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Determination of hydroxy polycyclic aromatic hydrocarbons by liquid chromatography–mass spectrometry Comparison of atmospheric pressure chemical ionization and electrospray¹

M.T. Galceran*, E. Moyano

Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain

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Abstract

Atmospheric pressure chemical ionization (APCI) and pneumatically assisted electrospray (ESP) mass spectrometry (MS) in both positive and negative ionization modes coupled to high-performance liquid chromatography (HPLC) have been used for the characterization of hydroxy polycyclic aromatic hydrocarbons (hydroxy-PAHs). A series of hydroxy-PAHs were separated using reversed-phase liquid chromatography and their ESP and APCI spectra were analyzed. Results showed that chemical ionization was the main process in APCI and, in contrast to what happened with ESP, no electrochemical reaction was observed. In order to optimize the response in ESP and to adjust the optimum flow-rate for the coupling of the HPLC system to the ESP source, the variation of the response versus flow-rate and the use of split systems were studied. LC–MS detection limits (signal-to-noise ratio 3:1) were determined for APCI and ESP in both ionization modes, ranging from 1.5 to 35 $\mu\text{g/ml}$ for ESP and from 0.3 to 50 $\mu\text{g/ml}$ for APCI.

Keywords: Ionization methods; Interfaces, LC–MS; Polynuclear aromatic hydrocarbons

1. Introduction

Liquid chromatography–mass spectrometry (LC–MS) is an area of analytical chemistry in which ionization techniques at atmospheric pressure are of increasing importance [1–6]. Although atmospheric pressure ionization–mass spectrometry (API–MS) is relatively new, the concept originated in the 1960s [7–9]. Nowadays, API instruments are available commercially and a large number of applications

using the API techniques have been published [6,10–18]. Moreover, a great deal of interest has also arisen in the mechanisms by which the gas-phase ions required for mass spectrometric detection are produced [5,6,10]. In some analytical mass spectrometric techniques, such as fast atom bombardment, plasma desorption and laser desorption, the energy is supplied by a complex high-energy collision cascade and highly localized heating, resulting in additional processes such as net ionization and the fragmentation of ions. In API techniques, due to the relatively low temperatures at which nebulization and ionization take place, decomposition of thermally labile compounds is not as important as in other ionization techniques.

*Corresponding author.

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Ionization in the gas phase in atmospheric pressure chemical ionization (APCI) takes place after the evaporation of the LC eluent. A corona discharge from a needle is used as primary ionization source and the ionization of the analytes takes place via processes identical to those occurring in chemical ionization sources, such as charge transfer, electron capture and ion–molecule reactions [5]. Electrospray (ESP) is adequate for compounds that exist as preformed ions in the LC eluent due to their characteristics and to the pH of the media, and also for non-ionic compounds which can associate with small ions such as Na^+ , NH_4^+ or Cl^- . In this ionization technique the LC eluent is dispersed into an electrically charged aerosol by applying a high potential to the capillary tip. Once electrically charged droplets are formed, evaporation of solvents takes place and the droplet sizes decrease. When the electrical field in the droplet exceeds a critical value the solute ions escape from the liquid phase into the gas phase (ion evaporation) in the API source. Although the mechanism by which the droplets are charged is assumed to be electrophoretic [11–13], the electrospray process may also involve electrochemical reactions at the metal–liquid interface of the ESP capillary tip [1,14–16].

Few data are available on LC–MS of hydroxy polycyclic aromatic hydrocarbons (hydroxy-PAHs); only two papers [16,17] deal with the analysis of these compounds using ESP. In this paper the potential of LC–MS using ESP and APCI for the separation and identification of hydroxy-PAHs is evaluated. APCI spectra in both positive and negative ionization modes are compared with those obtained with ESP previously published [16] and discussed in terms of ionization mechanisms. Also, the variation of response vs. flow-rate and the use of split systems for LC–ESP–MS are explored. Finally, the limit of detection in LC–ESP–MS and LC–APCI–MS are reported.

2. Experimental

2.1. Reagents

The compounds studied were 5-hydroxyindol (5-HI), 1-indanol (1-HI), 2-hydroxy-1,4-naphthoquin-

one (2-H-1,4-NQ), 9-hydroxyfluorene (9-HFL), 9-hydroxyphenanthrene (9-HF), 2-hydroxy-9-fluorenone (2-H-9-FLO), 2-nitro-1-naphthol (2-N-1-N) and 5,8-dihydroxy-1,4-naphthoquinone (5,8-DH-1,4-NQ) provided by Aldrich (Milwaukee, WI, USA). LC solvents, HPLC-grade methanol (Merck, Darmstadt, Germany) and water purified with a Culligan system (Barcelona, Spain) were used. All solvents were degassed in an ultrasonic bath for 15 min and passed through a 0.45- μm nylon filter before use.

