities, Brockville, Canada) in which the stationary phase had been modified with copper. The stationary phase modification (akin to the silver loading commonly used in lipid chemistry [14]) was achieved by sequentially pumping the following solutions through the column (flow-rate of 1 ml/min, unless otherwise stated): deionized water (250 ml); 1 *M* $CuSO_4$ (150 ml at a flow-rate of 0.1 ml/min); deionized water (250 ml); methanol (250 ml); acetone (150 ml); ethyl acetate (150 ml); chloroform (150 ml); and hexane–chloroform (5:1) (150 ml). This sequence is identical to that used in conditioning silver–SCX columns for lipid class separations, except for the substitution of 1 *M* $CuSO_4$ in place of 1 *M* $AgNO_3$, and for the final rinses with the chloroform-containing solvents. When not in use, the column was stored in hexane. It was found that when the column had been in use for some time (ca. 1 month), its ability to successfully fractionate the PAC mixtures diminished. However, this problem could be overcome by reconditioning the column with the following solvents (at 1 ml/min, unless stated otherwise): hexane (75 ml); dichloromethane (75 ml); methanol (75 ml); deionized water (75 ml); 0.1 *M* HNO_3 (150 ml); deionized water (500 ml); 1 *M* $CuSO_4$ (150 ml at a flow-rate of 0.1 ml/min); deionized water (700 ml); methanol (150 ml); acetone (75 ml); ethyl acetate (75 ml); chloroform (75 ml); and hexane (75 ml).

2.2. Sample preparation and clean-up

Tar Pond extract

The Tar Pond extract originated from the Sydney Tar Ponds, Sydney, Canada, resulting from 80 years of uncontrolled discharge of the byproducts of a metallurgical coking operation [15]. The sample was isolated using the following procedure:

(1) A 5-g amount of Tar Pond sediment was Soxhlet extracted in dichloromethane (175 ml) for 24 h. After this time, the dichloromethane was evaporated to a final volume of 1 ml using a rotary evaporator.

(2) A 7-g amount of 100–200 mesh silica gel (Fisher Scientific, Nepean, Canada), dried at 160°C, was placed into a 25 cm × 2 cm fractionating column, and an additional 1 cm of copper metal powder (Fisher Scientific) was added. The copper had been previously cleaned with 0.1 *M* HNO_3 (100 ml) followed by deionized water (3 × 100 ml), acetone (3 × 100 ml), hexane (3 × 100 ml), and subsequently vacuum filtered to dryness. A 20-ml volume of diethyl ether was passed through the column and discarded. The dichloromethane extract was conveyed to the column and eluted with 20% dichloromethane in diethyl ether (3 ml) followed by 40% dichloromethane in diethyl ether (20 ml). The extracts were combined and rotary evaporated to approximately 1 ml. The purpose of this step was to remove elemental sulfur by reaction with the copper, and to trap compounds that may compromise subsequent chromatographic separations on the silica [2].

(3) A 20-g amount of Sephadex LH-20 (Pharmacia, Uppsala, Sweden), pre-swollen in cyclohexane–methanol–dichloromethane (6:4:3) for 12 h, was placed into a 42 cm × 2 cm glass column. A 20-ml volume of the cyclohexane–methanol–dichloromethane solution was passed through the column and discarded. The extract from step 2 was added and eluted with 100 ml of cyclohexane–methanol–dichloromethane (6:4:3). The first 40 ml, which contained the aliphatic fraction, were discarded [16], the remaining 60 ml, which contained the PAC fraction [17], were collected. This fraction was rotary evaporated and dissolved in chloroform (10 ml) for analysis by immobilized copper ion chromatography (ICIC). The resulting solution contained approximately 75 mg of the original (5 g) mass of Tar Pond extract.

