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Supercritical fluid chromatography–atmospheric pressure chemical ionisation mass spectrometry for the analysis of hydroxy polycyclic aromatic hydrocarbons

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Abstract

Supercritical fluid chromatography in combination with atmospheric pressure chemical ionisation mass spectrometry has been used for the characterisation of hydroxy polycyclic aromatic hydrocarbons (hydroxy-PAHs). These compounds were separated on a reversed-phase column, and temperature, pressure and modifier were evaluated to optimise the chromatographic conditions. Mass spectra were obtained in both positive and negative ionisation modes. Proton addition and proton abstraction were the common routes of ionisation and losses of CO and H_2O also occurred. Transformation to the quinone structure was observed for some of the hydroxy-PAHs. Detection limits were at the ng level and run-to-run reproducibility ranging from 2 to 12% (R.S.D.) was obtained. © 1997 Elsevier Science B.V.

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1. Introduction

Although de la Tour [1] discovered critical phenomena in 1822 and Hannay and Hogarth [2,3] demonstrated the solvent power of supercritical fluids in 1879, industrial and analytical applications did not begin until the second half of this century. Supercritical fluid chromatography (SFC) is now used in many laboratories because compounds that are not volatile enough for gas chromatography can be analyzed [4–6]. In addition, SFC gives shorter analysis times than liquid chromatography.

Coupling SFC with mass spectrometry (SFC–MS) is easier than liquid chromatography (LC)–MS because the supercritical fluid is transformed to an easily removable gas when the pressure drops. As a consequence, packed-column SFC–MS is possible with a direct fluid-introduction interface, although it requires additional pumping or flow splitting to accommodate the greater flow-rate of the mobile phase, especially with a traditional 4.6 mm I.D. packed column [7,8]

The simplest splitting interface is the pre-expansion interface, in which a portion of the chromatographic eluent from the packed column is directed to the mass spectrometer [9]. The balance of the effluent goes to another detector, or is discarded. Other packed-column interfaces may be described as post-expansion splitting interfaces, in which the entire expanded eluent is directed to an ionisation

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region. Much of the eluent, as well as many of the ions formed, is pumped from the ionisation region. Examples of this type of interface are thermospray [10], atmospheric pressure chemical ionisation (APCI) [11,12] and electrospray [13]. Developments in the ion-sampling region of post-expansion interfaces may enhance the total number of ions directed to the mass spectrometer. The post-expansion interfaces are used most often, and they are reasonably simple and perform well. Because the electrospray and APCI work at atmospheric pressure, the chromatograph and mass spectrometer can operate independently, and the changes and adjustments of the interface are easier. Despite the advantages offered by SFC–MS, relatively few applications of this technique have been reported [14–17], although the range of potential applications warrants greater interest, particularly in industries, such as fossil fuels, food and pharmaceuticals.

In this paper, a pre-expansion split in combination with a post-expansion interface (APCI) has been used to couple SFC to the mass spectrometer for the analysis of the hydroxy-polycyclic aromatic hydrocarbons (hydroxy-PAHs). These compounds can be generated from PAHs by photochemical reactions in the atmosphere [18,19] and have been identified as PAH metabolites in biological fluids; some of them are considered as biomarkers of occupational exposure to PAHs [20,21]. The SFC–MS spectra of these compounds were obtained in both positive- and negative-ion mode and the SFC–MS system was evaluated.

2. Experimental

2.1. Chemicals

The compounds studied were 1-indanol (1-IOH), 9-hydroxyfluorene (9-HFL), 9-hydroxyphenanthrene (9-HF), 2-hydroxy-9-fluorenone (2-H-9-FLO), 1,4-dihydroxynaphthalene (1,4-DHN), 1,8-dihydroxy-9,10-anthraquinone (1,8-DH-9,10-AQ) and 2-nitro-1-naphthol (2-N-1-N), which were provided by Aldrich (Milwaukee, WI, USA) or Merck (Darmstadt, Germany). Stock standard solutions of 100 μg ml^{-1} in methanol were prepared and used for further dilutions.

Both gases, N_2 and SFC-grade CO_2, were obtained from BOC Gases (Manchester, UK). Methanol and water were of HPLC grade from Fisons (Loughborough, UK). All of the solutions were passed through a 0.2-μm nylon mesh from Phenomenex (Torrance, CA, USA) before use.