Individual stock solutions were prepared containing 1 mg/ml of each compound in methanol. The 60 $\mu\text{g/ml}$ individual solutions and a mixture of each compound (60 $\mu\text{g/ml}$) in mobile phase were obtained by appropriate dilution of the 1 mg/ml solutions.

2.2. Instrumentation and procedures

Mass spectrometry was performed using a VG Quattro (FISONS Instruments, VG Biotech, Altrincham, UK) triple quadrupole equipped with ESP and APCI interfaces. A nitrogen flow-rate of 10 l/h was used for the nebulization and the flow-rate for the drying nitrogen was 200 l/h. Ion source temperatures were optimized at the highest flow-rate. For the ESP ion source the temperature was 80°C and for the APCI source it was 200°C. The capillary in ESP was held at a potential of +3.5 kV and –3.5 kV relative to the potential of the counter electrode for positive and negative modes, respectively, and the corona potential in APCI was +3.2 kV for positive mode and –3.2 kV for negative mode. The first skimmer potential was 60 V for the ESP system and 30 V for the APCI source.

For data acquisition the mass spectrometer operated over the range m/z 100–600 in a centroid mode at a cycle time of 2.00 s and an inter-scan time of 0.10 s. Ion intensity was optimized using 2-hydroxy-9-fluorenone and calibration was performed with these clusters in both modes; in Fig. 1 the mobile phase spectra in APCI positive and negative modes are shown. To improve cluster formation a drying nitrogen flow of 50 l/h and a skimmer potential of 40 V were used.

Two HPLC systems were used to perform the coupling. With the ESP source two Phoenix 20

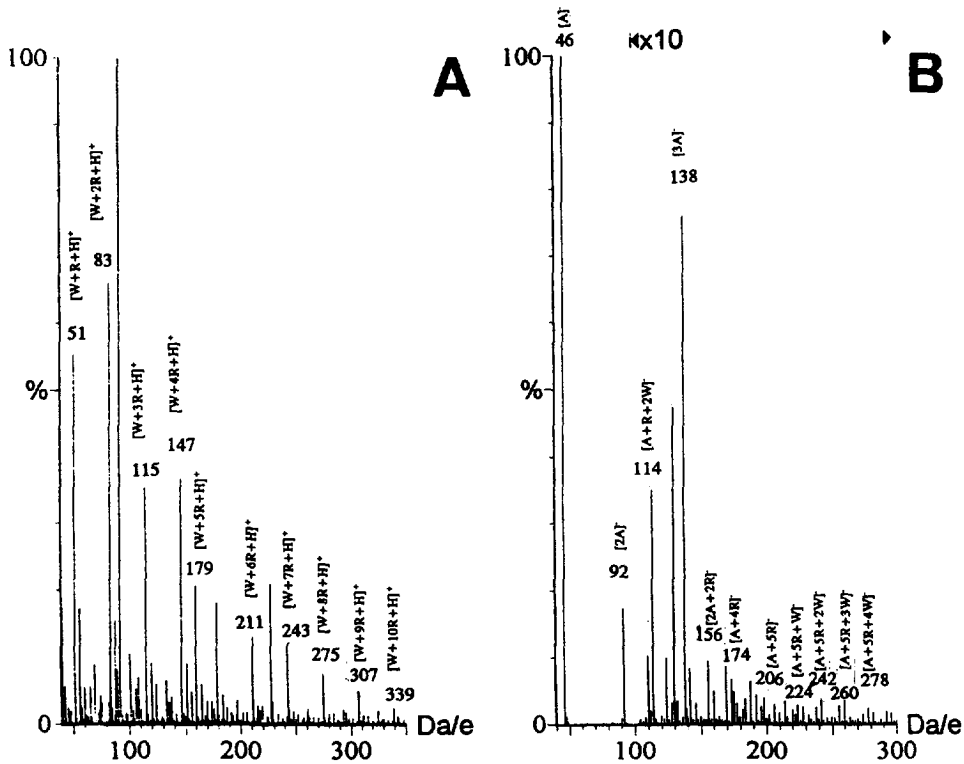


Fig. 1. APCI spectra of mobile phase in (A) positive ionization and (B) negative ionization mode. W=H₂O, R=CH₃OH, A=HCOOH.

