



Development of a sequential supercritical fluid extraction method for the analysis of nitrated and oxygenated derivatives of polycyclic aromatic hydrocarbons in urban aerosols

P. Castells, F.J. Santos, M.T. Galceran*

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

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Abstract

A two-step supercritical fluid extraction (SFE) method has been developed for the analysis of oxygenated and nitrated polycyclic aromatic hydrocarbons (oxy- and nitro-PAHs, respectively) present in urban aerosol samples. The proposed SFE procedure first involves an extraction step using pure CO₂ in order to remove the less polar compounds from the matrix and a second consecutive step using toluene-modified CO₂. The oxy- and nitro-PAHs are obtained in the second step. Parameters affecting both collection efficiencies and the selective extraction of oxy- and nitro-PAHs in the second SFE step were optimised. Analysis of the extracts was performed using gas chromatography with electron-capture detection and coupled to mass spectrometry. The proposed SFE method was compared with a conventional extraction technique such as sonication and good agreement in the results was obtained. Nevertheless, clean up of sonication extracts was needed, whereas no purification was necessary for SFE extracts. The SFE method was applied to the analysis of oxy- and nitro-PAHs in urban aerosol samples and 9-fluorenone, 9,10-anthraquinone, 2-methyl-9,10-anthraquinone, benzanthrone, benz[*a*]anthracene-7,12-dione and 1-nitropyrene were identified at concentrations ranging between 15 and 364 pg m⁻³.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and a large number of structurally related PAH derivatives are generated in the incomplete combustion of organic matter. These compounds constitute an important environmental hazard. Oxygenated and nitro-substituted polycyclic aromatic hydrocarbons (oxy-

and nitro-PAHs, respectively) are formed in the combustion of fuel, natural gas, wood, coke, and in residue incineration. They are present in tobacco smoke and also in the ambient air due to chemical reactions of PAHs with different atmospheric pollutants, such as NO_x or O_x [1–4]. Therefore, oxy- and nitro-PAHs are found in atmospheric aerosols.

Several oxy- and nitro-PAHs are known to be mutagenic, and it seems that some of them are potential human carcinogens. The Ames test has shown that up to 50% of total mutagenic activity of diesel particulate extracts is due to the presence of

*Corresponding author. Tel.: +34-93-4021-286; fax: +34-93-4021-233.

E-mail address: galceran@apollo.qui.ub.es (M.T. Galceran).

these moderately polar derivatives of PAHs [5,6]. Furthermore, the direct mutagenic activity of tobacco smoke or atmospheric aerosol extracts has been mainly correlated with the presence of oxy- and nitro-PAHs [7–9]. While PAHs have proved to be indirect mutagens, nitro-PAHs are direct-acting mutagens. Consequently, several nitro-PAHs have been included in the International Agency for Research on Cancer lists (2B and 3) of carcinogens [10].

Analysis of oxy- and nitro-PAHs has been performed by gas chromatography with flame ionisation (GC–FID) [11] or nitrogen and phosphorus selective detection (GC–NPD) [12], and also coupled to mass spectrometry (GC–MS) [13–16]. However, the most selective and sensitive techniques for the detection of oxy- and nitro-PAHs are based on electron capture processes occurring in the gas phase, due to the electronegative character of the nitro and oxy groups conjugated with the aromatic rings. This allows the detection of low concentrations and a higher degree of selectivity than in other types of common detectors. Thus, negative ion chemical ionisation mass spectrometry (NCI-MS) [17–21] and electron-capture detection (ECD) [22,23] are the most widely used techniques for the analysis of oxy- and nitro-PAHs, showing the lowest limits of detection (below the pg m^{-3} level in atmospheric aerosol samples). In addition, some authors have used liquid chromatography with on-line reduction to amino-PAHs and fluorescence detection [24–26] and coupled with mass spectrometry [27]. Micellar electrokinetic chromatography with UV detection (MEKC-UV) has also been reported for the analysis of nitro-PAHs [28].

The extraction methods used for the analysis of oxy- and nitro-PAHs in atmospheric aerosols usually involve Soxhlet and sonication techniques using dichloromethane [8,22,24] and toluene [11,21] as solvents. The aerosol extracts obtained with these conventional extraction techniques contain several hundred PAH derivatives, generally at the low pg m^{-3} range for the nitro-PAHs [21,29–35] and few hundreds of pg m^{-3} for the oxy-PAHs [29,36,37]. Therefore, individual compounds are present at very low levels with a large amount of interference substances at high concentrations, thus laborious clean up procedures are required to isolate the

compounds of interest before their chromatographic determination.