2.2. Supercritical fluid chromatography

For optimisation of SFC conditions, an SFC system with UV detection and an electrothermally cooled reciprocating pump, model G1205A from Hewlett-Packard (Palo Alto, CA, USA), was used. An HP Autosampler model 7673 was used to introduce the samples. An Ultrasphere ODS C_{18} column (Alltech Associates Applied Science, Lancashire, UK; 5 μm particle size, 25.0 cm×1 mm I.D.) was used. Different chromatographic conditions were tested and the optimum separation was obtained using CO_2 as the mobile phase and (methanol–water, 92:8, v/v) as the modifier. A 1.5-ml min^{-1} flow-rate and simultaneous pressure and modifier gradients were used. The modifier gradient was as follows: initially 5% modifier, hold for 30 s, rate 5% min^{-1} to 10% and hold for 2 min. Pressure gradient was: initially 1800 p.s.i., hold for 1 min, rate of 300 p.s.i. min^{-1} to 2500 p.s.i. and hold for 2 min (1 p.s.i.= 6894.76 Pa). The separation was carried out at 45°C and with a detection wavelength of 220 nm.

2.3. Mass spectrometry

A TSQ 700 triple-quadrupole mass spectrometer (Finnigan MAT, San José, CA, USA) was coupled to the HPSFC system using a modified Finnigan APCI interface. A pre-expansion splitting device, between the column and the UV detector, was used to introduce one third of the supercritical eluent into the APCI-MS system. A modified Finnigan APCI interface, which contained a sample inlet fitting, a manifol and an APCI nozzle, was used. The modification allowed a metal restrictor (30 cm long×1/16 in. O.D.×0.005 in. I.D.; 1 in.=2.54 cm) to be fitted. The restrictor protruded 5 mm from the end of the APCI nozzle. The modified Finnigan SFC–APCI source operated in the same way as a normal APCI source, except that the modifier created a reagent plasma to allow ionisation to take place. If no

modifier is present, a reagent plasma may be created by the introduction of a suitable solvent into the sheath gas (N_2).

Both ionisation modes (positive and negative) were used to characterise the hydroxy-PAHs. Sheath gas was used at a pressure of 30 p.s.i. and the corona current was 5 μA (4.5 kV) for positive ionisation and 5 μA (−1.7 kV) for negative ionisation. The vaporiser was held at 500°C and the capillary heater was at 250°C. Full-scan was used from *m/z* 50 to 250 using a scan time of 1 s and a mass scan step of 1 dalton in order to characterise these compounds. Then the mass spectrometer was set to multiple-ion detection (MID) mode. The ions used for monitoring in the positive ionisation mode were: *m/z* 117 and 115 (1-IOH), 135 and 131 (5-IOH), 159 and 131 (1,4-DHN), 165 and 181 (9-HFL), 197 (2-H-9-FLO), 195 and 167 (9-HF), 241 (1,8-DH-9,10-AQ), and 204, 156, 172 and 159 (2-N-1-N) at a scan time of 0.5 s. In negative ionisation mode, the ions used for monitoring were: *m/z* 133 (5-IOH), 173 and 158 (1,4-DHN), 195 (2-H-9-FLO), 193 (9-HF), 240 and 239 (1,8-DH-9,10-AQ), and 188, 172, 159 and 142 (2-N-1-N) at a scan time of 0.5 s.

3. Result and discussion

3.1. Supercritical fluid chromatographic conditions

In order to establish the chromatographic conditions for the separation of the hydroxy-PAHs on the ODS reversed-phase column, variables such as pressure, temperature and composition of mobile phase (CO_2 modifier) were studied, since the polarity and the solvent strength depend on these variables.

High retention and tailed peaks were obtained when CO_2 was used without methanol as the modi-