(Carlo Erba, Milan, Italy) syringe pumps, a master (A) and a slave (B) pump, with a Valco injection valve (200-nl loop) were used, and an Hypersil ODS C₁₈ (5 μm particle size, 100×1 mm I.D.) reversed-phase column (Shandon Scientific, Cheshire, UK) was used for the hydroxy-PAH liquid chromatographic separation. With the APCI system, Waters HPLC pumps (model 600-MS) with an autosampler (Waters, Model 717) and a C₁₈ RP-SELECT-B (5 μm particle size, 150×4 mm I.D.) (Merck, Darmstadt, Germany) were used for chromatographic separation. In both cases the mobile phase was 50% methanol–formic acid/ammonium formate 10 mM (pH 3) at 50 μl/min for ESP and 1 ml/min for APCI. Mixtures of standards were prepared in mobile phase; 10-μl aliquots were injected when the flow injection mode and chromatographic mode for APCI were used instead of 200 nl used for ESP chromatographic mode.

For post-column split a system with a Valco tee was used and the split ratio was 1:10.

3. Results and discussion

As mentioned before, the ionization of organic compounds with API techniques can take place in different ways depending on the source used. In the ESP interface the main ionization takes place in the liquid phase and the preformed ions are transferred from the solution to the gas phase. In contrast, in the APCI source, the main ionization takes place directly in the gas phase via chemical ionization, although some preformed ions can be transferred from the solution to the gas phase via ion evaporation.

Mass spectral data of the hydroxy-PAHs in both ionization modes with APCI and ESP sources are given in Table 1. Proton addition to form [M+H]⁺ is the common route of ionization in positive mode for amines using ESP and APCI [18,19] and it is related with high proton affinity of amines in both condensed and gas phase. In this work protonation was observed for 5-hydroxyindol in both techniques in positive mode (Table 1). Proton addition with high

Table 1
Mass spectra of hydroxy-PAHs in APCI and ESP

Compound (M_r)	Mass spectra (m/z (relative abundance) [ion])			
	APCI		ESP	
	Positive	Negative	Positive	Negative
5-Hydroxyindol (133)	134 (100) [M+H] ⁺	n.d.	134 (100) [M+H] ⁺ 117 (72) [M+H-OH] ⁺	n.d.
2-Hydroxy-9-fluorenone (196)	n.d.	196 (20) [M] ⁻ 195 (100) [M-H] ⁻	197 (100) [M+H] ⁺	196 (20) [M] ⁻ 195 (100) [M-H] ⁻
2-Hydroxy-1,4- -naphthoquinone (174)	189 (100) [M+CH ₃] ⁺ 161 (21) [M+CH ₃ -CO] ⁺ 175 (9) [M+H] ⁺ 174 (19) [M] ⁺	174 (100) [M] ⁻ 173 (32) [M-H] ⁻	189 (100) [M+CH ₃] ⁺ 175 (60) [M+H] ⁺ 161 (12) [M+CH ₃ -CO] ⁺	175 (40) [M+2H-H] ₂ ^b 174 (30) [M] ⁻ 173 (100) [M-H] ⁻
5,8-Dihydroxy-1,4- -naphthoquinone (190)	n.d.	190 (100) [M] ⁻	n.d.	n.d.
2-Nitro-1-naphthol (189)	160 (100) [M-COH] ⁺ 158 (72) [M-NOH] ⁺	189 (30) [M] ⁻ 188 (39) [M-H] ⁻ 173 (14) [M-O] ⁻ 172 (100) [M-OH] ⁻ 159 (27) [M-NO] ⁻	n.d.	n.d.
9-Hydroxyfluorene (182)	182 (40) [M] ⁺⁺ 165 (100) [M+H-H ₂ O] ⁺	181 (10) [M-H] ⁻ 180 (100) [M-2H] ⁻	181 (10) [M-2H+H] ⁺ ^a 165 (100) [M+H-H ₂ O] ⁺	n.d. n.d.
1-Indanol (134)	134 (12) [M] ⁺⁺ 117 (100) [M+H-H ₂ O] ⁺	n.d. n.d.	134 (2) [M] ⁺⁺ 117 (100) [M+H-H ₂ O] ⁺	n.d.
9-Hydroxyphenantrene (194)	n.d.	194 (12) [M] ⁻ 193 (100) [M-H] ⁻	n.d.	194 (15) [M] ⁻ 193 (100) [M-H] ⁻

n.d.: No spectra detected at working conditions.

^a Oxidation product.

