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# Determination of organic molecular markers in marine aerosols and sediments: one-step flash chromatography compound class fractionation and capillary gas chromatographic analysis

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## Abstract

*n*-Alkanes, polycyclic aromatic hydrocarbons (PAHs), *n*-alkanals, *n*-alkan-2-ones, long-chain alkenones, oxy-PAHs, *n*-alkanols, sterols and free alkanolic acids have been reliably determined in various environmental matrices by using one-step flash chromatography compound class fractionation and subsequent gas chromatography (GC)–MS and/or GC–flame ionization detection analysis. The obtained recoveries, for the whole analytical procedure were 62–98% for *n*-alkanes, 60–100% for PAHs, 75% for *n*-alkanals, 96–100% for nitroarenes, 65–73% for oxy-PAHs, 88–98% for *n*-alkanols, 80–89% for steroidal alcohols and 98% for alkanolic acids. This analytical protocol has been applied, and proved suitable, for the determination of more than 170 organic compounds in samples, such as aerosols and marine sediments, collected during a 1 year period in a coastal environment of Eastern Mediterranean. © 1998 Elsevier Science B.V.

**Keywords:** Aerosol analysis; Marine sediments; Sediments; Environmental analysis; Alkanes; Polynuclear aromatic hydrocarbons; Carbonyl compounds; Alkanols; Sterols; Organic acids

## 1. Introduction

Atmospheric transport and atmospheric deposition processes have been considered to be the major pathways for the introduction of land-derived natural and anthropogenic organic species in the open ocean [1]. Long-chain *n*-alkanes (of higher plant, marine and petrogenic origin), *n*-alkanols (of higher plant and marine origin), *n*-alkanals (of higher plant and photochemical origin), *n*-alkan-2-ones, free *n*-alkanoic acids,  $\alpha,\omega$ -dicarboxylic acids (of higher plant origin and/or photooxidation products of anthropogenic cyclic olefins and biogenic unsaturated fatty

acids), polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons from anthropogenic sources have been detected in remote marine aerosols [2–9]. In addition to atmospheric transport, dry and wet deposition both play an important role as sources of various natural and anthropogenic organic species introduced to the marine environment [10–14]. Thus, the reliable determination of specific molecular markers, used as source tracers in various environmental samples (e.g. atmospheric aerosols and marine sediments), is a subject of intensive research as it contributes to the understanding of the biogeochemical cycle of organic matter in open marine areas.

Various analytical methods for the determination

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of semi- and nonvolatile organic compounds in environmental samples have been reported in the literature [1–18]. By following most of these procedures, the total organic extract is saponified by KOH or transesterified by  $\text{BF}_3$  in methanol. The esterified fatty acids together with the neutral compounds, are fractionated into compound classes of different polarity using column liquid chromatography or thin-layer chromatography. Taking into consideration that the concentrations of various polar compounds differ often dramatically from each other, their quantification can be unreliable, if found in the same fraction. These concentration ranges differ especially when the polar compounds, such as alkanolic acids, *n*-alkanols, sterols, *n*-alkanals and *n*-alkan-2-ones, are considered. In most published analytical methods [3–6,16,18] the above compounds were collected at the same fraction, derivatized and then analysed. Possibly this is one of the reasons that *n*-alkanals and *n*-alkan-2-ones, generally in lower concentrations than alkanolic acids and *n*-alkanols, were only tentatively identified, and rarely quantified [3–6,16,18].

The purpose of this work was to establish a single analytical procedure, which can be applied for the analysis of specific molecular markers in various environmental samples. Such an analytical approach is useful for the study of the physicochemical and biological processes responsible for the fate of organic compounds during long-range atmospheric transport, wet and dry deposition on the sea surface, settling in the water column, and final incorporation in the deep sediments. In this article we present an improved (for comparison see Ref. [17]) flash chromatographic fractionation, in which the total extractable organic matter from various environmental samples, is fractionated by one-step silica gel flash chromatography into very distinct nonpolar, semipolar, polar and acidic compound classes. After derivatization of hydroxyl and carboxylic compounds, capillary gas chromatography, with flame ionisation detection (GC–FID) or mass spectrometry (GC–MS), was used for the identification and quantitation of more than 170 individual compounds. The molecular markers studied were chosen because of their significance as tracers for organic terrestrial, marine matter and as well as for their significance as pollution indicators. In this study we also included

the analysis of other organic compounds of environmental, geochemical and paleoclimatic significance such as compounds formed by photoinduced reactions of parent PAHs like nitroarenes and oxy-PAHs, steroidal alcohols and long-chain alkenones. This method proved to be extremely selective for reliable determination of very low compound concentrations in different environmental samples, such as aerosols and marine sediments, and allows the simultaneous study of both the atmospheric transport phenomenon and deposition of specific molecular markers in the marine environment using the same analytical protocol.

## 2. Experimental

### 2.1. Materials

All solvents were purchased from Merck (Suprasolv; Darmstadt, Germany). Standard compounds were obtained from Ehrenstorfer (Augsbourg, Germany). *n*-Alkanals were prepared by oxidation of the corresponding *n*-alkanols. Silica gel (0.040–0.063 mm) was also from Merck. Soxhlet thimbles and glass fibre filters were obtained from Whatman (Maidstone, UK).

All materials used (silica gel, glass and cotton wool, paper filters, anhydrous sodium sulphate etc.) were Soxhlet extracted with methanol–acetone (50:50) overnight, and twice with methylene chloride for 24 h, and kept dry (in desiccator) until use. Glass and quartz fibre filters were cleaned at 400°C and 550°C overnight, respectively. All glassware was cleaned by heating at 55°C, and rinsed with the applied organic solvent just before use.

### 2.2. Fractionation, derivatization and identification

The standard compounds, about 10 ng each, were applied on quartz fibre filters dissolved in methylene chloride (it corresponds to an air concentration of 4  $\mu\text{g}/\text{m}^3$  and a sediment concentration of 0.2 ng/g). Methylene chloride was evaporated. The compounds were extracted from the filters, cut into small pieces, in a flask by refluxing methylene chloride–methanol (4:1, v/v) for 20 h. The total extractable organic matter containing neutral and acidic compounds,

were evaporated by the Kuderna–Danish method, transferred to a 1 ml vial, and further evaporated by a gentle nitrogen stream. Then the extract was fractionated into individual compounds classes by flash chromatography on silica gel as follows:

The mixture of standard compounds was dissolved in a small aliquot of *n*-hexane and applied on the top of a 30×0.7 cm column, containing 1.5 g of silica gel (Merck, 0.040–0.063 mm, activated at 150°C for 3 h). Nitrogen pressure was used in order to obtain a flow of 1.4 ml/min at the bottom of the column. The following solvent systems were used to elute the different compound classes: (1) 15 ml *n*-hexane (fraction F1, aliphatics); (2) 15 ml toluene–*n*-hexane (5.6:9.4) (fraction F2, PAHs and nitro-PAHs); (3) 15 ml *n*-hexane–methylene chloride (7.5:7.5) (fraction F3, carbonyl compounds such as *n*-alkanals, *n*-alkan-2-ones, long-chain alkenones and oxy-PAHs); (4) 20 ml ethyl acetate–*n*-hexane (8:12) (fraction F4, *n*-alkanols and sterols); and (5) 20 ml solution of (4%, v/v) pure formic acid in methanol (fraction F5, free fatty acids).

*n*-Alkanols and steroidal alcohols were derivatized to the corresponding trimethylsilyl ethers prior to GC–FID and GC–MS analysis, as follows: the fraction containing the *n*-alkanols and sterols was initially evaporated in a rotary evaporator until about 1 ml volume, and then under a gentle nitrogen stream to dryness; 100 µl of N,O-bis-(trimethylsilyl)trifluoroacetamide and 100 µl of iso-octane were added; the reaction mixture was shaken vigorously for 1–2 min and allowed to stand for 1 h at 80°C. After that, the reaction mixture was evaporated under nitrogen to dryness. The last fraction containing the free alkanolic acids (fraction F5) was methylated with freshly distilled diazomethane, and the corresponding methyl esters were analysed by GC–FID and GC–MS.

A scheme of the whole analytical procedure is presented in Fig. 1.