Supercritical fluid extraction (SFE) has been shown to be suitable for the analysis of relatively non-polar compounds such as aliphatic hydrocarbons and PAHs using pure CO_2 as extraction fluid [38–41]. In addition, SFE reduces the extraction time and solvent consumption compared with Soxhlet extraction. However, the recoveries obtained are dramatically low for the more polar compounds present in atmospheric aerosols, such as oxy- and nitro-PAHs. Experiments with these matrices indicate that the limiting factor is the ability of the extraction fluid to overcome matrix–analyte interactions, instead of the solubility of these polar analytes in the SFE fluid. High extraction efficiencies for nitro-PAHs in diesel exhaust particulates have been reported using pure CHClF_2 , although CO_2 modified with toluene has also provided good recoveries [15,42]. On the other hand, the ability of SFE for selective extractions of groups of compounds has been reported in the literature [43–45]. In particular, a consecutive two-step SFE procedure using pure CO_2 has been applied to the sequential separation of aliphatic hydrocarbons from PAHs present in diesel exhaust particulates [40].

The present work reports on the applicability of SFE for the analysis of oxy- and nitro-PAHs in urban particulate matter using a simultaneous extraction and clean up procedure. The developed SFE method was applied to the analysis of these PAH derivatives in urban aerosol samples using GC–ECD and GC–MS. Results were compared with those obtained with ultrasonic extraction.

2. Experimental

2.1. Standards and reagents

Toluene, *n*-hexane, dichloromethane, acetone (of organic trace analysis grade) and anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany). Seven oxy-PAHs and six nitro-PAHs were selected for this study. 1,4-Naphthoquinone (1,4-NQ), 9-fluorenone (9-Flu), 9-nitroanthracene (9-NA) and 2-methyl-9,10-anthraquinone (2-M-9,10-AQ), were purchased from Fluka (Buchs, Switzerland);

acenaphthenequinone (AnQ), 1,5-dinitronaphthalene (1,5-dNN) and 2-nitrofluorene (2-NF) from Merck (Darmstadt, Germany); 2-nitronaphthalene (2-NN) and 1-nitropyrene (1-NP) from Sigma–Aldrich (Milwaukee, WI, USA); 2-methyl-1-nitronaphthalene (2-M-1-NN), 9,10-anthraquinone (9,10-AQ), benzanthronone (BzA) and benz[*a*]anthracene-7,12-dione (Bz(a)A-7,12-d) were provided by Ega Chemie (Steinheim, Germany). 1,2,4,5-tetrabromobenzene was used as internal standard and it was obtained from Fluka.

Standard solutions with concentrations ranging from 5 to 1000 ng g⁻¹ for each analyte were prepared by weight in *n*-hexane for calibration purposes. Solutions containing 200 ng g⁻¹ were used for spiking experiments. The concentration of the internal standard in the calibration solutions and sample extracts was 20 ng g⁻¹ for GC–ECD and 200 ng g⁻¹ for GC–MS.

2.2. Analytical procedure

SFE was performed with a Suprex SFE apparatus Model 0159 (Suprex, Pittsburgh, PA USA) using SFC-grade CO₂. Collection of the extracted analytes was carried out with an Accutrap collection system, filled with a Supelclean LC-18 C₁₈ adsorbent purchased from Supelco. An incorporated HPLC pump was used to generate toluene-modified CO₂. Samples were extracted in 5-ml extraction cells. All SFE extractions were performed with a 5-min period on static mode, followed by 30 min of dynamic extraction. Supercritical fluid flow on dynamic step was held at 1 ml min⁻¹ by a variable-flow restrictor. Optimisation of several SFE parameters such as pressure, temperature and dynamic extraction time, as well as the volume and type of solvent used for the elution of the sorbed analytes, was carried out.