^b Reduction product.

relative abundance was also observed for α,β -unsaturated ketones (2-hydroxy-9-fluorenone, 2-hydroxy-1,4-naphthoquinone) in positive mode ESP, although in positive APCI only the last compound gave a peak with low relative abundance. The distinct behaviour of these compounds in ESP and APCI may be due to differences in their basic properties in the condensed and gas phase. In negative ionization, proton abstraction to form [M-H]⁻ was observed for most of the hydroxy-PAHs. In APCI (Table 1) 2-hydroxy-9-fluorenone, 2-hydroxy-1,4-naphthoquinone, 2-nitro-1-naphthol, 9-hydroxyfluorene and 9-hydroxyphenantrene lost a proton but only three of them, 2-hydroxy-9-fluorenone, 2-hydroxy-1,4-naphthoquinone and 9-hydroxyphenantrene, gave proton abstraction in ESP. Since the hydroxy groups of these compounds were

not deprotonated at the LC working conditions, proton abstraction would take place via gas phase and this process may be enhanced by the corona discharge in APCI.

For these compounds molecular ions [M]⁺⁺ and [M]⁻ were obtained (Table 1). The relative abundances of the ions in APCI were generally higher than in ESP, for instance the ion [M]⁺⁺ for 9-hydroxyfluorene (m/z 182) and 2-hydroxy-1,4-naphthoquinone (m/z 174) only appeared in APCI in the positive mode. The ion [M]⁻ for this last compound (m/z 174) also gave a higher abundance in APCI in negative mode, although similar values were observed for 2-hydroxy-9-fluorenone (m/z 196) and 9-hydroxyphenantrene (m/z 194) in both APCI and ESP. For 5,8-dihydroxy-1,4-naphthoquinone the ion [M]⁻ (m/z 190) is the base peak in APCI and no

ionization was observed using ESP. These results can be explained taking into account that the molecular ions $[M]^{+}$ and $[M]^{-}$ were obtained by charge transfer and electron capture, respectively, and both processes take place in the gas phase, therefore, they may be enhanced in APCI.

Methylation via chemical ionization with the methanol of the mobile phase was observed for 2-hydroxy-1,4-naphthoquinone in ESP and APCI in positive ionization. Both spectra are given in Fig. 2. The relative abundance of the m/z 189 ion increased when the APCI source was used, because chemical ionization was favoured in the APCI source. Moreover, the ion at m/z 161 can be explained as the COH loss from the methylated product (m/z 189); this loss has been described as a common fragmenta-

tion for hydroxy aromatic compounds with electron impact [20].

Other characteristic fragmentations were also observed. For instance, the characteristic fragmentation of the nitro group was observed for 2-nitro-1-naphthol when APCI was used in negative and positive ionization modes (Table 1); using ESP no ionization was observed for this compound. For the non-aromatic hydroxy compounds (9-hydroxyfluorene and 1-indanol) the base peak obtained with both techniques in the positive mode corresponded to the ion $[M+H-H_2O]^+$ (Table 1). This fragmentation is frequently observed in chemical ionization with methane as reactive gas and can be explained by the loss of water from the unstable protonated cyclic alcohol ions [20]. In the negative ionization mode only 9-hydroxyfluorene gave response and the base peak is $[M-2H]^-$.

In ESP, electrochemical reactions can be produced. In a previous paper [16] we reported reduction of 2-hydroxy-1,4-naphthoquinone to 1,2,4-trihydroxynaphthalene and oxidation of 9-hydroxyfluorene to 9-fluorenone using this technique. In the present study the APCI spectra of these compounds did not show the ions of electrochemical origin observed in ESP, as can be seen in Fig. 3 where both spectra are given. The ion m/z 175 in negative ESP for 2-hydroxy-1,4-naphthoquinone corresponded to the proton abstraction of the reduction product (1,2,4-trihydroxynaphthalene) and the m/z 181 in positive ESP for 9-hydroxyfluorene corresponded to the proton addition of the oxidation product (9-fluorenone).

3.1. HPLC-MS

Few studies are available on the separation of hydroxy polycyclic aromatic hydrocarbons by HPLC. In a previous paper [21], we used reversed-phase liquid chromatography with electrochemical detection for the analysis of hydroxy aromatic compounds with methanol-phosphoric acid/sodium dihydrogenphosphate (pH 3). In this paper the buffer has been changed to formic acid/ammonium formate (pH 3), compatible with MS.