The individual fractions were spiked with internal standards (1-chlorohexadecane for *n*-alkanes, *n*-alkanals and *n*-alkan-2-ones, *n*-alkanol silyl ethers and for fatty acid methyl esters; cholestane for sterol silyl ethers; hexamethylbenzene for PAHs and *n*-hexacosane and long-chain alkenones) for quantitative determinations.

Relative response factors, in both GC–FID and

GC–MS in selected ion monitoring (SIM) mode, were calculated for 3–10 standard compounds, representing each compound class, of increasing molecular mass. Relative response factors for PAHs were calculated for each single compound individually.

Control of procedural blanks has been performed to assess possible contamination. The total blank weight never exceeded 2% of the individual sample extracts (except for the *n*-alkanoic acids, where the maximum contamination represented 10% of the total fraction extract, especially for the homologues C<sub>14</sub>, C<sub>16</sub> and C<sub>18</sub>). The contaminants were characterised by GC–MS analysis and comparison with standard mixtures. The most frequent contaminants were phthalate esters. All quantities given here were corrected, taking into consideration the application of the analytical methodology with standard compounds (column chromatography performance and relative response factors in GC–FID and GC–MS in SIM mode).

Compound identification was performed by GC–MS analysis and coinjection with authentic standard compounds or compound mixtures (PAHs, oxy-PAHs, nitro-PAHs).

### 2.3. GC–MS and GC–FID

The GC–MS analyses were carried out on a Hewlett–Packard mass-selective detector with the appropriate data system. A Hewlett–Packard Model 5890 GC equipped with a Grob-type split–splitless injector, was directly coupled with the fused-silica capillary column (HP-5 MS with 0.25 µm film thickness, 30 m×0.25 mm I.D.), to the ion source. Helium was used as the carrier gas with a back pressure of 55 kPa. The electron impact ionisation mode conditions were the following: ion energy 70 eV; ion source temperature 195°C; mass range 35–590 *m/z* or in the SIM ion monitoring mode for quantitative determinations; electron multiplier voltage 1700–1800 V.

The GC–FID analyses were performed on a Hewlett–Packard GC, Model 5890 with Hewlett–Packard Chemstation data system, fitted with an on-column injector, and a fused-silica capillary column coated with HP-5 (0.17 µm film thickness, 50 m×0.32 mm I.D.). Helium was used as carrier gas with a back pressure of 80.5 kPa.

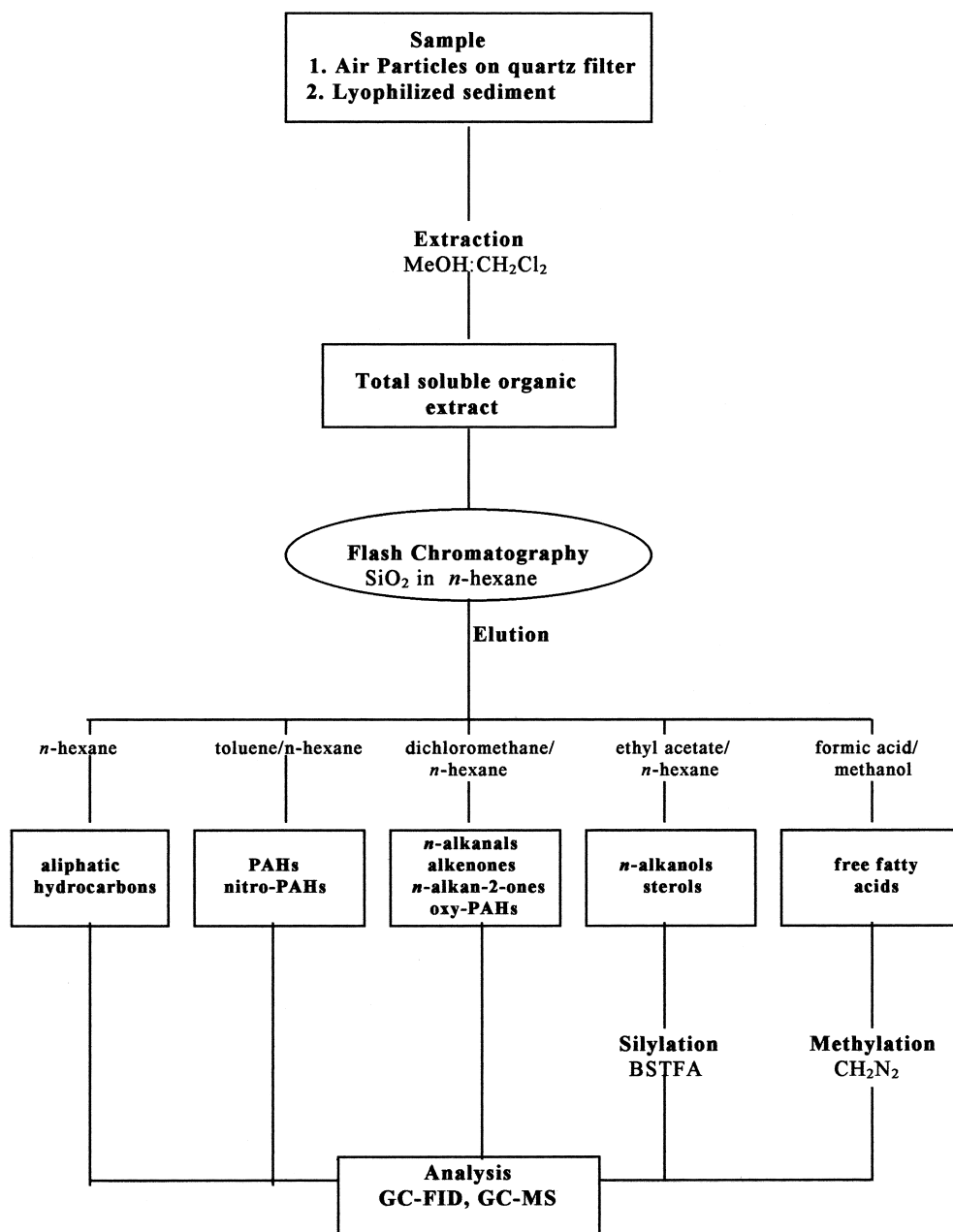


Fig. 1. Scheme of the entire analytical procedure.

The chromatographic conditions were the following. GC-MS (temperature program A): injector temperature 270°C; temperature program, 70°C (1 min), 70–150°C (10°C/min), 150–290°C (5°C/min), and 290°C (30 min); 1 µl of each sample was

injected, in the splitless mode (split valve closed for 35 s), and the hot needle technique. GC-FID (temperature program B): detector temperature 290°C; temperature program, 40°C (1 min); 40–150°C (10°C/min) and 150–290°C (5°C/min), and 290°C

(30 min). GC–FID for the long-chain alkenones (temperature program C): detector temperature 300°C; temperature program, 50°C (1 min); 50–220°C (25°C/min), 220–300°C (5°C/min), and 300°C (30 min).

#### 2.4. Sampling

Airborne particles were collected on 20×25 cm quartz fibre filters, having a collection efficiency higher than 99% for particles with radius higher than 0.3 µm at the 68–80 m<sup>3</sup>/h flow-rate used. Filters were mounted in a high volume air sampling system (Model GMWL-2000; General Metals Works, OH, USA). Samples were collected for 18–24 h (1500–2000 m<sup>3</sup> air sampled) on a 15 m high building situated in a rural coastal area of Eastern Mediterranean [Finokalia, north coast of the Island of Crete (N35° 20', E25° 42'), Greece]. The samples were stored frozen (–30°C), in order to avoid microbial reworking of organic matter, in precleaned glass flasks sealed with PTFE tape and covered with aluminium foil. Compound isolation and determination from samples was performed as above.

Marine sediment cores, corresponding to six different water column depths (100 m, 200 m, 540 m, 700 m, 940 m and 1540 m) and two different transects, were collected with the use of a Multicorer sampler on a seasonal basis (May and September 1994). The sampling area has been chosen in the north coast of Crete, close to the aerosol sampling station. The undisturbed upper part (ca. 1 cm) of each core was sealed with aluminium foil, frozen at –30°C on board ship (to avoid bacterial reworking), and later freeze-dried.