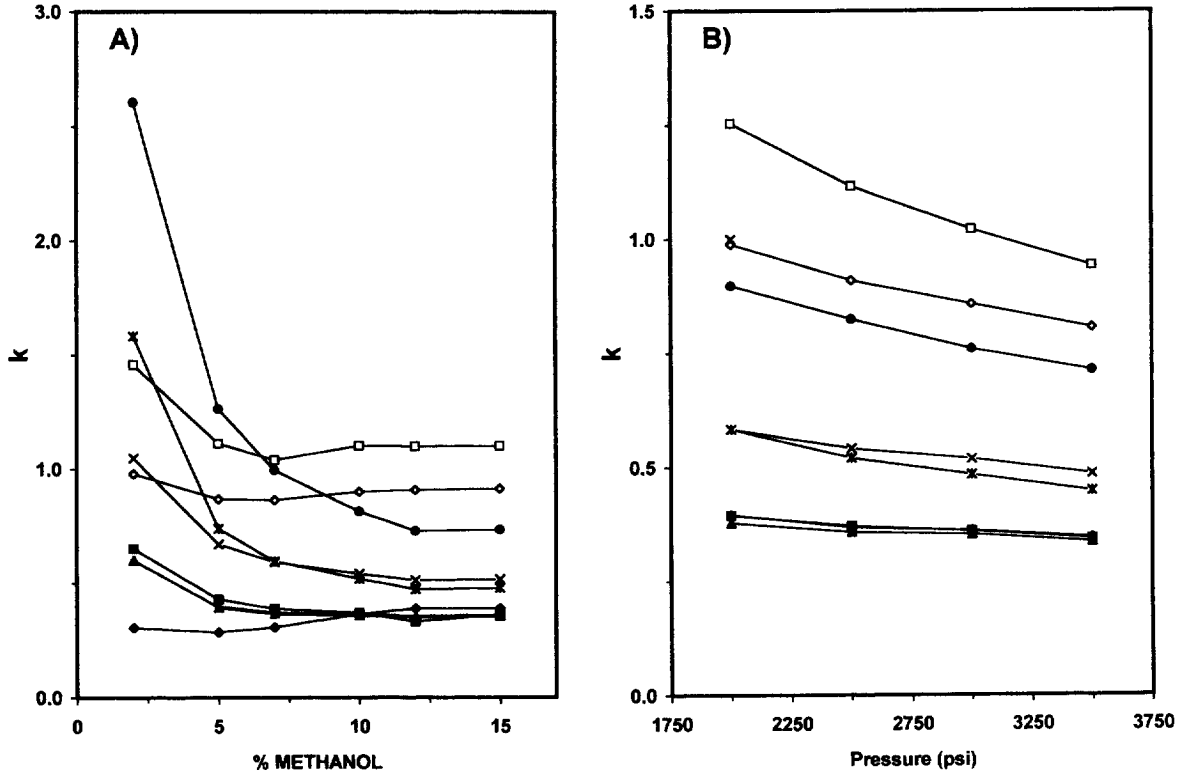

Fig. 1. Variation of the *k* values vs. (A) the percentage of methanol and (B) the pressure for standard solutions of several hydroxy-PAHs injected in the SFC–UV (220 nm) system. (♦) 1,4-DHN, (■) 5-IOH, (▲) 1-IOH, (×) 9-HFL, (*) 2-H-9-FLO, (●) 9-HF, (◇) 2-N-1-N and (□) 1,8-DH-9,10-AQ.