Harbour sediment, HS-3

HS-3 is a harbour sediment reference material that contains PAHs (Marine Analytical Chemistry Standards Programme, NRC, Halifax, Canada). A 35-g amount of this material was Soxhlet extracted and subjected to the first and second steps as described for the Tar Pond extract. For the third step, 6 g of 70–230 mesh neutral alumina (Merck, Darmstadt, Germany), which had been stored in an oven at 160°C were placed into a 25 cm × 2 cm fractionating column. To this were added 4 g of 60–120 mesh silica gel (BDH, Toronto, Canada). A 20-ml volume of pentane was passed through the column and discarded. The sample was added and eluted with pentane (35 ml), 5% benzene in pentane (40 ml), 15% benzene in pentane (40 ml) and methanol–benzene–diethyl ether (3:1:1) (25 ml). The first three fractions, which contained the aliphatic, monocyclic aromatic and bicyclic aromatic fractions, respectively [17], were discarded. The final fraction containing the PACs [18] was rotary evaporated and dissolved in 10 ml of chloroform for subsequent analysis. The resulting chloroform solution contained 50 mg of the original (35 g) HS-3 extract.

Heavy gas oil

Heavy gas oil (HGO) is one of the end-products of a refining process carried out by Syncrude Canada Ltd. for the production of naphtha and light and heavy gas oils from the Alberta Oil Sands. The PAC fraction was isolated using the following procedure:

(1) A 5-g amount of KOH was added to 400 ml of isopropanol and stirred for 1 h. A 75-ml volume of this solution was added to 5 g of 60–120 mesh silica gel (BDH), and the resulting slurry stirred for 24 h. The slurry was placed into a 42 cm × 2 cm glass column and the isopropanol solution was allowed to pass through the column, followed by 25 ml of chloroform which were discarded. A 250-mg amount of the HGO was added to the column and eluted with 70 ml of chloroform. The KOH-treated silica removed terpenoic acids which could interfere in subsequent chromatographic separations [18,19]. The chloroform was removed by rotary evaporation, and the resulting residue dissolved in 1 ml of pentane.

(2) The HGO extract was passed through a silica over alumina column as described for the harbour sediment. The methanol–benzene–diethyl ether (3:1:1) fraction was rotary evaporated, and the extract dissolved in chloroform (100 μl) in hexane (10 ml). The resulting solution contained 50 mg of the original (250 mg) HGO sample.

2.3. Instrumentation

A Hewlett-Packard (Palo Alto, CA, USA) 1090 Series II liquid chromatographic system was used for sample profiling. Hexane, chloroform and acetonitrile were used as the eluting solvents. A 25-μl volume of each extract was injected onto the copper-coated SCX column and eluted with the gradient described in Table 1 at a flow-rate of 1 ml/min. Detection in the first instance was performed using a Hewlett-Packard 1040 diode array detection (DAD) system set at a wavelength of 254 nm. For the HPLC–UV experiments the LC was controlled, and data collected and processed, by Hewlett-Packard 3365 Chemstation software installed on a Hewlett-Packard Vectra 486/33U computer.

The on-line ICIC–MS fractionation experiments were conducted using a Sciex API III triple quadrupole mass spectrometer (Sciex, Thornhill, Canada) equipped with an atmos-

Table 1
Mobile phase gradient employed for the separation of extracts containing PACs by ICIC–DAD and ICIC–APCI-MS using a copper-coated SCX column

Time (min)	Hexane (%)	Chloroform (%)	Acetonitrile (%)
0.00	100	0	0
8.00	85	15	0
15.00	0	100	0
17.00	0	100	0
22.00	0	50	50
27.00	0	100	0
29.00	0	100	0
31.00	100	0	0