A Sonorex RK100 sonicator from Bandelin (Berlin, Germany), was used for ultrasonic extractions. The sample was kept in an ultrasonic bath with 75 ml of dichloromethane for 30 min, the extracts were concentrated to approximately 2 ml by rotary evaporation and the final volume was adjusted to 0.5 ml in *n*-hexane. Clean up of the extracts was carried out with Bond Elut silica cartridges (500 mg/3 ml) purchased from Varian (Harbor City, CA, USA). A Visiprep SPE vacuum manifold (Supelco, Bellefonte,

PA, USA) was used for manipulations with solid-phase extraction cartridges. For preconditioning, 10 ml of *n*-hexane was used, then the sample extract was added and three fractions were obtained by elution with 2 ml of *n*-hexane (aliphatic hydrocarbons), 3 ml of hexane–dichloromethane 4:1 (PAHs), and 6 ml of dichloromethane (oxy- and nitro-PAHs). This last fraction was reduced to 1 ml of *n*-hexane and analysed by GC–ECD.

2.3. Chromatographic conditions

A Carlo Erba HRGC-5300 Mega Series gas chromatograph (Carlo Erba, Milan, Italy) equipped with an AS200S autosampler and a ⁶³Ni electron-capture detector (ECD) was used for GC–ECD analyses. A DB-17 (50% phenyl, 50% methylpolysiloxane), 30 m×0.25 mm I.D., fused-silica capillary column (J&W Scientific, Folsom, CA, USA) of 0.25-μm film thickness was used. The carrier gas was helium (1 ml min⁻¹) and nitrogen was used as ECD make-up gas at 30 ml min⁻¹. Injector and detector temperatures were kept at 260 and 330 °C, respectively. Samples were injected in the splitless injection mode (1 min). The oven temperature programme was: 60 °C (held for 2 min), to 180 °C at 10 °C min⁻¹ (held for 1 min), and to 270 °C (held for 5 min) at 2.5 °C min⁻¹. Chrom-Card version 3.2 software (Fisons Instruments, Spain) was used for data acquisition.

GC–MS experiments for confirmation and quantification purposes were carried out using a ThermoFinnigan Trace GC 2000 Series gas chromatograph coupled to a GCQ/Polaris ion-trap mass spectrometer (ThermoFinnigan, Austin, TX, USA). The column and chromatographic conditions were the same as described for GC–ECD separation. Xcalibur version 1.2 software was used for data acquisition. An AS2000 autosampler was used to perform the injections. The mass spectrometer was operated in the electron ionisation (EI) mode at an electron energy of 70 eV. The transfer line and ion source temperatures were 270 and 200 °C, respectively. A mass range of *m/z* 100–400 was used in the full-scan mode at a scan rate of 0.73 s/scan (6 μscans). For the selective ion-monitoring (SIM) mode, the chromatogram was divided into four retention time windows. For each compound, the

Table 1
Ions monitored for each compound in MS-SIM mode

Window	Compound	Quantification ion (<i>m/z</i>)	Confirmation ion (<i>m/z</i>)
1	1,4-Napthoquinone	158	130
	2-Methyl-1-nitronaphthalene	187	115
	2-Nitronaphthalene	173	127
2	1,2,4,5-Tetrabromobenzene (I.S.)	394	396
	9-Fluorenone	180	152
	Acenaphthenequinone	182	154
	9,10-Anthraquinone	208	180
	1,5-Dinitronaphthalene	218	114
3	2-Methyl-9,10-anthraquinone	222	165
	2-Nitrofluorene	211	165
	9-Nitroanthracene	223	176
4	Benzanthrone	230	202
	Benz[<i>a</i>]anthracene-7,12-dione	258	230
	1-Nitropyrene	247	217

molecular ion was monitored for quantification and the most intense fragment ion was used for confirmation (see Table 1).

2.4. Sampling

Several urban aerosol samples were collected during the winter and summer of 2001 in Barcelona (Spain). Urban air particulate matter was collected on 20.3×25.4-cm Whatman GF/A glass-fibre filters using a Sierra Misco model 650 (Pt. Richmond, CA, USA) high volume sampler. In order to have enough amounts of sample for optimisation and to perform replicate analyses, several samples were collected and pooled. For all samples, the sampling period was 24 h at a flow-rate of 60 m³ h⁻¹. The filters were thermally treated (500 °C for 6 h) before use in order to eliminate potentially sorbed interferences. After collection, the filters were folded in order to avoid the loss of particles and kept in a dessicator for 2 h. They were then wrapped in aluminium foil and stored in the dark at -20 °C before analysis.