### 3. Results and discussion

In Table 1 are shown the recoveries for each compound class, when standard compounds are used for the assessment of the efficiency of the overall analytical protocol (Fig. 1) including extraction, fractionation and derivatization steps. Tables 2 and 3 summarise the results of the application of this analytical protocol for the determination of various molecular markers of environmental, geochemical

Table 1

Mean values (threefold measurements with standard deviations <±3%) of recoveries for molecular markers representative for the main compound classes determined in rural and urban aerosols and marine sediments; the recoveries correspond to the entire analytical procedure

Compound class	Recoveries (%)
I. <i>n</i> -Alkanes	
C <sub>19</sub>	61.54
C <sub>24</sub>	95.28
C <sub>26</sub>	98.23
II. PAHs	
Phenanthrene	60.28
Methylphenanthrene	61.32
Dimethylphenanthrene	63.54
Chrysene	100.00
Benzo[ <i>a</i> ]pyrene	96.21
Perylene	98.51
Benzo[ <i>ghi</i> ]perylene	100.00
III. <i>n</i> -Alkanals	
C <sub>22</sub>	75.22
IV. <i>n</i> -Alkanols	
C <sub>16</sub>	88.15
C <sub>18</sub>	95.91
C <sub>22</sub>	97.69
V. Sterols	
Cholest-5-en-3β-ol	80.40
5α-Cholestan-3β-ol	88.63
24-Ethylcholesta-5,22-dien-3β-ol	78.45
24-Ethylcholest-5-en-3β-ol	81.63
VI. Fatty acids	
C <sub>19</sub>	98.25
VII. Nitroarenes	
9-Nitroanthracene	96.34
1-Nitropyrene	98.46
9-Nitrochrysene	100.00
VIII. Oxy-PAHs	
4H-Cyclopenta[ <i>def</i> ]phenanthren-4-one	72.64
6H-Benzo[ <i>cd</i> ]pyren-6-one	64.75

and paleoclimatic significance in atmospheric particles and marine sediments of the same area.

In Fig. 2 are shown a representative GC–FID chromatogram of a sediment aliphatic hydrocarbons fraction (Fig. 2A) and a characteristic ion chromatogram of the corresponding PAH fraction (Fig. 2B). Fig. 3 shows a representative ion chromatogram of

Table 2  
Quantitative results of the analyses of rural and urban aerosols

I. Aliphatics	
Homologues range; $C_n$ max	( $C_{15}$ – $C_{40}$ ); $C_{29}$ , $C_{31}$
Concentration ( $\text{ng}/\text{m}^3$ )	5.09–35.24
CPI ( $C_{21}$ – $C_{36}$ )	1.26–6.49
UCM/NA	0.0–4.91
Pristane ( $\text{ng}/\text{m}^3$ )	0–0.51
Phytane ( $\text{ng}/\text{m}^3$ )	0–0.18
II. PAHs	
Concentration ( $\text{ng}/\text{m}^3$ )	0.07–2.0
CPAHs/TPAHs	0.85 $\pm$ 0.08
MP/P	0.82 $\pm$ 0.74
BA/BA+CT	0.15 $\pm$ 0.05
BeP/BeP+BaP	0.82 $\pm$ 0.10
Fl/Fl+Py	0.65 $\pm$ 0.11
IP/IP+BgP	0.47 $\pm$ 0.12
III. <i>n</i> -Alkanals	
Homologues range; $C_n$ max	( $C_{15}$ – $C_{30}$ ); $C_{26}$ , $C_{28}$
Concentration ( $\text{ng}/\text{m}^3$ ); CPI	0.9–16.85; 2.34–15.84
IV. Alkanones	
(1) <i>n</i> -Alkan-2-ones	
Homologues range; $C_n$ max	( $C_{10}$ – $C_{31}$ ); $C_{29}$ , $C_{27}$
Concentration ( $\text{ng}/\text{m}^3$ ); CPI	0.4–2.1; 2.2–6.2
(2) IK ( $\text{ng}/\text{m}^3$ )	0–3.02
V. <i>n</i> -Alkanols	
Homologues range; $C_n$ max	( $C_{12}$ – $C_{30}$ ); $C_{26}$ , $C_{28}$
Concentration ( $\text{ng}/\text{m}^3$ ); CPI	2.74–94.50; 11.28–60.68
VI. Fatty acids	
(1) Saturated	
Homologues range; $C_n$ max	( $C_8$ – $C_{32}$ ); $C_{16}$ , $C_{18}$
Concentration ( $\text{ng}/\text{m}^3$ ); CPI	22.06–112.63; 2.12–6.14
(2) Unsaturated	
Homologues $C_{n:1}$ ; $C_n$ max	$C_{16:1}$ , $C_{18:1}$ ; $C_{18:1}$
Concentration ( $\text{ng}/\text{m}^3$ )	0.0–1.31
(3) Oxo- and di-acids	
Concentration ( $\text{ng}/\text{m}^3$ )	2.22–10.36
VII. Oxy-PAHs	
(in urban aerosols)	
PAKs ( $\text{ng}/\text{m}^3$ )	0.4–1.9
PAQs ( $\text{ng}/\text{m}^3$ )	0.3–4.0
Carboxyaldehydes ( $\text{ng}/\text{m}^3$ )	2.1–4.4

CPI (carbon preference index), odd-to-even for *n*-alkan-2-ones and even-to-odd for the other compound classes; UCM, unresolved complex mixture; NA, *n*-alkanes; CPAHs/TPAHs, concentrations of nine major nonalkylated compounds to the total concentration of PAHs; MP/P, ratio methyl-phenanthrenes to phenanthrene; BA, benzo[*a*]anthracene; CT, chrysene, triphenylene; BeP, benzo[*e*]pyrene; BaP, benzo[*a*]pyrene; Fl, fluoranthene; Py, pyrene; IP, indeno[1,2,3-*cd*]pyrene; BgP, benzo[*ghi*]perylene; IK, 6,10,14-trimethylpentadecan-2-one; PAKs, polycyclic aromatic ketones; PAQs, polycyclic aromatic quinones.

oxy-PAHs, fraction F3, from an urban aerosol. Fig. 4 contains ion chromatograms for *n*-alkanals ( $m/z$  82) and *n*-alkan-2-ones ( $m/z$  59) (Fig. 4A) and a portion of a gas chromatogram (with FID) for the long-chain alkenones (Fig. 4B) of fraction F3 of a marine sediment extract. The chromatogram of silyl ethers of *n*-alkanols and sterols of the F4 fraction of a marine sediment extract is shown in Fig. 5. Fig. 6 presents gas chromatograms of the methyl esters of carboxylic acids (F5 fraction) determined in an aerosol sample (Fig. 6A) and a marine sediment (Fig. 6B).

### 3.1. Analytical performance

The efficiency of the whole procedure (extraction, fractionation and derivatization) is very satisfactory, if one considers the low concentrations (4  $\text{pg}/\text{m}^3$  for aerosols and 0.2  $\text{ng}/\text{g}$  for sediments) of the compounds studied. In our previous work on aerosols [17], we obtained recoveries of the same order of magnitude or lower by using a two-phase fractionation (McCarthy–Dutie column for the separation of carboxylic acids and subsequent flash chromatography fractionation of the neutrals). In this work by using a single fractionation procedure for both neutral and acidic compounds, we improved the recoveries (see mean values in Table 1; three fold measurements and standard deviation  $< \pm 3\%$ ) of higher homologues of *n*-alkanes, PAHs (a lower recovery than in Ref. [17] was observed only for phenanthrene), *n*-alkanals and *n*-alkanols. The other compound classes included in this study, such as nitroarenes, oxy-PAHs, and sterols gave also very satisfactory results (Table 1). They were well fractionated and their recoveries were high, 96–100% for nitroarenes, 65–73% for oxy-PAHs and 78–89% for sterols. This improvement of the recovery especially for the higher homologues of *n*-alkanes and *n*-alkanols is also due: (a) to the use of on-column injection technique during GC analysis and (b) especially for *n*-alkanols and free fatty acids, to the derivatization (silylation instead of acetylation and the use of diazomethane instead of solution of  $\text{BF}_3$  in methanol, respectively). The recovery of fatty acids was definitely enhanced (almost by 10%) by using the single flash chromatography procedure compared to separation with the McCarthy–Dutie column [17].