pheric pressure chemical ionization (APCI) source. A 5-μl volume of each sample was injected onto the column and the eluent from the LC was conveyed into the ion source of the mass spectrometer through a heated pneumatic nebulizer interface. The interface has been described elsewhere [20]. However, due to the inherent danger of using hexane with the heated nebulizer interface [21], two modifications to the original design of the interface and source housing were used. First, a 200 MΩ high-voltage resistor was installed to limit the current through the corona discharge needle, and second, an over-pressure relief vent was added to the front end of the interface. Ultra-high-purity nitrogen was used as both nebulizer gas and make-up gas. The interface was operated at 400°C, with a nebulizer pressure of 60 p.s.i. (1 p.s.i. = 6894.76 Pa) and a make-up flow-rate of 1 l/min. The corona discharge needle was maintained at a current-controlled 3 μA discharge. Mass spectrometer control, data acquisition and data processing were accomplished using a Macintosh Quadra 950 microcomputer. The quadrupole mass spectrometer was operated using a dwell time of 2 ms per 1 u step for the mass range m/z 150–1000.

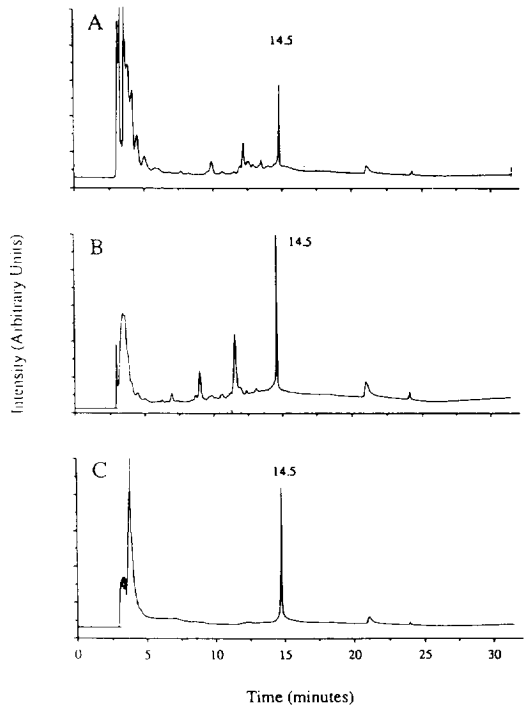

Fig. 1. Chromatograms obtained by the separation of (A) Tar Pond extract, (B) HS -3 and (C) HGO by ICIC–DAD (254 nm fixed) using a copper-coated SCX column.

3. Results and discussion

The extracts were initially passed through the copper-coated SCX column using the mobile phase gradient shown in Table 1, and were profiled using DAD. The resulting ICIC–DAD chromatograms for the extracts of the Tar Pond sediment, HS-3 and HGO, are shown in Fig. 1. It can be seen from the chromatograms that each extract has been separated into several distinct fractions, with some peaks common to all three extracts. Most of the fractionation takes place in the early part of the mobile phase gradient (100% hexane going to 100% chloroform). The use of hexane as a fractionating solvent was critical to the success of the technique. Other potential fractionating solvents were used [e.g., methanol, hexane–chloroform (1:1)], but it was found that all of the components of the extract eluted in one unresolved peak. To evaluate the reproducibility of the technique each fraction was passed through the column eight times. It was found that the retention time for the peak labelled at 14.5 min in Fig. 1 had an average retention time of 14.54 min with a relative standard deviation (R.S.D.) of 1.1%. However, when the column had been in use for a long time (1 month), its ability to resolve the components of the extracts diminished somewhat. Reconditioning of the column with solvent alone was not found to sufficiently improve resolution. However, when the column was reconditioned with the solvents and $CuSO_4$ solution, as described in the Experimental section, its performance returned to the original without any change in peak retention times.

In order to be able to identify the compounds present in the chromatographic peaks, either as impurities generated from the column or as components of the extracts, the extracts were injected into the HPLC system and detected by

APCI-MS. The total ion chromatogram (TIC) obtained by ICIC–APCI-MS for the Tar Pond extract is shown in Fig. 2A. It can be seen that the introduction of the HPLC eluent into the ion source of the mass spectrometer has been achieved without any loss of chromatographic resolution. It was found that the copper-coated SCX column was able to separate the Tar Pond extract into fractions containing components of various homologous series. This is exemplified in Fig. 2B which shows the mass spectrum obtained for the peak indicated in the inset of Fig. 2A. The mass spectrum indicates the presence of protonated molecules $[M + H]^+$ of pyrene, M_r 202, and alkylated pyrene derivatives. The peak marked "1" in each fraction (see Fig. 2, and Figs. 3 and 4 later) arises from copper compounds which elute from the column at the onset of the acetonitrile portion of the gradient. It is