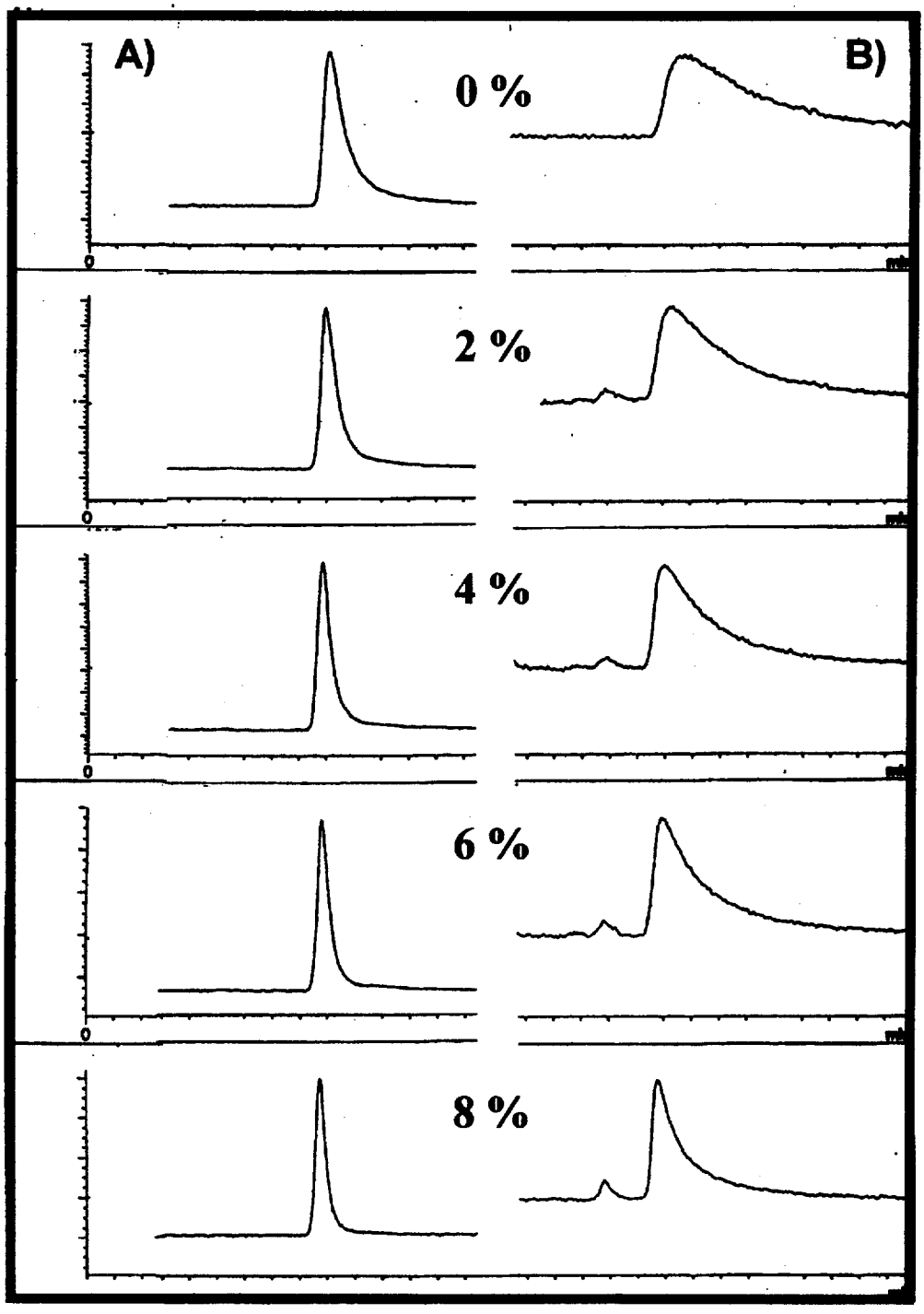

Fig. 2. SFC–UV chromatograms at different percentages of water modifier for (A) 2-N-1-N and (B) 1,8-DH-9,10-AQ at 3500 p.s.i., 45°C, 2 ml min^{-1} and 10% modifier (methanol–water).

fier. This may be due to the interactions of these moderately polar compounds with the silica support of the column, as reported with LC [22]. This effect was enhanced when a non-polar mobile phase, such as CO_2, was used. To study the influence of a modifier on the separation, standard solutions containing 50 μg l^{-1} of the hydroxy-PAHs were injected using several percentages of methanol in the CO_2. Fig. 1A shows the variation of the k values vs. the percentage of methanol. While no important differences in k values were observed for 2-N-1-N and 1,4-DHN, a marked decrease was obtained for 9-HF and 2-H-9-FLO. Shorter analysis times and changes in the elution order took place when the percentage of methanol was increased from 2 to 15%.

To study the effect of temperature and pressure on the separation, CO_2 with 10% methanol was used as the mobile phase. A decrease in k values was observed when the temperature was increased from 25 to 75°C and when the pressure decreased from 3500 to 2000 p.s.i., due to the lower CO_2 density at high temperature and lower pressure, which leads to lower polarity and solvent strength. As a consequence, these conditions are recommended, although high pressure is needed to increase the resolution between 9-HFL and 2-H-9-FLO, as can be seen in Fig. 1B, where the k values vs. pressure are given.

Even with 10% methanol in the CO_2, tailing peaks were obtained for some of these compounds. The tailing was reduced by the addition of water to the modifier (methanol), as can be seen in Fig. 2A–B, where the chromatograms using UV detection for 1,8-DH-9,10-AQ and 2-N-1-N, respectively, at different percentages of water in the methanol, are given as an example.

From Fig. 1A–B, it can be deduced that it is difficult to achieve a good separation between the eight hydroxy-PAHs studied without applying a gradient. So, different gradients (temperature, pressure and modifier) were tested in order to improve the separation. The optimum separation was achieved using simultaneous pressure and modifier gradients. The temperature chosen was 45°C, since higher temperatures produced an increase in k and in base width. The modifier was methanol–water (92:8, v/v); higher water contents produced a high back pressure and no stable base line. Simultaneous gradients, pressure (between 1800 and 2500 p.s.i.) and modifier (from 5 to 10%), gave the best separation. Fig. 3 shows the chromatogram with UV detection under these working conditions.