The ion chromatograms for each mass in LC-APCI-MS with positive and negative ionization are

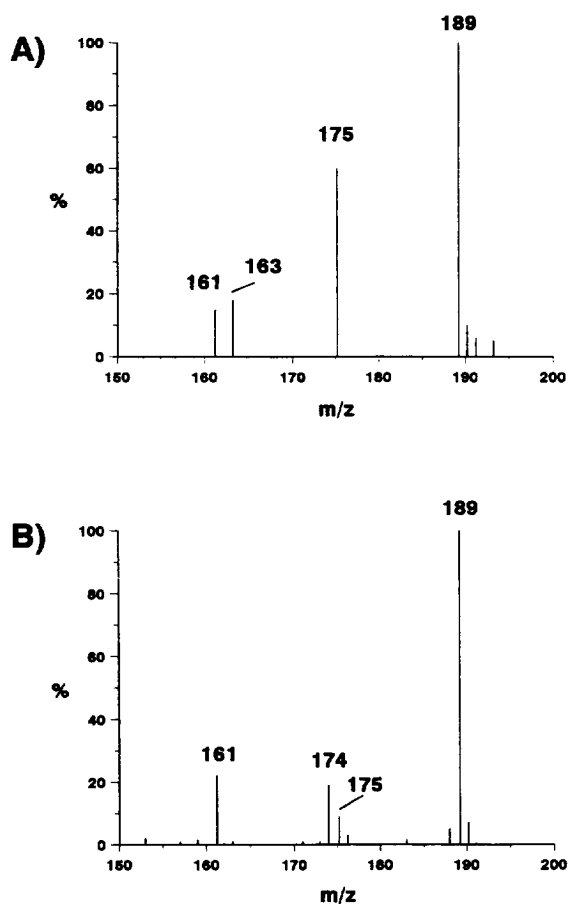


Fig. 2. Spectra of 2-hydroxy-1,4-naphthoquinone (M_r 174) in (A) ESP and (B) APCI in positive ionization mode.

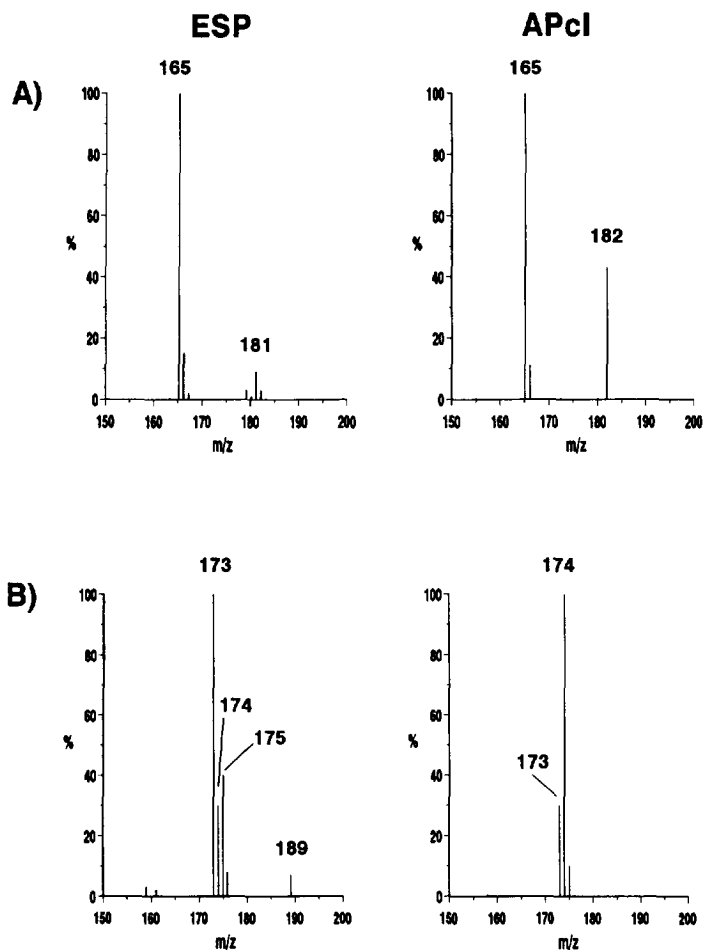


Fig. 3. Spectra of (A) 9-hydroxyfluorene (M_r 182) with ESP and APCI in positive ionization mode and (B) 2-hydroxy-1,4-naphthoquinone (M_r 174) with ESP and APCI in negative ionization mode.

given in Fig. 4 and Fig. 5, respectively. The ions selected have been the more intense ones.

Classical electrospray gives optimal responses at a very low effluent flow-rate (5 $\mu\text{l}/\text{min}$). Higher values can be obtained using the mega-flow system and pneumatically assisted electrospray. The first accepts flow-rates up to 1 ml/min and the second up to 200 $\mu\text{l}/\text{min}$. The flow-rate that gives the best sensitivity still seems to be discussed but Bruins [5] reported that this is observed at approximately 5 $\mu\text{l}/\text{min}$. To study the effect of the flow-rate on the MS response the intensities for the ions m/z 197 in positive ESP and 195 in the negative mode for

2-hydroxy-9-fluorenone versus flow-rate were compared. The variation obtained using flow injection is given in Fig. 6. For the positive mode a marked decrease in the intensity was obtained when flow-rate increased. In contrast, a nearly constant response was obtained for the negative mode. The different behaviour in positive and negative modes can be related with the spectra obtained for this compound in each mode. In the positive mode a high number of clusters appeared at high flow-rates, as can be seen in Fig. 7, which disappeared at lower flow-rates, while in the negative mode no significant differences in the spectra were observed.