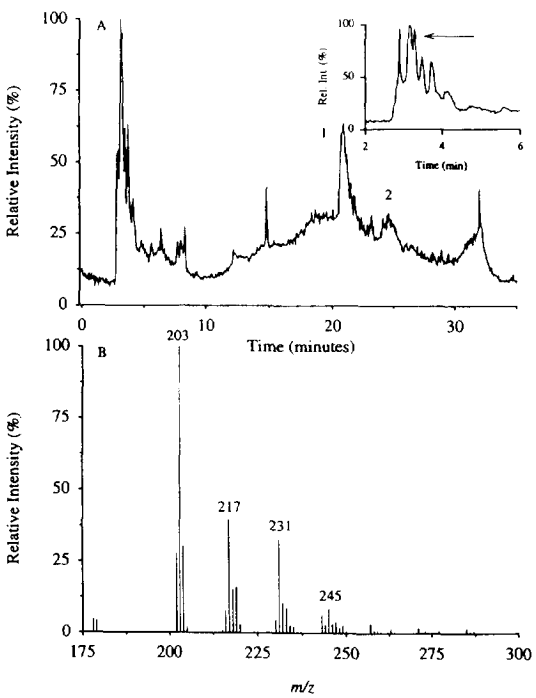

Fig. 2. (A) Total ion chromatogram (m/z 150–1000) obtained by the separation of the Tar Pond extract by ICIC–APCI-MS using a copper-coated SCX column. (B) Mass spectrum, obtained from the peak marked in the inset of (A), showing the components of the peak to be an homologous series of pyrene (M_r 202) and alkylated pyrene compounds.

unclear whether these compounds arise from previous clean-up steps or are formed by a reaction between the copper on the column and components of the sample. The mass spectrum (not shown) of this peak shows two major sets of double peaks, at 2 u intervals as a result of the copper isotopes (^{63}Cu and ^{65}Cu) forming oxides (m/z 158 $[^{63}Cu_2O_2]^{+\cdot}$ and m/z 160 $[^{63}Cu^{65}CuO_2]^{+\cdot}$) as well as complexes with acetonitrile {m/z 170 $[^{63}Cu^{65}Cu(CH_3CN) + H]^+$ and m/z 172 $[^{65}Cu^{65}Cu(CH_3CN) + H]^+$}. For both the oxide and the acetonitrile cluster ion, peaks attributable to the remaining possible isotopic combinations {$[^{65}Cu_2O_2]^{+\cdot}$ and $[^{63}Cu_2(CH_3CN) + H]^+$} were not detected. None of the other peaks in the chromatograms exhibited the presence of copper complexes, indicated by the absence of regular series of mass spectral peaks separated by 2 u.

It has been found previously that the relative responses of low- and high-molecular-mass (>200) PACs in APCI-MS are qualitatively different, when the sample is introduced under supercritical fluid chromatography conditions [22,23], and the analysis of PACs by the present ICIC–APCI-MS technique yielded similar results. The mass spectra of the lower-molecular-mass compounds are characterized by the formation of both molecular ions $[M]^{+\cdot}$ and protonated molecules $[M + H]^+$. Typically, the higher-molecular-mass PACs form only protonated molecules in an APCI environment (see Fig. 2B). The reasons for these phenomena have been discussed in previous publications [22,23].