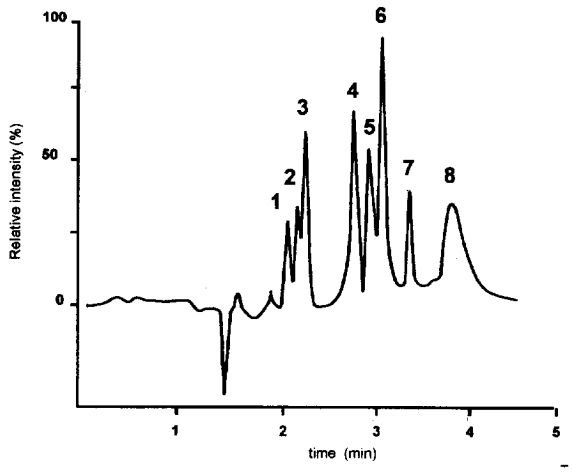

Fig. 3. SFC–UV chromatogram of a standard solution of hydroxy-PAHs using the working conditions described in Section 2. (1) 1,4-DHN, (2) 1-IOH, (3) 5-IOH, (4) 9-FLO, (5) 2-H-9-FLO, (6) 2-N-1-N, (7) 9-HF and (8) 1,8-DH-9,10-AQ.

3.2. Mass spectra

Full scan mass spectra in positive and negative modes were obtained for the characterisation of the hydroxy-PAHs by injecting standard solutions of 10 and 50 μg l^{-1}, respectively. Mass spectral data for these compounds are given in Table 1. Proton addition to form $[M+H]^+$ is the common route of ionisation in positive mode for 2-H-9-FLO, 9-HF, 1,8-DH-9,10-AQ and 5-IOH. Losses of the H_2O to give $[M+H-H_2O]^+$ and of CO to give $[M+H-CO]^+$ were also observed. As an example, Fig. 4A shows the spectrum for 5-IOH. The protonated molecular ion at m/z 135 and the ion at m/z 107, originated by the loss of CO from the $[M+H]^+$, can be observed. Moreover, ions at m/z 131 and 103 were also observed. MS–MS experiments for the ion at m/z 135 gave only m/z 107 and 77, showing that the ions at m/z 131 and 103 did not come from the $[M+H]^+$ (m/z 135) (Fig. 4B). The compounds 5-IOH, 9-HFL and 1,4-DHN gave, by chemical ionisation, the corresponding quinone structures shown in Fig. 5, which lost the CO to give the ions at m/z 103, 153 and 131, respectively.

Table 1
Mass spectral data of hydroxy-PAHs in SFC–APCI-MS

Compound	M_r	Mass spectra [m/z (relative abundance) [ion]]			
		Positive		Negative	
1,4-Dihydroxynaphthalene (1,4-DHN)	160	159 (100) 131 (28) 105 (9) 103 (8)	$[M-H]^+$ $[M-H-CO]^+$ $[M-H-C_3H_2O]^+$ $[M-H-(CO)_2]^+$	173 (23) 158 (100) 145 (8)	$[M-H_2+CH_3]^-$ $[M-H_2]^{-\cdot}$ $[M-H_2+CH_3-CO]^-$
1-Indanol (1-IOH)	134	117 (100) 115 (60) 91 (40)	$[M+H-H_2O]^+$ $[M+H-H_2O-2H]^+$ $[C_7H_7]^+$	–	–
5-Indanol (5-IOH)	134	135 (65) 131 (66) 107 (100) 103 (23)	$[M+H]^+$ $[M-3H]^+$ $[M+H-CO]^+$ $[M-3H-CO]^+$	133 (100)	$[M-H]^-$
9-Hydroxyfluorene (9-HFL)	182	181 (74) 165 (100) 153 (8)	$[M-H]^+$ $[M+H-H_2O]^+$ $[M-H-CO]^+$	–	–
2-Hydroxy-9-fluorenone (2-H-9-FLO)	196	197 (100) 169 (6) 141 (6)	$[M+H]^+$ $[M+H-CO]^+$ $[M+H-(CO)_2]^+$	196 (13) 195 (100)	$[M]^{-\cdot}$ $[M-H]^-$
2-Nitro-1-naphthol (2-N-1-N)	189	204 (100) 172 (20) 159 (28) 156 (49) 128 (22) 115 (10)	$[M+CH_3]^+$ $[M-OH]^+$ $[M-NO]^+$ $[M-NO-H3]^{+\cdot}$ $[M-NO-CO-H_3]^{+\cdot}$ $[M-CO-NO_2]^+$	188 (55) 172 (100) 159 (60) 158 (33) 142 (82)	$[M-H]^-$ $[M-OH]^-$ $[M-NO]^-$ $[M-NOH]^-$ $[M-OH-NO]^-$
9-Hydroxyphenanthrene (9-HF)	194	195 (100) 177 (14) 167 (45)	$[M+H]^+$ $[M+H-H_2O]^+$ $[M+H-CO]^+$	194 (16) 193 (100)	$[M]^{-\cdot}$ $[M-H]^-$
1,8-Dihydroxy-9,10-anthraquinone (1,8-DH-9,10-AQ)	240	241 (100)	$[M+H]^+$	240 (100) 239 (38)	$[M]^{-\cdot}$ $[M-H]^-$