Table 3  
Quantitative results of the analyses of marine sediment samples

I. Aliphatics	
Homologues range; $C_n$ max	( $C_{15}$ – $C_{40}$ ); $C_{31}$ , $C_{29}$
Concentration (ng/g)	80.12–896.17
CPI ( $C_{21}$ – $C_{36}$ )	2.67–4.70
UCM/NA	2.37–10.00
Pristane (ng/g)	1.59–8.64
Phytane (ng/g)	0.91–10.94
II. PAHs	
Concentration (ng/g)	14.74–161.47
CPAHs/TPAHs	0.81±0.03
MP/P	1.38±0.52
BA/BA+CT	0.28±0.05
BeP/BeP+BaP	0.64±0.04
Fl/Fl+Py	0.61±0.09
IP/IP+BgP	0.61±0.04
III. <i>n</i> -Alkanals	
Homologues range; $C_n$ max	( $C_{15}$ – $C_{28}$ ); $C_{22}$ , $C_{28}$
Concentration (ng/g); CPI	6.62–29.66; 1.49–4.65
VI. Alkanones, alkenones	
(1) <i>n</i> -Alkan-2-ones	
Homologues range; $C_n$ max	( $C_{15}$ – $C_{33}$ ); $C_{29}$ , $C_{27}$
Concentration (ng/g); CPI	14–112.07; 4.16–7.39
(2) IK (ng/g)	
	3.02–28.35
(3) Me- and ethyl-alkenones	
Homologues range	( $C_{37}$ – $C_{39}$ )
Concentration (ng/g)	12.60–153.92
V. Hydroxyl compounds	
(1) <i>n</i> -Alkanols	
Homologues range; $C_n$ max	( $C_{12}$ – $C_{30}$ ); $C_{28}$ , $C_{26}$
Concentration (ng/g); CPI	118.0–1063.2; 2.77–18.06
(2) Phytol (ng/g)	
	1.35–164.66
(3) 1,15-Triacontadiol (ng/g)	
	9.81–76.40
(4) 1-Hydroxy-15-triacontanone (ng/g)	
	0.6–52.74
(5) Sterols (ng/g)	
	379.3–7691.1
Cholest-5-en-3 $\beta$ -ol (ng/g)	96.07–1353.43
24-Methylcholsta-5,22-dien-3 $\beta$ -ol (ng/g)	30.49–1143.50
24-Ethylcholest-5-en-3 $\beta$ -ol (ng/g)	73.83–654.20
24-Ethylcholsta-5,22-dien-3 $\beta$ -ol (ng/g)	20.68–418.34
Dinosterol (ng/g)	16.39–490.88
VI. Fatty acids	
(1) Saturated	
Homologues range; $C_n$ max	( $C_8$ – $C_{32}$ ); $C_{16}$ , $C_{18}$
Concentration (ng/g); CPI	1471.4–11152.5; 1.51–3.30
(2) Unsaturated	
Homologues $C_{n:1}$ ; $C_n$ max	$C_{16:1}$ , $C_{18:1}$ , $C_{22:x}$ , $C_{20:x}$ ; $C_{18:1}$
Concentration (ng/g)	109.7–1463.0
(3) Branched	

(Cont.)

Table 3. Continued

Homologues iso and anteiso	C <sub>15</sub> , C <sub>17</sub>
Concentration (ng/g)	54.9–592.5
(4) Oxo- and di-acids	
(a) Homologues range; C <sub>n</sub> max	(C <sub>6</sub> –C <sub>12</sub> ); C <sub>9</sub>
Concentration (ng/g)	4.15–59.47
(b) Homologues range; C <sub>n</sub> max	(C <sub>20</sub> –C <sub>28</sub> ); C <sub>22</sub>
Concentration (ng/g)	100.6–723.2
(5) Phytanic acid (ng/g)	3.47–61.26

CPI (carbon preference index), odd-to-even for *n*-alkanes and *n*-alkan-2-ones and even-to-odd for the other compound classes; UCM, unresolved complex mixture; NA, *n*-alkanes; CPAHs/TPAHs, concentrations of nine major nonalkylated compounds to the total concentration of PAHs; MP/P, ratio methyl-phenanthrenes to phenanthrene; BA, benzo[*a*]anthracene; CT, chrysene, triphenylene; BeP, benzo[*e*]pyrene; BaP, benzo[*a*]pyrene; Fl, fluoranthene; Py, pyrene; IP, indeno[1,2,3-*cd*]pyrene; BgP, benzo[*ghi*]perylene; IK, 6,10,14-trimethylpentadecan-2-one.

A remark should be made concerning the effect of volatility of the compounds examined, on the entire procedure efficiency. All compounds having higher volatility were less recovered than these with lower volatility, probably due to the losses during solvent removal steps.

The efficiency of our protocol can also be demonstrated when the gas chromatograms of all fractions are examined (Figs. 2–6). We obtained reliable fractionation of the different compound classes, by applying this analytical protocol at the same time to both aerosol and marine sediment samples. In our preceding article [17] we provided gas chromatograms, of all fractions, obtained by using the two-step fractionation procedure for the analysis of aerosol samples. As the GC results for aerosols, in the present study, do not differ from those obtained in our former one [17], we present here only those achieved for oxy-PAHs and carboxylic acids and as well as those obtained for marine sediments. Oxy-PAHs were included in the present study because of their importance to the study of atmospheric photochemical reactions. The ion chromatogram in Fig. 3 shows that oxy-PAHs can be efficiently fractionated in the F3 fraction of an urban aerosol extract and analysed by capillary GC–MS in the SIM mode. In this sample polycyclic aromatic ketones, polycyclic aromatic quinones and carboxyaldehydes were the most representative compounds. In rural aerosol samples only benz[*a*]anthracene-7,12-dione was determined in very low concentration (Table 2), while nitro-PAHs were not detected.

Figs. 2,4 and 5 demonstrate the efficiency of this protocol for the fractionation of the nonpolar ali-

phatic fraction (Fig. 2A) from the semipolar polycyclic aromatic fraction (Fig. 2B). The same is true for carbonyl compounds, such as *n*-alkanals and *n*-alkan-2-ones (Fig. 4), which are separated in a very distinct fraction from the more polar *n*-alkanols and sterols (Fig. 5). Also the elution with the solution of formic acid in methanol was very efficient for the separation of free fatty acids from the other lipids in both aerosol (Fig. 6A) and sediment (Fig. 6B) extracts.

In spite of the efficiency of fractionation the SIM technique was very useful to determine some molecular markers, rather difficult to identify with only GC–FID. Especially for the PAHs and oxy-PAHs, the use of SIM eliminated possible interference from other compound classes. The GC–MS analysis, in the SIM mode, of carboxylic acids (*m/z* 74), of *n*-alkanals (*m/z* 82, 96), of *n*-alkan-2-ones (*m/z* 59), and of *n*-alkanols (*m/z* 75, 103 and molecular ion) did not offer any advantage compared to their GC–FID analysis.

The analysis of the homologues of the several compound classes in aerosol and marine sediment samples, allowed the determination of compound relative distributions and concentrations. Subsequently, parameters which have been successfully used in organic geochemistry as source indicators [9], were evaluated allowing increased insight into the fate of organic matter in the marine environment. Among these parameters, one can distinguish: (I) The carbon preference index (CPI), a measure of carbon number predominance of homologous compound series, useful to estimate the biogenic or anthropogenic contribution; *n*-alkanes from epicuticular wax of higher