3. Results and discussion

3.1. SFE optimisation

In order to determine the collection efficiencies for the extracted analytes on the C₁₈ trap and to optimise

the elution method, SFE experiments were carried out using anhydrous Na₂SO₄ (7.5 g), which was previously spiked in the extraction cell with 1 ml of a standard solution containing 200 ng g⁻¹ of each analyte in *n*-hexane. Initially, the following extraction conditions were used: pressure 350 atm, temperature 90 °C and dynamic extraction time 30 min. CO₂ modified with toluene (10% v/v) was selected as the extraction fluid according to literature data [15]. Nevertheless, under these conditions condensation of the modifier on the C₁₈ trap occurred and a reduction of the retention capability of the sorbent was observed. To avoid this problem the percentage of toluene was optimised and toluene-CO₂ (5:95, v/v) was used for all subsequent extractions.

The solvent and the volume used for the elution of the analytes retained in the C₁₈ trap after the extraction were also optimised. *n*-Hexane, acetone and dichloromethane were tested, and recoveries obtained with elution volumes ranging from 1 to 5 ml were determined. For *n*-hexane, more than 5 ml was necessary for the adequate elution of trapped analytes, whereas for acetone 3 ml was enough. Dichloromethane gave the best results, and no significant differences were observed between 2 and 5 ml. Therefore, 2 ml of dichloromethane was chosen as the optimal elution volume. In these conditions, collection efficiencies ranged from 75 to 100% for all the compounds, with relative standard deviations (*n*=3) lower than 9%.

3.2. Selective SFE extraction

A two-step SFE procedure was used in order to study the applicability of this technique to achieve a selective separation of oxy- and nitro-PAHs from other compounds present in the urban air particulates, such as aliphatic hydrocarbons and PAHs. To extract the less polar compounds pure CO₂ was used as SFE fluid, whereas for oxy- and nitro-PAHs toluene-modified CO₂ was tested. For this purpose, experiments using different spiked materials were performed.

Initially, materials expected to show a low degree of interaction with oxy- and nitro-PAHs were used. For this purpose, anhydrous Na₂SO₄ and glass-fibre filters were spiked with 1 ml of a solution containing 200 ng g⁻¹ of these compounds in *n*-hexane, keeping the analytes and the matrix in contact for 12 h at 4 °C. Both spiked matrices were subjected to a two-step SFE procedure, consisting on a first extraction with pure CO₂ and a second consecutive step with toluene–CO₂ (5:95, v/v). SFE conditions for both steps were the same as described in Section 3.1, and the final extracts were analysed by GC–ECD. However, under these conditions the oxy- and nitro-PAHs were mainly extracted in the first step using pure CO₂. In the second SFE step, percentages over the total amount extracted were lower than 15%. This fact can be explained by the low degree of interaction between the analytes and the materials tested. Due to the lack of urban particulate reference materials with certified values for oxy- and nitro-PAHs, experiments with spiked urban aerosol samples were performed. The aim of these experiments was to use a material with a behaviour similar to real samples and subsequently find the SFE conditions for the extraction of the oxy- and nitro-PAHs in the second SFE step.

In order to have a pooled and homogeneous urban aerosol sample without detectable quantities of the analytes under study, several samples were collected, ground, pooled and extracted twice with CO₂–toluene. This sample was analysed by GC–ECD to assure that no compounds of interest were present. The resulting materials were spiked with the analytes, incubated for 12 h, and subjected to the two-step SFE method as described previously. In order to check the effect of the spiking solvent, different

volumes (0.5–2 ml) of *n*-hexane and dichloromethane (DCM) were tested. The highest recoveries for the oxy- and nitro-PAHs extracted in the second SFE step were obtained using 0.5 ml of DCM, because, unlike DCM, the complete evaporation of *n*-hexane during the incubation process was not accomplished and the remaining solvent could act as a modifier in the first SFE step with pure CO₂. This fact could favour the extraction of certain amounts of oxy- and nitro-PAHs that should be retained in the first SFE step. The time of contact between the matrix and the spiked analytes was also studied (12, 24 and 48 h), and a significant improvement on the results was observed up to 24 h. Table 2 shows the recoveries obtained for all the analytes in these conditions. As shown, the total recoveries ranged from 70 to 93%. The percentages of oxy- and nitro-PAHs extracted in the second SFE step were higher than those obtained with spiked Na₂SO₄ or glass-fibre filters, and ranged from 25 to 46%.