For the non-aromatic hydroxy compounds (9-HFL and 1-IOH), the base peak corresponded to the ion $[M+H-H_2O]^+$. This fragmentation is frequent in chemical ionisation and was also observed in LC–MS using electrospray and APCI as interfaces [23–25] and it is due to the unstable protonated cyclic alcohol ions.

Chemical ionisation with methanol may be the reason for the presence of the ion at m/z 204 for 2-N-1-N. Typical losses for nitro-compounds were also observed. Nevertheless, in negative ionisation mode, only the $[M-H]^-$ ion and the typical fragment ions for nitro-compounds were observed for this compound. The $[M-H]^-$ ion was the base peak for 2-H-9-FLO, 9-HF and 5-IOH. Moreover, electron capture to give $[M]^{-\cdot}$ was observed for 2-H-9-FLO, 9-HF and 1,8-DH-9,10-AQ. No signal in the negative ionisation mode was obtained for 1-IOH and 9-HFL. Mass spectra for 1,4-DHN (Fig. 6) exhibited a based peak at m/z 158, probably due to electron capture by the quinone structure produced by chemical ionisation. The ion at m/z 173 may be due to chemical ionisation with methanol and the ion at m/z 145 was produced by the subsequent loss of CO.

3.3. SFC–MS

Since no studies have been reported on the separation of these compounds by SFC–MS, a synthetic mixture of the eight hydroxy-PAHs was

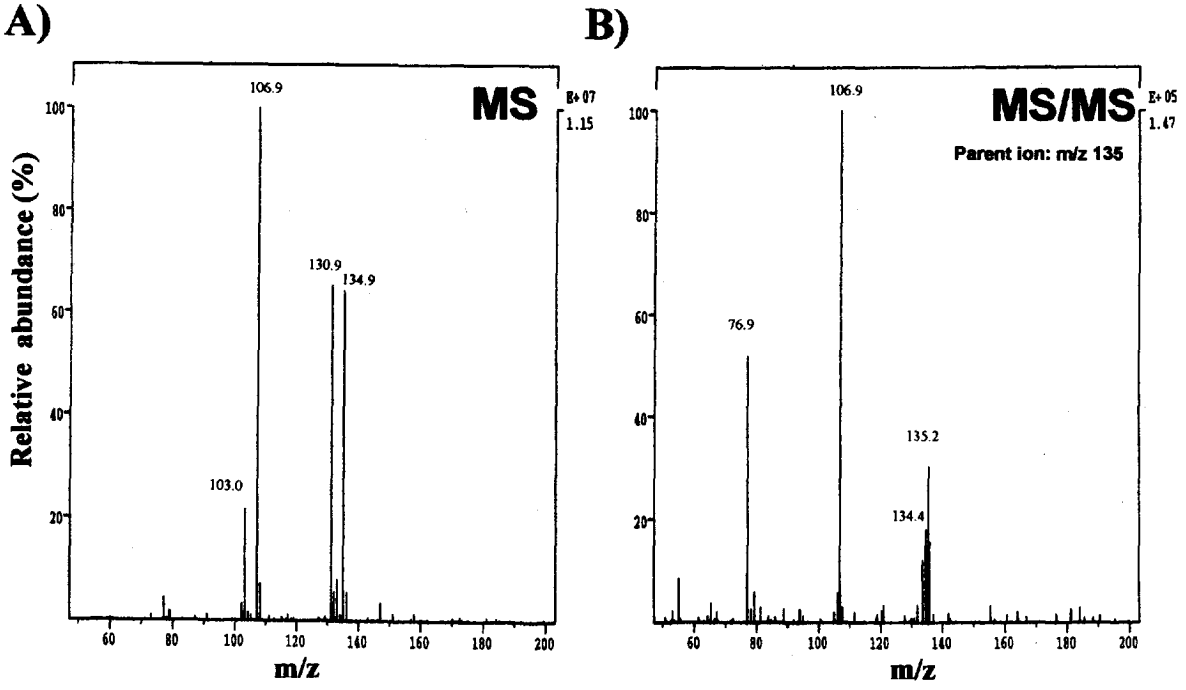

Fig. 4. SFC–APCI-MS spectra for 5-IOH. (A) MS spectra. (B) MS–MS spectra using m/z 135 as the parent ion, the collision cell energy was 40 V and the argon pressure was 1.8 mTorr (1 Torr=133.322 Pa).

used for the evaluation of the SFC–APCI-MS system. Improved resolution and sensitivity was obtained by using MID of the most intense ions in the spectra of the compounds. The reconstructed ion chromatograms for each mass in SFC–APCI-MS with positive ionisation are given in Fig. 7.