The TIC obtained by ICIC–APCI-MS for the harbour sediment HS-3 is shown in Fig. 3A. Comparison of the ICIC–DAD chromatogram (Fig. 1B) and the TIC shows that the ICIC–APCI-MS system has only been partially successful in matching the HPLC–DAD chromatogram. However, the components of each peak in the profile could be detected by extracting reconstructed ion chromatograms for selected masses. Fig. 3B shows the mass spectrum obtained from the major peak indicated in the TIC. This mass spectrum is rather uninformative, consisting of a peak at every mass over the range scanned. However, the intensity profile of the envelope of

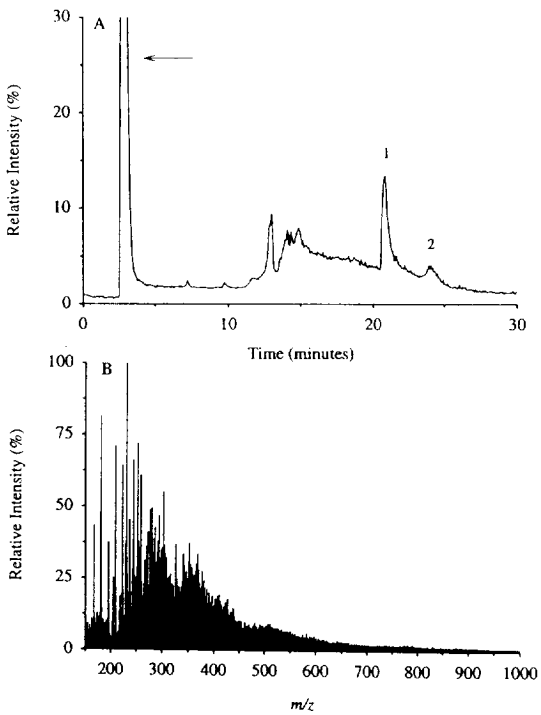

Fig. 3. (A) Total ion chromatogram (m/z 150–1000) obtained by the separation of the HS-3 extract by ICIC–APCI-MS using a copper-coated SCX column. (B) Mass spectrum obtained from the peak marked in the TIC above showing the components of the peak to include PACs of molecular masses that encompass the whole mass range scanned by the mass spectrometer.

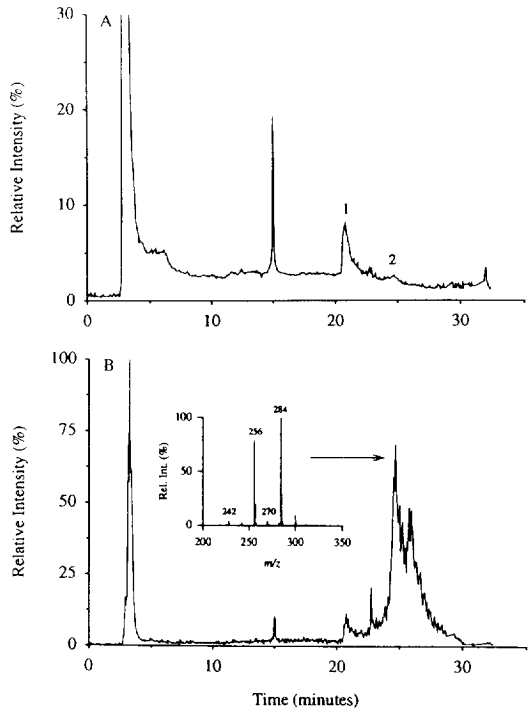

Fig. 4. (A) Total ion chromatogram (m/z 150–1000) obtained by the separation of the HGO extract by ICIC–APCI-MS using a copper-coated SCX column. (B) Reconstructed ion chromatogram of m/z 284 with the inset showing the mass spectrum of the indicated peak. The mass spectrum of this peak shows the possible presence of an homologous series of acridinones.

mass spectral peaks arising from this chromatographic peak is qualitatively very different for each of the three extracts.