To check whether this material reproduced the analyte–matrix interactions of real samples, a non-spiked urban aerosol was extracted under the same SFE conditions described previously and the extracts corresponding to both SFE steps were analysed by GC–ECD. The percentages obtained in the second SFE step for the detected compounds were compared with those obtained for the spiked samples. As shown in Table 2, no significant differences were obtained between spiked and non-spiked samples. Nevertheless, the recoveries of oxy- and nitro-PAHs for the second SFE step were still low, and experiments were performed in order to find softer conditions for the first SFE step (lower pressure and temperature) to allow the analytes to remain in the matrix while the less polar compounds were extracted. For these studies, the same two-step SFE procedure was applied to real samples except for the conditions of pressure and temperature of the first extraction step. In this case, temperature was lowered to 45 °C, whereas pressure was set to 350, 250 and 150 atm, respectively. Table 3 shows the percentages over the total amount extracted obtained in the second SFE step (toluene-modified CO₂) for each analyte. As can be seen, these values progressively increased as pressure used in the first step was reduced. When temperature and pressure were 45 °C and 150 atm, respectively, percentages of oxy- and

Table 2

Recoveries obtained for two-step SFE extraction of spiked urban aerosol samples (spiking conditions: 0.5 ml dichloromethane, incubation time 24 h)

Compound	Spiked samples			Non-spiked samples
	Total recovery (%)	RSD ^a (%)	Second SFE step ^b (%)	Second SFE step ^b
1,4-Naphtoquinone	84	4	25	n.d.
2-Methyl-1-nitronaphthalene	68	6	29	n.d.
2-Nitronaphthalene	70	7	26	n.d.
9-Fluorenone	76	7	25	28
Acenaphthenequinone	86	3	27	n.d.
9,10-Anthraquinone	70	7	33	37
1,5-Dinitronaphthalene	89	8	29	n.d.
2-Methyl-9,10-anthraquinone	78	3	28	26
2-Nitrofluorene	93	5	40	n.d.
9-Nitroanthracene	89	6	32	n.d.
Benzanthrone	70	5	37	35
Benz[<i>a</i>]anthracene-7,12-dione	78	5	46	48
1-Nitropyrene	72	7	42	39

n.d., not detected.

^a *n* = 3.

^b Percentages over the total amount extracted.

nitro-PAHs obtained in the second step were high, ranging between 87 and 98% of the total extracted quantities, and these conditions were selected as optimum for the first SFE step.

The extracts obtained in the first step using pure CO₂ were analysed by GC–MS in order to identify the non-polar compounds removed from the matrix. This fraction contained mainly aliphatic hydrocarbons and certain amounts of PAHs. The GC–MS chromatogram of these extracts (Fig. 1) showed a typical profile consisting of a broad unresolved band of aliphatic hydrocarbons and series of intense peaks identified as high-molecular-mass alkanes from C₁₆

to C₃₆. In addition, several PAHs were identified in these extracts, including benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, benzo[*a*]anthracene, pyrene, fluoranthene, anthracene and phenanthrene. In this fraction, PAHs were found at levels ranging between 10 and 20% of the total amount extracted for the whole two-step SFE procedure. The extracts obtained in the second SFE step (using toluene-modified CO₂) contained the PAHs mentioned above (between 80 and 90% of the total) and the oxy- and nitro-PAHs. No aliphatic hydrocarbons were found in this fraction.

In summary, the optimised two-step SFE pro-

Table 3

Percentages extracted in the second SFE step (toluene–CO₂, 5:95, v/v) at different pressure conditions

Compound	Pressure in the first extraction step (pure CO ₂)					
	350 atm		250 atm		150 atm	
	Mean	RSD ^a (%)	Mean	RSD ^a (%)	Mean	RSD ^a (%)
9-Fluorenone	39	12	51	10	87	10
9,10-Anthraquinone	45	10	52	8	90	7
2-Methyl-9,10-anthraquinone	40	9	52	9	98	6
Benzanthrone	48	11	61	9	92	8
Benz[<i>a</i>]anthracene-7,12-dione	56	11	67	9	99	9
1-Nitropyrene	53	12	68	11	98	9

^a *n* = 3.