To determine the run-to-run reproducibility, five

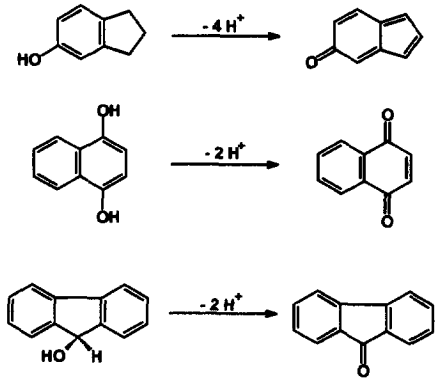

Fig. 5. Transformations of 1,4-DHN, 5-IOH and 9-HFL into their corresponding quinone structures.

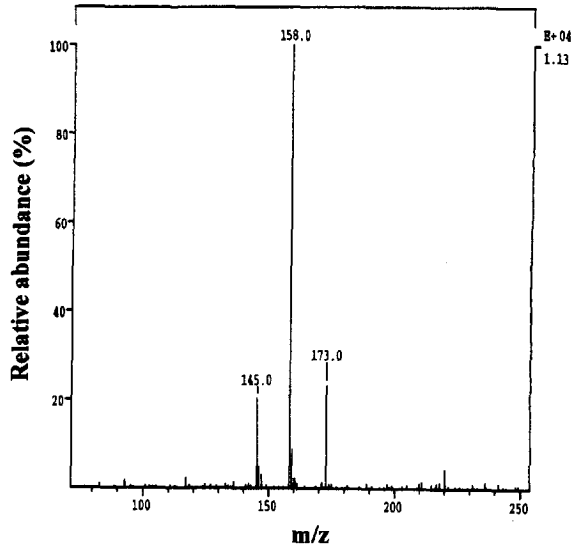

Fig. 6. MS spectra for 1,4-DHN using SFC–APCI-MS in the negative ionisation mode.