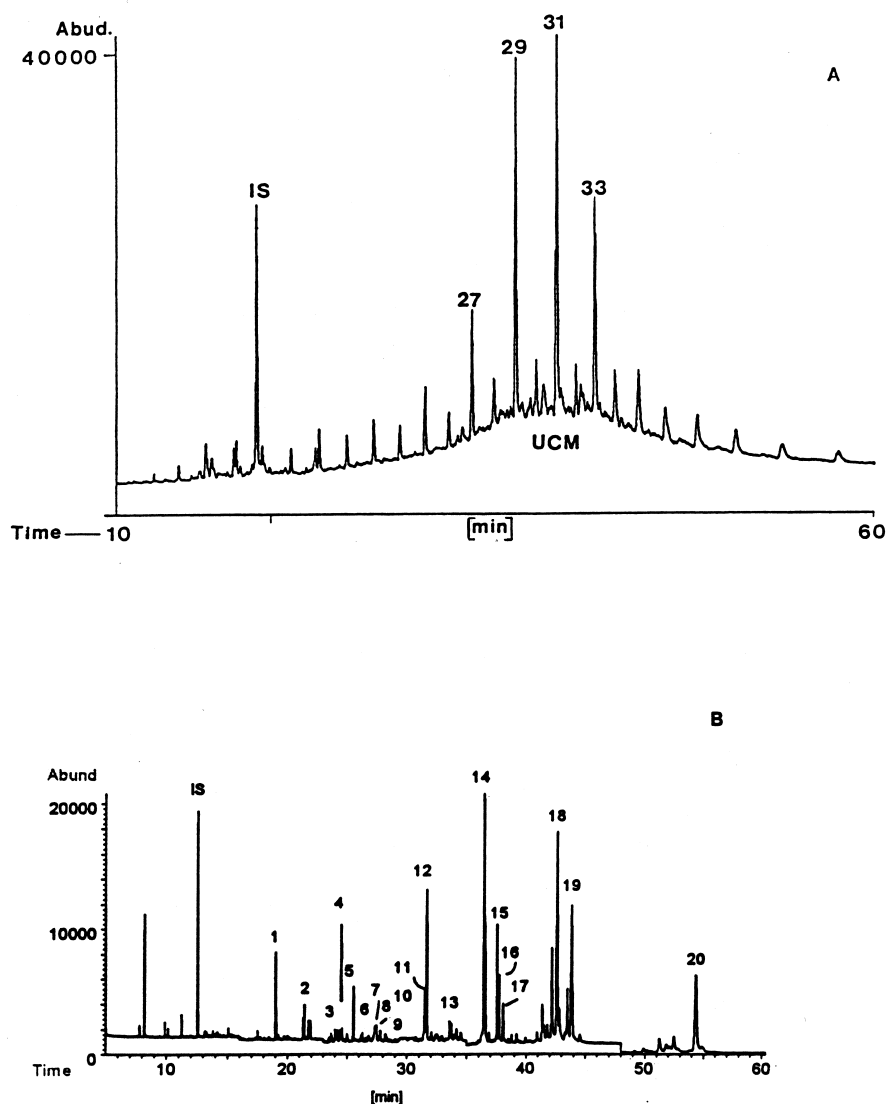


Fig. 2. (A) FID chromatogram (temperature program B) of the aliphatic hydrocarbon fraction (F1) determined in marine sediments. Numbers correspond to the carbon atoms number for the *n*-alkanes homologues; UCM, unresolved complex mixture; I.S., internal standard. (B) Selected ions chromatogram (temperature program A) of the polyaromatic hydrocarbons fraction (F2) determined in marine sediments. Compound number for individual PAHs: 1, phenanthrene ( $m/z$  178); 2, methylphenanthrene ( $m/z$  192); 3, dimethylphenanthrene ( $m/z$  206); 4, fluoranthrene ( $m/z$  202); 5, pyrene ( $m/z$  202); 6, trimethylphenanthrene ( $m/z$  220); 7, methyl-202 and benzo[*a*]fluoranthrenes ( $m/z$  216); 8, retene ( $m/z$  234); 9, dimethyl-202 ( $m/z$  230); 10, 4(H)-cyclopenta-[*cd*]-pyrene ( $m/z$  226); 11, benzo[*a*]anthracene ( $m/z$  228); 12, chrysene/triphenylene ( $m/z$  228); 13, methylchrysene ( $m/z$  242); 14, benzo[*b*]-, -[*j*]- and -[*k*]fluoranthene ( $m/z$  252); 15, benzo[*e*]pyrene ( $m/z$  252); 16, benzo[*a*]pyrene ( $m/z$  252); 17, perylene ( $m/z$  252); 18, indeno[1,2,3-*cd*]pyrene ( $m/z$  276); 19, benzo[*ghi*]fluoranthene ( $m/z$  276); 20, coronene ( $m/z$  300).

plants show a pronounced odd carbon number predominance or  $CPI > 1$  [19,20], while fossil fuel and microbial hydrocarbons exhibit a  $CPI \sim 1$ . In contrast, *n*-alkanoic acids, *n*-alkanols and *n*-alkanals from the

plant wax show an even carbon number predominance, due to their different biosynthetic pathway. (II) The unresolved complex mixture of branched and cyclic hydrocarbons, related to unburned pet-

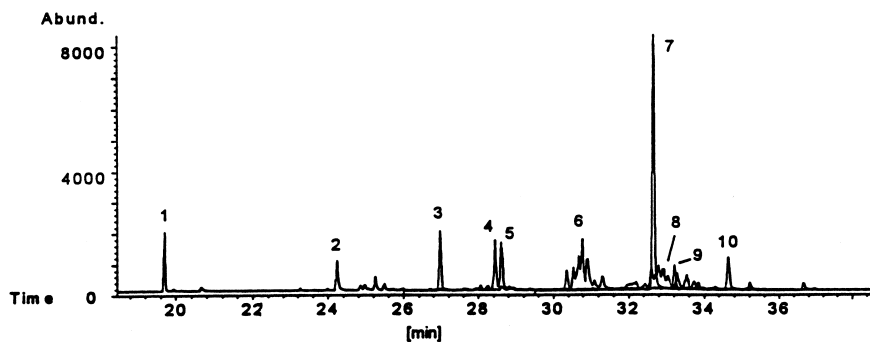


Fig. 3. Selected ions chromatogram (temperature program A) of the oxygenated polyaromatic hydrocarbons (F3) determined in urban aerosols. Compound number for individual oxy-PAHs: 1, 9H-fluoren-9-one ( $m/z$  180); 2, anthracene-9,10-dione ( $m/z$  208); 3, 4H-cyclopenta[*def*]phenanthren-4-one ( $m/z$  204); 4, anthracene-9-carboxyaldehyde ( $m/z$  206); 5, phenanthrene-9-carboxyaldehyde ( $m/z$  206); 6, Cl-phenanthrenecarboxyaldehyde ( $m/z$  220); 7, 7H-benz[*de*]anthracene-7-one ( $m/z$  230); 8, C2-phenanthrenecarboxyaldehyde ( $m/z$  234); 9, 1-pyrenecarboxyaldehyde ( $m/z$  230); 10, benz[*a*]anthracene-7,12-dione ( $m/z$  258).

rogenic hydrocarbon inputs from vehicular traffic [1,4–6]. (III) Diagnostic concentration ratios of PAHs, which have been used to reconcile their presence with potential emission sources: the ratio of the sum of concentrations of nine major nonalkylated compounds (fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*a*]fluoranthene, benzo[*a*]pyrene, benzo[*e*]pyrene, indeno[*cd*]pyrene and benzo[*ghi*]perylene) expressed as CPAHs, to the total concentration of PAHs (CPAHs/TPAHs) has often been used as a characteristic value for PAHs produced by combustion processes [21,22]. The ratio methyl-phenanthrenes to phenanthrene (MP/P), has been used for source identification of PAHs [21,22]. MP/P ratios between 1 and 8 represent evidence for enhanced mobile sources or unburned fossil organic material contribution. Ratios below 1 are typical for emissions from stationary combustion sources where fuel is burning at higher temperatures.

Diagnostic PAHs concentration ratios such as, benzo[*a*]anthracene to [benzo[*a*]anthracene+chrysene, triphenylene] (BA/BA+CT), benzo[*e*]pyrene to [benzo[*e*]pyrene+benzo[*a*]pyrene] (BeP/BeP+BaP), fluoranthene to [fluoranthene+pyrene] (Fl/Fl+Py) and indeno[1,2,3-*cd*]pyrene to [benzo[*ghi*]perylene+indeno[1,2,3-*cd*]pyrene] (IP/IP+B<sub>g</sub>P), are also used for source reconciliation [23–25].