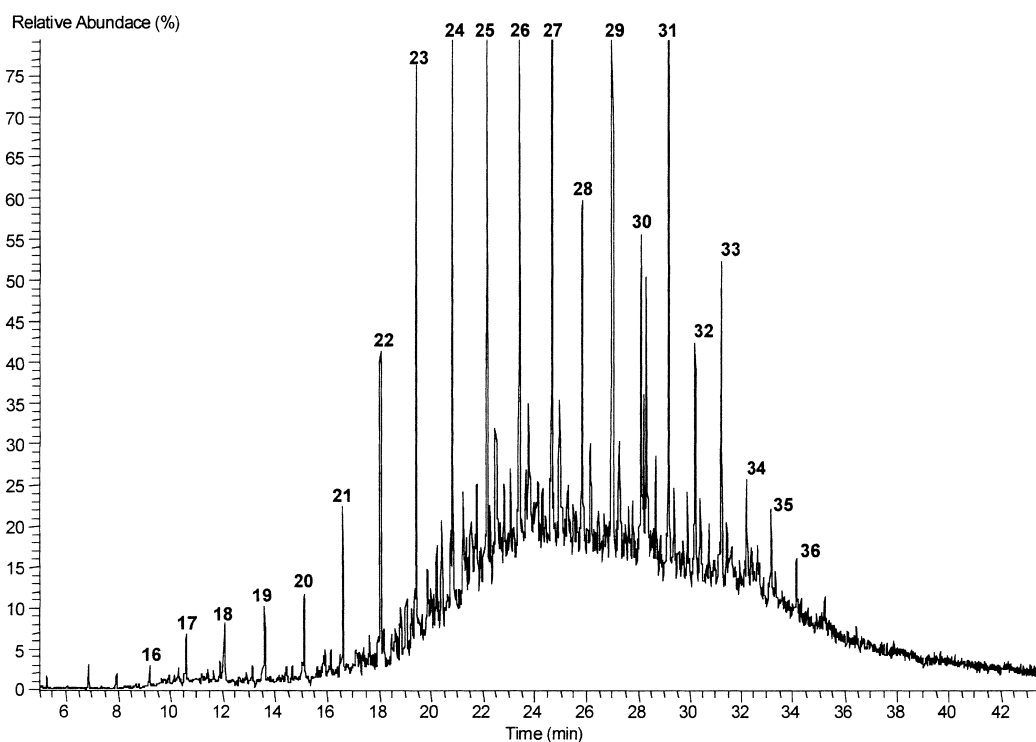


Fig. 1. GC–MS chromatogram of the SFE extracts corresponding to the pure CO₂ SFE step. The intense peaks correspond to *n*-alkanes with the indicated number of carbon atoms.

cedure consisted of a first pure CO₂ extraction step (150 atm, 45 °C) and a second consecutive extraction with toluene–CO₂ (5:95, v/v), at 350 atm and 90 °C. The analytes collected in the C₁₈ trap were eluted with 2 ml of dichloromethane. The sorbent was then

rinsed with 10 ml of dichloromethane and was ready for the next extraction.

In order to evaluate the applicability of the method to the analysis of oxy- and nitro-PAHs in urban aerosol samples, results with SFE were compared

Table 4
Quantitation results for oxy- and nitro-PAHs in urban aerosol samples by the proposed SFE method and ultrasonic extraction

Compound	SFE		Ultrasonic extraction		Significance level (<i>P</i> value) ^b
	Mean (pg m ⁻³)	SD ^a	Mean (pg m ⁻³)	SD ^a	
9-Fluorenone	18	3	n.d.	–	–
9,10-anthraquinone	143	14	138	11	0.144
2-Methyl-9,10-anthraquinone	213	19	174	26	0.105
Benzanthrone	241	29	210	18	0.196
Benz[<i>a</i>]anthracene-7,12-dione	59	12	77	16	0.195
1-Nitropyrene	67	9	81	7	0.107

n.d., not determined due to interference.

^a *n* = 3.

^b Significant differences between methods for *P* < 0.05 (at the 95% confidence level).