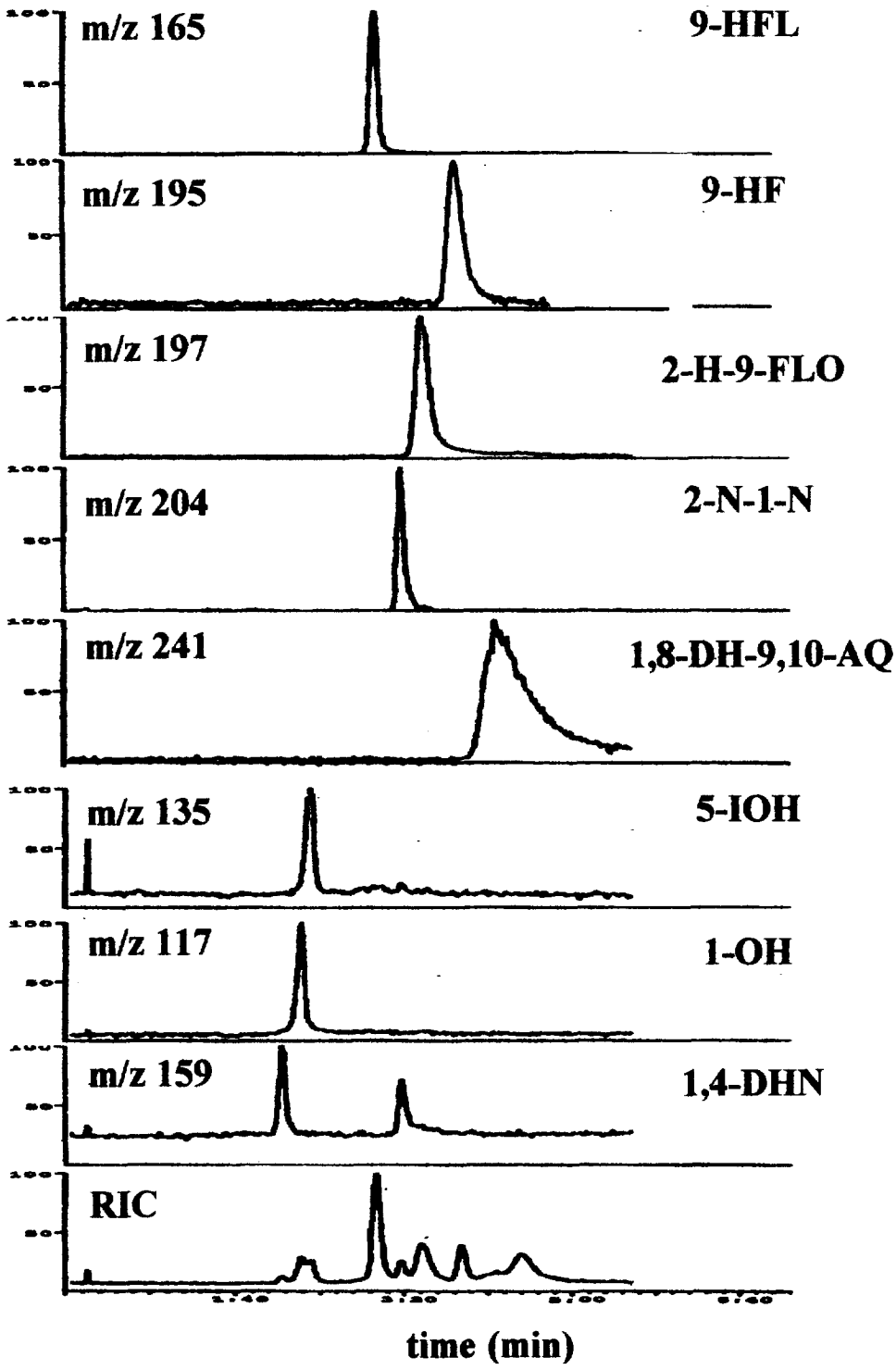

Fig. 7. SFC–APCI-MS reconstructed ion chromatograms for a standard solution of hydroxy-PAHs of 20 μg ml^{-1}.

Table 2
Detection limits (ng) of hydroxy-PAHs in SFC–UV and SFC–APCI-MS

Compounds	UV	MS	
		Positive	Negative
1,4-DHN	2.5	43.5	45.0
1-IOH	6.0	3.2	n.d.
5-IOH	3.9	18.1	24.2
9-HFL	2.8	1.0	n.d.
2-H-9-FLO	5.3	1.0	1.0
2-N-1-N	1.7	6.1	5.5
9-HF	2.9	2.1	20.7
1,8-DH-9,10-AQ	8.0	4.1	110.1

replicate determinations of 50 ng (5 μg ml^{-1}, 10 μl) of each hydroxy-PAH in methanol were carried out under the optimum conditions. Relative standard deviations (R.S.D.s) in the range 0.2 to 5% for UV detection and slightly higher, from 2 to 12%, for SFC–APCI-MS, based on peak heights, were obtained.

Detection limits based on a signal-to-noise ratio of 3:1, using standard solutions at concentrations down to 1 μg ml^{-1} and multiple ion detection, were determined (Table 2). Moreover, values for UV detection were also obtained. Detection limits in MS-positive mode were similar to those obtained with UV detection, except for 1,4-DHN and 5-IOH, which gave higher values, probably due to the lower ionisation of these compounds under the MS working conditions used. SFC–APCI-MS with negative ionisation usually gave higher detection limits, although for 2-H-9-FLO and 2-N-1-N, the detection limits were similar to those obtained using positive ionisation, probably due to the higher electronegativity of these compounds, which enhances the response in negative mode.

4. Conclusions

SFC–APCI-MS has been used for the characterisation of several hydroxy-PAHs. Addition of water (8%) to the modifier (methanol) was needed to improve the peak shape, preventing interaction with the silica support. Simultaneous pressure and modifier gradients, using supercritical CO_2 as the mobile phase, were applied to achieve the optimum chromatographic separation. Proton addition and proton abstraction to form $[M+H]^+$ in the positive ionisation mode and $[M-H]^-$ in the negative mode, respectively, were the common routes of ionisation. Losses of CO and H_2O were the origin of the most significant fragment ions. Transformation to the quinone structure was observed for 5-IOH, 9-HFL and 1,4-DHN. Detection limits at the ng level using SFC–APCI-MS were similar to those obtained with UV detection. The run-to-run reproducibilities were slightly higher than for UV detection, and ranged from 2 to 12% (R.S.D.).

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