### 3.2. Analysis of molecular markers in aerosol and marine sediment samples

#### 3.2.1. F1: aliphatic hydrocarbons

All gas chromatograms of the aliphatic fraction (e.g. Fig. 2A) were dominated by *n*-alkanes (Table 2). For the aerosol samples, the concentrations of *n*-alkanes varied from 5.09 to 35.24 ng/m<sup>3</sup>. These concentrations are similar to those measured over the Western Mediterranean [26], but higher than those measured in remote marine areas [27,28]. Sampling season and air masses origin influenced the distribution and the concentrations of *n*-alkanes in the aerosol samples of this nonurban coastal area. For the samples collected under south winds, *n*-alkanes ranged from C<sub>15</sub> to C<sub>40</sub> and showed a unimodal distribution, which maximised at C<sub>31</sub>. *n*-Alkanes in samples collected under intensive north winds and under north-west winds ranged from C<sub>15</sub> to C<sub>40</sub>, exhibited a bimodal distribution, which maximised at C<sub>17</sub> for the first mode and at C<sub>29</sub> or C<sub>31</sub> for the second one. The homologues C<sub>15</sub> and C<sub>17</sub> presumably of marine origin [29] were more abundant in the samples collected during the phytoplankton bloom (late April). The second C<sub>max</sub> for these samples apparently depends on the air mass origin. For example C<sub>29</sub> was the most abundant homologue for north-originated aerosols and C<sub>31</sub> for the south-origi-

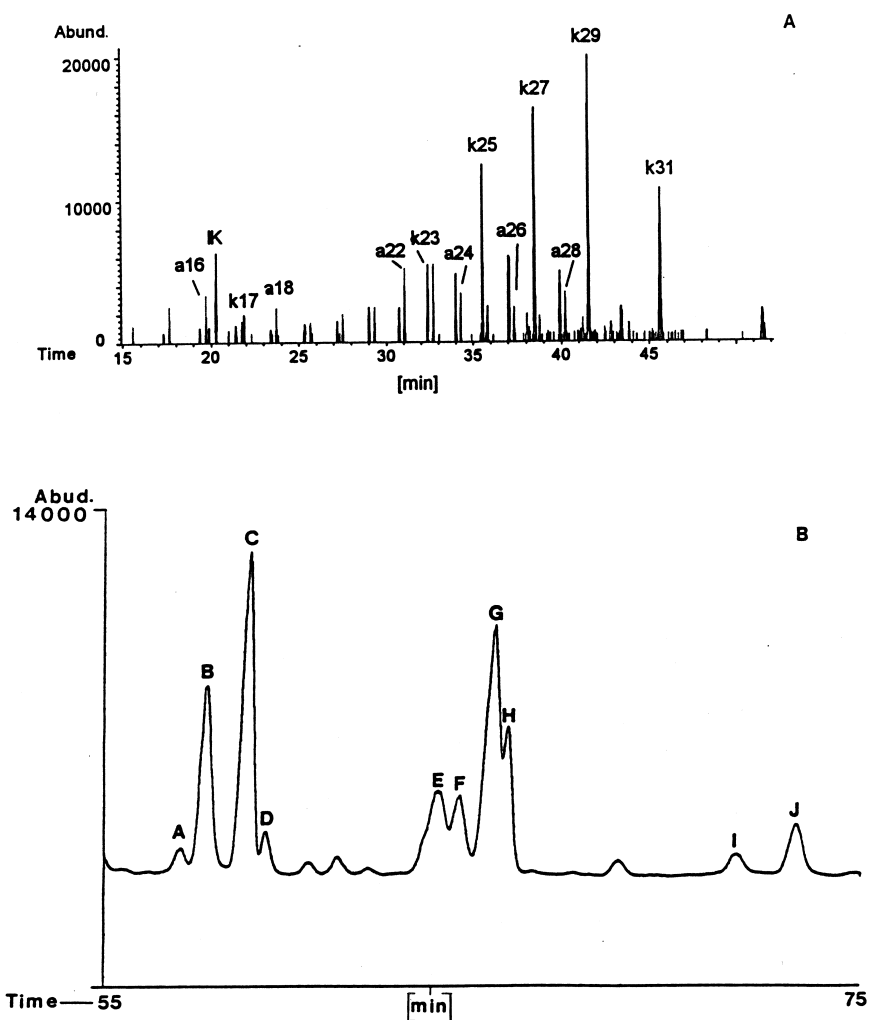


Fig. 4. (A) Selected ion chromatogram (temperature program A;  $m/z$  82 for *n*-alkanals and  $m/z$  59 for *n*-alkan-2-ones) of the carbonyl compound fraction (F3) determined in a marine sediment extract. The carbon atom numbers are indicated with a for *n*-alkanals and k for *n*-alkan-2-ones; IK, 6,10,14-trimethylpentadecan-2-one. (B) Section of the FID chromatogram (temperature program C) of the same fraction (F3) with the long chain alkenones determined in the above marine sediment extract. Compounds are indicated as follows: A, heptatriaconta-8E,15E,22E,29E-tetraen-2-one; B, heptatriaconta-8E,15E,22E-trien-2-one; C, heptatriaconta-15E,22E-dien-2-one; D, methylhexatriaconta-14E,21E-dienoate; E, octatriaconta-9E,16E,23E-dien-3-one; H, octatriaconta-16E,23E-dien-2-one; I, nonatriaconta-17E,24E-dien-3-one; J, nonatriaconta-10E,17E,24E-trien-3-one.

nated ones. Homologues with more than 21 carbons, with odd to even predominance ( $CPI > 1$ ), suggest predominant vascular plant wax *n*-alkane inputs [30] while the homologues with less than 20 carbons may have a mixed origin [31,32] e.g. marine, (for  $CPI > 1$ ), bacterial and/or petrogenic (for  $CPI \sim 1$ ). The

ratio of unresolved complex mixture to total resolvable aliphatics (UCM/NA) indicated a minor anthropogenic contribution for the south-originated samples (0–0.88), and a major anthropogenic contribution for the samples collected under intensive north winds (1.10–4.91). We observed that, likewise the wind

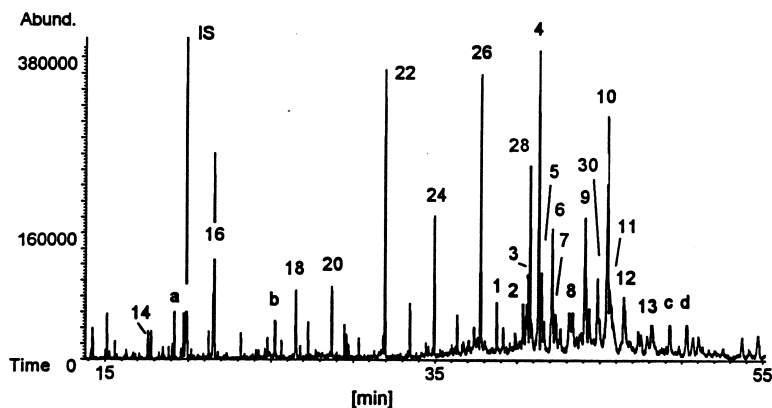


Fig. 5. Total ion chromatogram (temperature program A) of the hydroxyl compound fraction (F4) determined in a marine sediment extract. *n*-Alkanols homologues are annotated with their carbon atoms number from 14 to 30. Sterols are annotated as follows: 1, 24-norcholesta-5,22(E)-dien-3 $\beta$ -ol; 2, 27-nor-24-methylcholesta-5,22(E)-dien-3 $\beta$ -ol; 3, cholesta-5,22(E)-dien-3 $\beta$ -ol; 4, cholest-5-en-3 $\beta$ -ol; 5, 5 $\alpha$ (H)-cholestan-3 $\beta$ -ol; 6, 24-methylcholesta-5,22(E)-dien-3 $\beta$ -ol; 7, 24-methyl-5 $\alpha$ (H)-cholesta-22(E)-en-3 $\beta$ -ol; 8, 24-methylenecholest-5-en-3 $\beta$ -ol; 9, 24-ethylcholesta-5,22(E)-dien-3 $\beta$ -ol; 10, 24-ethylcholesta-5-en-3 $\beta$ -ol; 11, 24-ethyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol; 12, 4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)cholest-22(E)-en-3 $\beta$ -ol; 13, 4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)cholestan-3 $\beta$ -ol. a, Phytol; b, 1,15-triacontadiol; c, 1-hydroxy-15-triacontanone; I.S., internal standard.

direction, the wind speed was an important factor for the long-range transport of organic compounds to the sampling area. Samples collected under intensive north winds exhibit the highest CPI values for *n*-alkanes ranging from  $C_{21}$ – $C_{36}$  (higher plant waxes) (3.51–6.49). Pristane and phytane were present in all analysed samples (Table 2). Their presence was generally, attributed to petroleum residues [33]. The high concentration of pristane at the samples collected under north and north-west wind conditions during spring (phytoplankton bloom period), suggested that this molecular marker had two sources of origin under these conditions, namely marine biogenic and terrestrial anthropogenic [34,35]. This observation was in agreement with high concentrations of other marine molecular markers observed in the same samples. To conclude, the aliphatic fraction of these marine aerosols contained three main compound categories: (a) *n*-alkanes from higher plants (depending mainly on the origin of air masses, the wind speed, showing seasonal variability), (b) aliphatic compounds of marine origin (seasonal dependence) and (c) a minor contribution from petroleum residues (depending mainly on the origin of air masses and the wind speed).