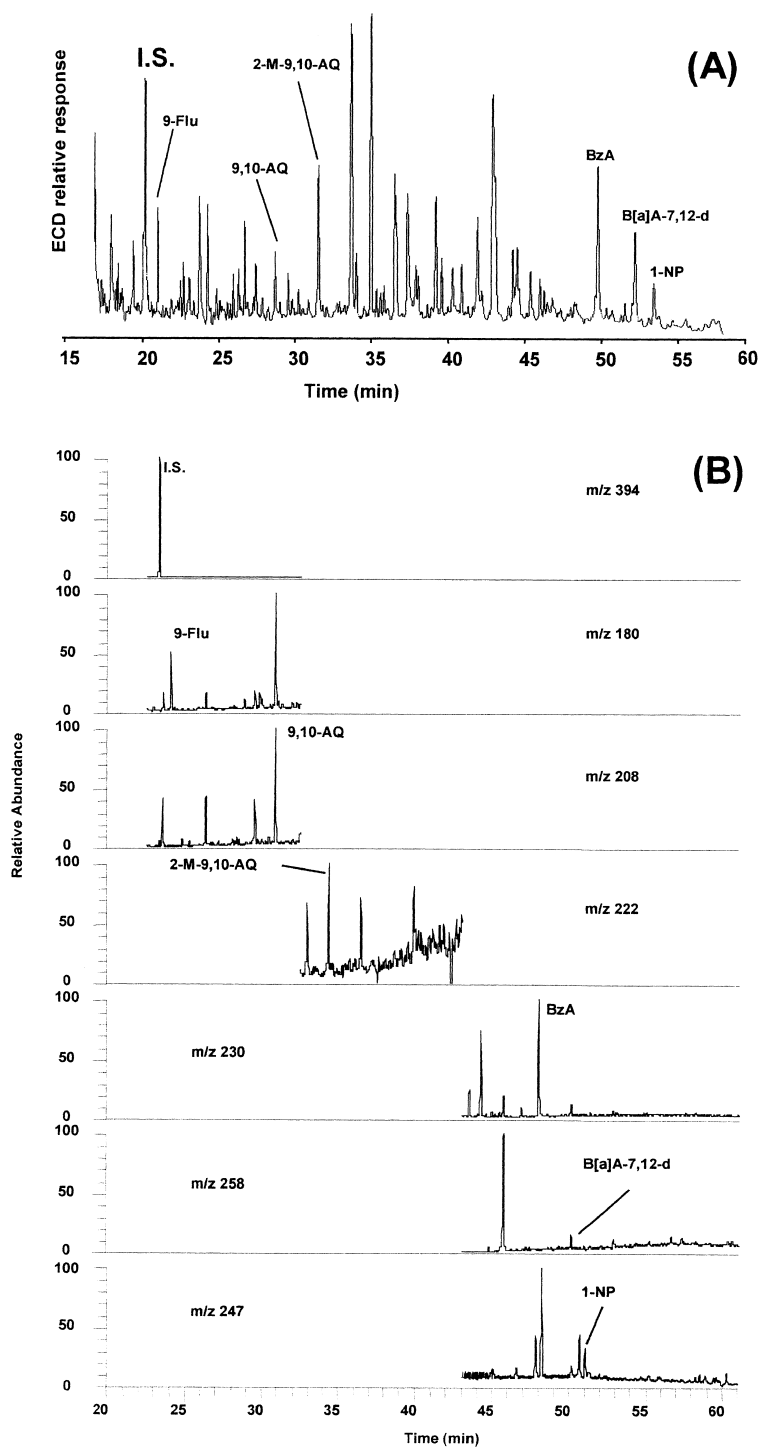


Fig. 2. (a) GC-ECD and (b) GC-MS-SIM chromatograms of an urban aerosol sample. Peak identification: I.S., 1,2,4,5-tetrabromobenzene; 9-Flu, 9-fluorenone; 9,10-AQ, 9,10-anthraquinone; 2-M-9,10-AQ, 2-methyl-9,10-anthraquinone; BzA, benzanthrone; B[a]A-7,12-d, benzo[a]anthracene-7,12-dione; 1-NP, 1-nitropyrene.