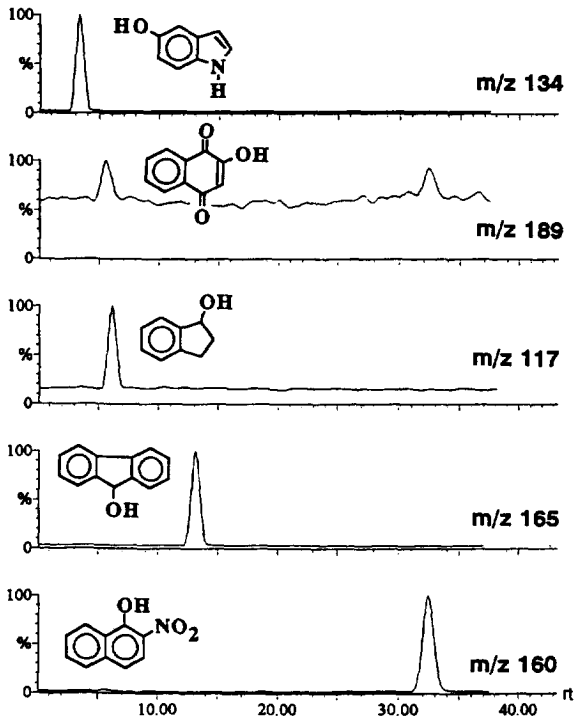


Fig. 4. LC-MS ion chromatograms of a hydroxy-PAHs mixture with APCI in positive ionization ($10 \mu\text{l}$ standard solution of $50 \mu\text{g/ml}$). Mobile phase, methanol–acid formic/ammonium formate 10 mM (pH 3) (50:50). Flow-rate, 1 ml/min . Acquisition data, SCAN. Retention time in min.

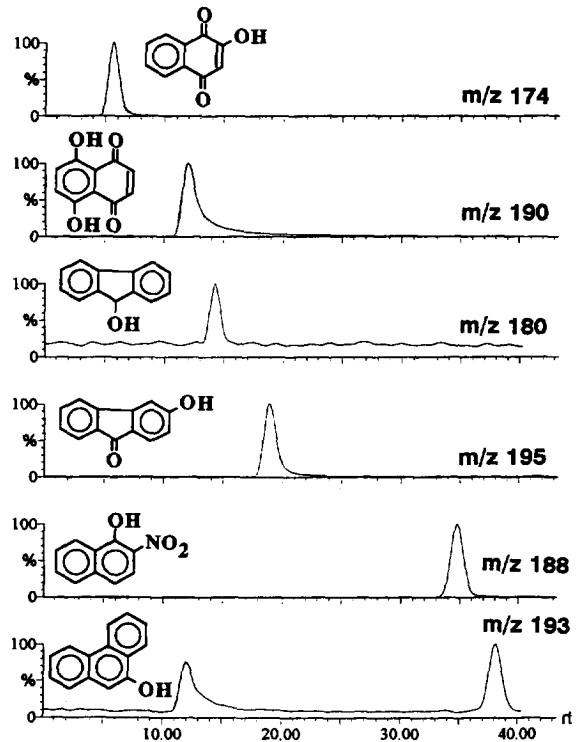


Fig. 5. LC-MS ion chromatograms of a hydroxy-PAHs mixture with APCI in negative ionization ($10 \mu\text{l}$ standard solution of $50 \mu\text{g/ml}$). Mobile phase, methanol–formic acid/ammonium formate 10 mM (pH 3) (50:50). Flow-rate, 1 ml/min . Acquisition data, SCAN. Retention time in min.