The relative *n*-alkanes distribution for offshore superficial sediments, is given in Fig. 2A, and

indicated an important higher plant input. For sediments, CPI values ( $C_{21}$ – $C_{36}$ ) ranged from 2.67 to 4.70 (Table 3) and support the hypothesis, that airborne, land-derived biogenic organic matter, contribute significantly to deep sediments in open marine regions [36,37]. Low-molecular-mass *n*-alkanes ( $C_{max}$  at  $C_{17}$ ; Table 3) with an algal or microbial origin [31] were also present, but in lower concentrations. This fact implies their preferential removal with respect to the higher-molecular-mass homologues, during sedimentation. According to some authors, terrestrially derived lipids seem more protected from biodegradation processes, due to their resistant plant wax coating [38,39]. The concentrations of *n*-alkanes ranged from 80.12 to 896.17 ng/g (Table 3) and increased from the shallower to the deeper stations. No significant seasonal variations have been observed, probably due to the low sedimentation rate in the area (the upper cm of each core represents 80–100 years approximately).

### 3.2.2. F2: aromatic hydrocarbons

Nitro-PAH compounds were not detected in any examined samples. The qualitative composition of PAHs present in the aerosol samples was very similar to those of the sediments and contained predominantly 4-, 5- and 6-ring compounds of pyrolytic origin

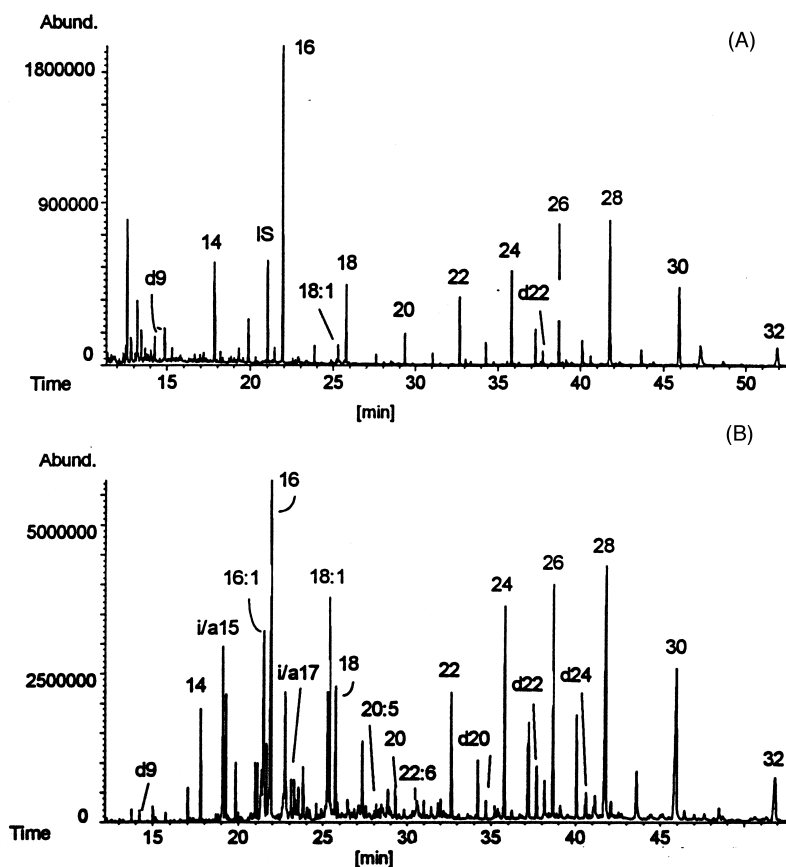


Fig. 6. (A) Total ion chromatogram (temperature program A) of the methylated fatty acids fraction (F5) determined in a rural aerosol sample. (B) Total ion chromatogram (temperature program A) of the methylated fatty acids fraction (F5) determined in a marine sediment extract. *n*-Alkanoic acids are annotated with the number of carbon atoms for each homologue (from 14 to 32); the corresponding unsaturated fatty acid are indicated with the number of their carbon atoms and the number of double bonds ( $C_n:x$ ); the carbon atoms numbers of dicarboxylic and iso- and anteiso are indicated with d and i/a accordingly; I.S., internal standard.

with benzo[*b*]-, -[*j*]- and -[*k*]fluoranthenes, fluoranthene, chrysene(+triphenylene), indeno[*cd*]pyrene and benzo[*ghi*]perylene being the major individual compounds.

Concentrations of PAHs for aerosol samples are given in Table 2 and for marine sediment samples in Table 3. Aerosol samples collected during north and north-west are enriched in PAHs (0.9–1.72 ng/m<sup>3</sup>; Table 2), compared to those collected during south and south-west wind events (0.07–0.32 ng/m<sup>3</sup>; Table 2). The concentrations of PAHs in sediments (14.74–161.47 ng/g; Table 3) showed a decreasing tendency from the shallower to the deeper stations. Aerosol (Table 2) and sediment (Table 3) samples were characterised by relatively stable diagnostic

concentration ratios. The mean value of CPAHs/TPAHs was 0.85 in aerosol samples (Table 2) and 0.81 in superficial sediments (Table 3). These values point out that combustion PAHs are the major components, while petrogenic PAHs are not very abundant in both aerosol and sediment samples. The corresponding MP/P values were generally <1 for aerosols (Table 2) and >1 respectively for sediments (Table 3). MP/P values for aerosol were within the characteristic range for combustion derived products [40,41], with some exceptions when the corresponding air masses had a very clear influence from urban areas located western from the sampling station. For sediments, MP/P ratios >1 indicated an additional petrogenic contribution (Table 3).

In Tables 2 and 3, some more diagnostic ratios are given: BA/BA+CT, BeP/BeP+BaP, Fl/Fl+Py and IP/IP+BgP. These ratios should be taken into consideration with caution, since a number of experimental studies have demonstrated that the various PAHs show different tendencies to undergo photochemical and/or chemical oxidation reactions under simulated atmospheric conditions [42]. Furthermore, bioavailability and biodegradation rates appear to vary for different PAHs that enter the marine environment [42,43]. The BA/BA+CT ratio ranged from 0.03 to 0.23 for aerosol samples (Table 2), and from 0.23 to 0.40 for sediments (Table 3). BeP/BeP+BaP ranged from 0.58 to 0.86 for aerosols (Table 2) and from 0.56 to 0.71 for sediments (Table 3). The above ratios are characteristic of a faster decay of benzo[*a*]pyrene and benzo[*a*]anthracene [24,25,42,43], and indicate an origin from a distant source. Fl/Fl+Py ranged from 0.53 to 0.9 for aerosols (Table 2), and from 0.51 to 0.82 for sediments (Table 3). IP/IP+BgP ranged from 0.38 to 0.54 for aerosols (Table 2) and from 0.52 to 0.65 for sediments (Table 3). These values indicate mixed combustion [23–25] sources for both kind of samples.

The study of distributions and diagnostic ratios for aerosols and sediments suggests that PAHs of combustion origin, seem more resistant to transformation processes (photolysis) during long range transport of aerosols, and flux of sinking material (biodegradation, etc.) through the water column, on their way to deep marine sediments.

### 3.2.3. F3: carbonyl compounds and oxy-PAHs

*n*-Alkanals and *n*-alkan-2-ones are the compound classes dominating this fraction. Nevertheless their concentration ranges are at least one order of magnitude lower than those of the other polar compounds, such as *n*-alkanols and fatty acids for both aerosols and sediments (see Tables 2 and 3).