Table 5

Concentrations in air of oxy- and nitro-PAHs found in urban aerosol samples, collected during winter and summer of 2001 in Barcelona

Compound	Concentration ^a (pg m ⁻³)			
	Winter		Summer	
	GC-ECD	GC-MS	GC-ECD	GC-MS
9-Fluorenone	21±4	28±3	9±3	15±4
9,10-Anthraquinone	310±27	65±14	n.d.	23±5
2-Methyl-9,10-anthraquinone	218±21	184±15	260±19	144±12
Benzo[a]anthracene	337±31	364±21	106±14	127±11
Benz[<i>a</i>]anthracene-7,12-dione	132±12	112±10	51±14	66±11
1-Nitropyrene	104±10	98±7	24±4	23±3

n.d., not detected.

^a Concentrations expressed as: mean value±standard deviation (*n*=3).

with those obtained using a conventional technique such as ultrasonic extraction, which has often been used for the extraction of these compounds [14,19,46]. For this purpose, several samples were collected, pooled and extracted using both methods, and the extracts were analysed by GC-ECD. Chromatograms of the extracts corresponding to the second SFE step, which contained the oxy- and nitro-PAHs, were clean enough and any purification step had to be performed before injection into the GC-ECD system. In contrast, clean up was necessary when ultrasonic extraction was used. Results obtained with both methods are given in Table 4. The significance of the mean values was studied statistically using Student's *t*-test or Cochran's test when unequal variances (*F*-test) were obtained. As can be seen, no significant differences were observed between both methods for the detected compounds, except for 9-fluorenone, which showed an important interference when ultrasonic extraction was used. Therefore, the proposed SFE method can be successfully applied to the analysis of oxy- and nitro-PAHs in urban particulate matter. This method has the advantage that no clean up step has to be performed, in contrast to conventional and less selective techniques such as ultrasonic extraction.

3.3. Analysis of urban aerosol samples

The proposed method was applied to the analysis of oxy- and nitro-PAHs in urban aerosol samples collected during winter and summer of 2001 in Barcelona (Spain). The extracts corresponding to the second SFE step were analysed by GC-ECD and

GC-MS. As an example, Fig. 2 shows the GC-ECD and GC-MS-SIM chromatograms of an urban aerosol sample collected in winter, where 9-fluorenone, 9,10-anthraquinone, 2-methyl-9,10-anthraquinone, benzo[a]anthracene, benz[*a*]anthracene-7,12-dione and 1-nitropyrene were identified. As expected, a higher selectivity in the detection of the compounds of interest was achieved using GC-MS. Concentrations in air (pg m⁻³) for the identified compounds are given in Table 5. The values presented agree with those reported in the literature, which are in the range of approximately 10–100 pg m⁻³ for the nitro-PAHs [21,29–35] and up to 1 ng m⁻³ for the oxy-PAHs [29,36,37].

In some cases, for 9,10-anthraquinone in winter and 2-methyl-9,10-anthraquinone in summer, higher results were obtained with GC-ECD than using GC-MS. This is probably due to the presence of compounds that interfered in the ECD. On the other hand, a general decrease in the concentrations of oxy- and nitro-PAHs in the samples collected in summer was observed. This is due to the high temperatures that increase the degree of volatilisation of the compounds from the particles to the atmosphere.

4. Conclusions

The suitability of a two-step SFE procedure for the analysis of oxy- and nitro-PAHs in urban aerosol samples has been demonstrated. The proposed method is based on a first SFE extraction step using pure CO₂ as extraction fluid (at 150 atm and 45 °C), in

which non-polar compounds such as aliphatic hydrocarbons and some PAHs present in the matrix are removed. A second consecutive extraction step with toluene–CO₂ (5:95, v/v; at 350 atm and 90 °C), allowed the effective extraction of oxy- and nitro-PAHs. The applicability of pre-extracted urban aerosol samples spiked with the analytes to reproduce the matrix–analyte interactions observed in real samples has been demonstrated. An evaluation of the SFE method has been performed by comparison of the results with those obtained with a conventional procedure such as ultrasonic extraction. Similar results were obtained, but with the advantage that no clean up step was needed for the SFE extracts.

The developed two-step SFE method has been successfully applied to the analysis of oxy- and nitro-PAHs in urban aerosol samples collected in Barcelona. The SFE method can be proposed as an alternative to conventional extraction techniques such as Soxhlet or sonication, which involve laborious clean up procedures.

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