In order to optimize flow-rates in ESP, a post-column split (1:10) was introduced in the system. As a result an increase in signal was observed in the positive mode while a decrease was obtained in the negative mode for all the hydroxy aromatic compounds studied. As an example, in Fig. 8 the LC-ESP-MS chromatograms in positive and negative modes with and without split are given. Post-column split produced a decrease in both the flow-rate and the amount of compound introduced in the ion source of the mass spectrometer, giving a decrease in the signal when ESP was used in the negative mode. The increase in the signal when the positive mode was used can be explained by the reduction in the abundance of the cluster ions (Fig. 7) which compensates the lower amount of compound in the source. This allowed an advantage in sensitivity for

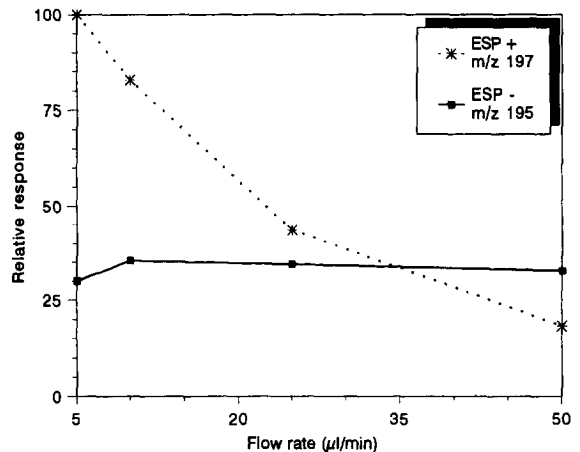


Fig. 6. Variation of relative responses in ESP versus mobile phase flow-rate for m/z 197 in positive ionization and m/z 195 in negative ionization for 2-hydroxy-9-fluorenone.

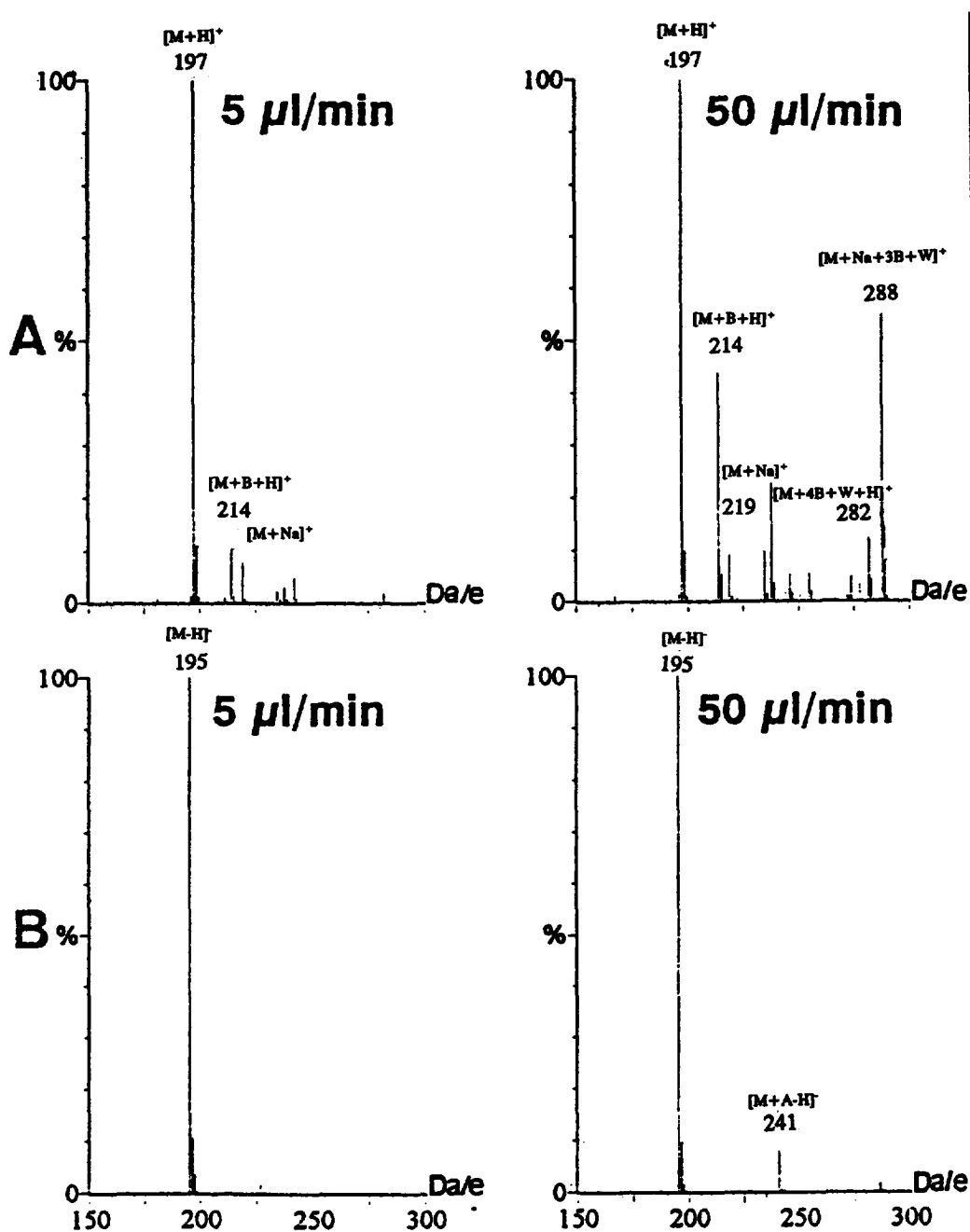


Fig. 7. ESP mass spectra at different flow-rates for 2-hydroxy-9-fluorenone. (A) positive ionization, (B) negative ionization.

this compounds using a split system in positive ESP detection.