The *n*-alcanals especially those with carbon atoms number higher than 20, are of biogenic origin [44]. The chain length distribution of *n*-alcanals in the range C<sub>20</sub>–C<sub>32</sub> is usually very similar or identical to that of *n*-alcanols [9], suggesting a close relationship [44]. Aerosol CPI values (2.34–15.84, Table 2) for homologues with carbon chain C<sub>20</sub>–C<sub>32</sub> indicate a clear biogenic input. The homologues with lower

carbon atoms number, from C<sub>9</sub> to C<sub>19</sub> may originate from the oxidation of *n*-alcanes [44].

*n*-Alcan-2-ones are considered to originate through in situ microbial oxidation of *n*-alcanes [45]. Their homologues distribution and C<sub>max</sub>, within the range C<sub>25</sub>–C<sub>33</sub> (see Tables 2 and 3), was very similar to the corresponding *n*-alcanes one [9] suggesting thus the above hypothesis (Fig. 2A and Fig. 4A).

The presence of the C<sub>18</sub> isoprenoid ketone (IK, Fig. 4A) 6,10,14-trimethylpentadecan-2-one, in all rural aerosol samples, collected during north and north-west wind events is of particular interest (Table 2). This compound has been positively identified through electron impact and isobutane chemical ionisation mass spectra [9]. Its presence has been reported in recent lacustrine and marine sediments [46,47], but to our knowledge has been reported only once [9] in marine aerosols. In the aerosol samples corresponding to air masses of a clear marine origin, this isoprenoid ketone was by far the most abundant compound. In all sediment samples, 6,10,14-trimethylpentadecan-2-one (IK in Fig. 4A), phytol (a in Fig. 5) and phytanic acid (Table 3), having the same origin (phytyl chain of chlorophyll), have been determined (Table 3).

Long-chain C<sub>37</sub>–C<sub>39</sub> alkenones (methyl and ethyl unsaturated ketones), derived from various phytoplanktonic species of the class Prymnesiophyceae [48,49] were detected in superficial sediments (Fig. 4B and Table 3). These compounds are of particular interest due to their use as paleoclimatic indicators [48,49].

### 3.2.4. F4: hydroxyl compounds

The most abundant neutral compound class, determined in this aerosol fraction was, by far, the *n*-alcanols (Table 2). In marine sediments *n*-alcanols and steroidal alcohols were the most common (Table 3). *n*-Alcanols in aerosol and sediment samples (Tables 2 and 3; Fig. 5) ranged from C<sub>11</sub> to C<sub>33</sub>, with a strong even carbon number preference (CPI 11.3–60.7 for aerosols and 2.8–18.1 for sediments; Tables 2 and 3). This very strong even-to-odd carbon number predominance and C<sub>max</sub> at C<sub>26</sub> or C<sub>28</sub> (Fig. 5, Tables 2 and 3) suggest higher plant waxes as source of these lipids [19,31] determined in the marine aerosol and sediment of this area. The distributions obtained here were similar to those

obtained in aerosol and sediments from other marine sites, such as the North Pacific [27] or in many locations in the USA [1].

The distribution of free sterols, in the various superficial sediments (Table 3 and Fig. 5), was very similar even though the sampling sites corresponded to different water depths and distance from the coast. On the other hand, their concentrations decreased from shallow to deep sampling stations. Cholest-5-en-3 $\beta$ -ol (4) and 24-ethylcholest-5-en-3 $\beta$ -ol (10) are the dominant sterols in all sediment samples (Fig. 5 and Table 3). 24-Methylcholsta-5,22-dien-3 $\beta$ -ol (6), 24-ethylcholsta-5,22-dien-3 $\beta$ -ol (9), and 4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)-cholest-22-en-3 $\beta$ -ol (12) were also present in all the samples (Fig. 5 and Table 3). Sterols 9 and 10 (Fig. 5 and Table 3) are common in terrestrial higher plants, but they can also have an algal origin [50]. The high abundance of wax lipids (long-chain *n*-alkanes, *n*-alcohols and fatty acids) in various sediment samples studied, indicate mostly a terrestrial origin for these sterols. Cholest-5-en-3 $\beta$ -ol (4 in Fig. 5) is the major sterol in zooplankton species and benthic fauna [51], while 24-methylcholesta-5,22-dien-3 $\beta$ -ol (6 in Fig. 5) can be ascribed to phytoplankton, as it is a common sterol of diatoms and prymnesiophytes [51–53]. 4 $\alpha$ ,23,24-Trimethyl-5 $\alpha$ (H)-cholest-22-en-3 $\beta$ -ol (12 in Fig. 5), known as dinosterol, is a dinoflagellate marker [54].

Two biological molecular markers of marine origin, namely the 1,15-triacontadiol (b in Fig. 5) and 1-hydroxy-15-triacontanone (c in Fig. 5) have been determined in all superficial sediments (Table 3). They are considered to derive from Cyanophyceae [55,56] but they have also been detected in cultures of Eustigmatophyceae (Chromatophyta) [57].

### 3.2.5. F5: carboxylic compounds

The chromatographic patterns of this fraction, for aerosol and sediments (Fig. 6A and B), show that homologues range from C<sub>10</sub> to C<sub>32</sub>. The compounds distribution was characterised by a strong even-to-odd CPI (see Tables 2 and 3), indicating a definite biogenic origin. The homologues <C<sub>20</sub> are attributed to marine or microbial sources, while those >C<sub>20</sub> to higher terrestrial plants [1]. A bimodal distribution that maximised at C<sub>16</sub> and C<sub>26</sub> or C<sub>28</sub> has been observed for both aerosol and sediment samples (Fig. 6A and B). Lower-molecular-mass free fatty acids, were the more abundant homologues indicating

marine (algal/microbial) origin [58,59], in aerosol samples (Fig. 6A). On the other hand, the higher concentration of the long-chain terrestrial plant fatty acids in sediments may be explained by the fact that they are more resistant to biodegradation compared to short-chain ones [38,39]. Unsaturated fatty acids are more susceptible to degradation in the marine environment than the saturated homologues [60]. These degradation processes occur during sedimentation. This is in agreement with our results, which showed a significant decrease of this ratio from the shallow to the deep sampling sites. The same trend has been also observed for branched-chain fatty acids (i/a15 and i/a17 in Fig. 6B; see also Table 3) although these have a bacterial origin [61]). This fact can be explained by the preference of microorganisms for “fresh” organic sinking material.

In all aerosol samples analysed in this study, a series of  $\alpha,\omega$ -dicarboxylic acids and  $\omega$ -oxocarboxylic acids were determined (Fig. 6A and Table 3). It has been proposed that these compounds are photooxidation products of cyclic olefins [62], and of unsaturated fatty acids [8]. The mass spectra of these compounds have been thoroughly presented [8]. The C<sub>5</sub> and C<sub>6</sub> homologues are formed by the oxidation of anthropogenic cyclic olefins, while the C<sub>8</sub> and C<sub>9</sub> ones (which are the most abundant) are formed by the photooxidation of unsaturated carboxylic acids such as oleic (C<sub>18:1</sub>) and linoleic (C<sub>18:2</sub>) acids. The unsaturated compounds were, in all samples, in very low concentration or even absent (Fig. 6A and Table 3). It is noticeable that the highest  $\alpha,\omega$ -dicarboxylic acids concentrations (summer), and at the same time the lowest concentration of their precursors (oleic and linoleic acids), were related to high ozone concentrations determined in the sampling area [8,63]. A bimodal distribution has been observed for the  $\alpha,\omega$ -dicarboxylic acids found in sediment samples (Fig. 6B and Table 3), maximised at C<sub>9</sub> (atmospheric deposition), and C<sub>22</sub> (marine/microbial origin) for the first and the second mode, respectively.

## 4. Conclusions

A one-step fractionation procedure for the analysis of specific molecular markers in various environmental samples was developed. This procedure was

successfully applied to marine aerosols and marine superficial sediments, permitting the determination of specific molecular markers which were used as source tracers allowing insight into the biogeochemical cycle of organic matter in open marine areas. The present analytical procedure was especially useful for the isolation of oxygen containing molecular markers which, although in lower concentration than hydroxyl containing compounds and fatty acids, are important source tracers. Furthermore, in contrast to other related analytical methods which identify the total lipidic content (free and esterified) of the organic matter after hydrolysis reaction, this method allows the determination of the free lipidic content. By using our method and a separate step of hydrolysis, it will be possible to determine separately free and esterified lipid categories and thus further enhance the quality of information on the biological and chemical transformations of organic matter in various environmental samples.

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