Fig. 4A shows the TIC obtained by ICIC–APCI-MS of the HGO extract. This extract showed good correlation between the ICIC–DAD chromatogram (Fig. 1C) and the TIC. The reconstructed ion chromatogram of m/z 284 (Fig. 4B) shows a peak corresponding to that indicated by "2" in all of the TICs shown (see Figs. 2–4). The inset of Fig. 4B shows the mass spectrum obtained from this peak, which is dominated by four peaks at m/z values each separated by 14 u. This sequence of mass spectral peaks could possibly be explained as arising from an homologous series of compounds of the general formula ($C_{14+a}H_{11+2a}NO_3$), for $a = 0$–3, consistent with the acridinones, although their identities could not be confirmed due to a lack of authentic standard compounds.

The column, like the copper-coated silica open column described previously [12], effects a partial separation of the PAC fraction into distinct compound classes. This may be demonstrated by considering the mass chromatogram for m/z 209, shown in Fig. 5A. This mass is typical of a family of alkylated fluorenones with three substituent carbons. The mass spectrum acquired at the crest of the peak at a retention time of 7.9 min, shown in Fig. 5B, indicates that there are several members of a homologous series of (possibly) alkylated fluorenones present in this peak (peaks at m/z 209, 223, 237) as well as some other unidentified compounds. This group of compounds has been well resolved from the PAH peak, eluting at 3 min.

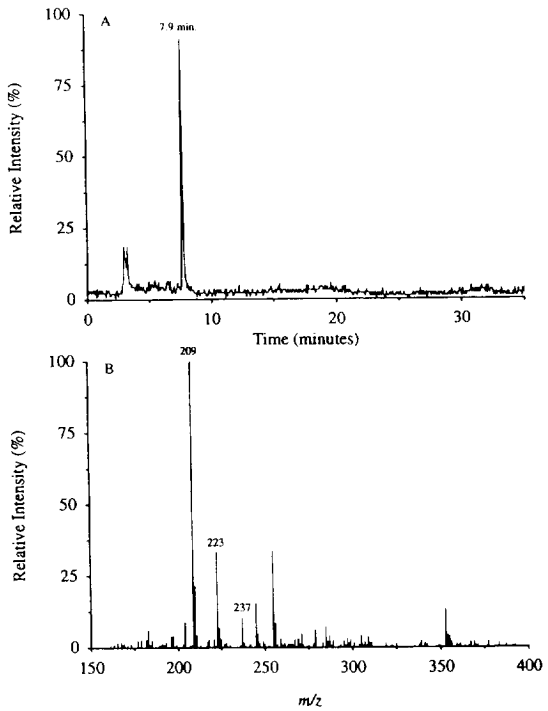

Fig. 5. (A) Mass chromatogram for m/z 209, extracted from the total ion chromatogram of Fig. 2A. (B) The background subtracted mass spectrum obtained at the crest of the peak at 7.9 min in (A).

4. Conclusions

The ICIC profiling technique appears to be a useful rapid method for revealing the relative amounts of some different classes of PACs, in complex mixtures of PACs from environmental and petroleum-derived sources. Using this method it is possible to demonstrate the presence or absence of several different classes of PACs in a mixture. The HPLC–ICIC–DAD profiles may be used to gain insight into the overall complexity of the PAC fraction, and thus to act as a guide to subsequent analytical procedures. Depending upon the needs of the analyst, it is possible to collect the fraction(s) of interest and submit them to further steps. As in the earlier work [12], the separation of other classes of PACs from the PAHs allows collection of high-quality mass spectra, sometimes permitting compound identification in suitable cases, in subsequent chromatographic–MS analyses. Work in progress in our laboratory has shown that the combination of ICIC fractionation with subsequent HPLC–MS–MS analysis can provide unambiguous identification of sulfur [24] and nitrogen [25] heterocycles in different fractions of the Tar Pond extract.

The three samples studied in this work have very different HPLC–UV profiles, which indicated significant differences in the types and amounts of various classes of PAC present. These samples were further examined using HPLC–ICIC–APCI-MS, indicating that the differences indeed derive from differences in the amounts of compounds of various PAC classes present in the mixtures of PACs present in the extracts. Using this technique, it is possible to obtain considerable information about the types and relative amounts of these minor components.

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