The limits of detection based on a signal-to-noise

ratio of 3:1, using standard solutions at concentrations down to 0.5 µg/ml for ESP and APCI in both ionization modes using selected ion monitoring

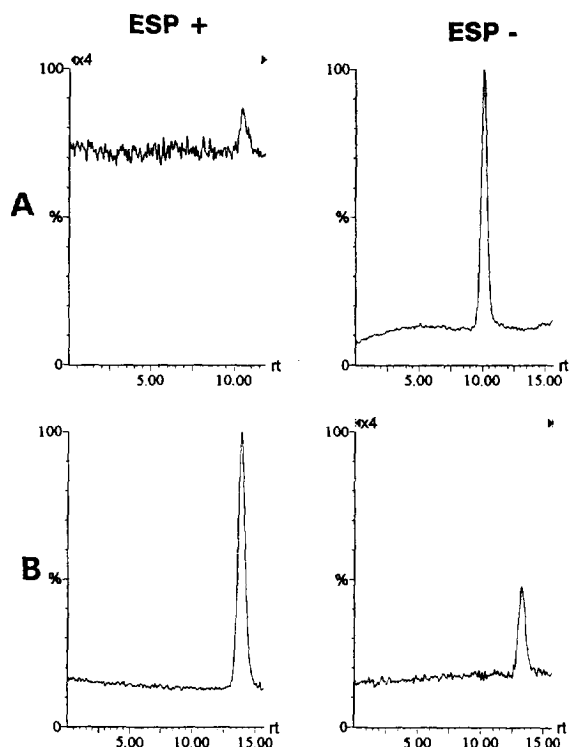


Fig. 8. LC-MS chromatograms (50 μ l/min) for 2-hydroxy-9-fluorenone (50 μ g/l, 200 nl) in ESP with positive (m/z 197) and negative (m/z 195) modes. (A) without split, (B) with split. The retraction time delay in B with respect to A corresponds to the lower flow-rate through the capillary post-split. Retention time in min.

(SIM) with a split when ESP system was used in positive mode, were determined and their values are given in Table 2. Similar values for both techniques, and sometimes better data for APCI, were observed. Considering the absolute amount injected lower values were obtained for ESP, from 0.5 to 7 ng, but this is only due to differences in the injection volume.

4. Conclusions

Mass spectra for eight hydroxy-PAHs were obtained with ESP and APCI techniques. Proton addition and proton abstraction were observed for most of the compounds and the differences in the responses in both techniques have been related with the acid-base properties in both gas and liquid phases. Electron capture, charge transfer and chemical ionization were enhanced in APCI. Moreover, electrochemical reactions were only observed in ESP. To improve sensitivity a split was used and higher responses were obtained in ESP with positive ionization but a loss in sensitivity was observed in the negative ionization mode. So, for these compounds, the use of split system is recommended for LC-ESP-MS in the positive ionization mode. Similar LC-MS limits of detection (μ g/ml) were obtained for both ESP and APCI sources.

Table 2
Limits of detection for hydroxy-PAHs in LC-ESP-MS and LC-APCI-MS

Compound	Positive ionization		Negative ionization	
	ESP (μ g/ml)	APCI (μ g/ml)	ESP (μ g/ml)	APCI (μ g/ml)
1-Indanol	1.5	5	–	–
5-Hydroxyindol	6	0.3	–	–
2-Hydroxy-1,4-naphthoquinone	10	50	15	0.5
5,8-Dihydroxy-1,4-naphthoquinone	–	–	–	3
9-Hydroxyfluorene	2.5	1	–	5
2-Hydroxy-9-fluorenone	4.0	–	5	0.5
9-Hydroxyphenanthrene	–	–	35	20
2-Nitro-1-naphthol	–	0.5	–	0.5

Injection volume: 200 nl in ESP and 10 μ l in APCI. LC column: ESP: Hypersil ODS C₁₈, 5 μ m, 100 \times 1 mm I.D. APCI: RP-SELECT-B, 5 μ m, 150 \times 4 mm I.D.

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