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Polycyclic aromatic hydrocarbon $^{13}C/^{12}C$ ratio measurement in petroleum and marine sediments Application to standard reference materials and a sediment suspected of contamination from the Erika oil spill

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Abstract

This paper describes a simple and rapid sample preparation procedure allowing to measure the stable carbon isotopic composition of polycyclic aromatic hydrocarbons (PAHs) in petroleum and in sediments. The aromatic fraction is first purified and isolated on alumina and silica micro-columns. A high-performance liquid chromatography fractionation allows one then to isolate each aromatic family in order to limit coelutions between PAHs. Moreover, this purification step reduces the importance of the unresolved complex mixture which otherwise contribute to the GC–isotope ratio MS background signal. The application of this analytical procedure has allowed one to determined PAH isotopic composition in a reference material crude oil (SRM 1582) and a marine sediment (SRM 1944) with good reproducibility as uncertainties between three independent assays performed were lower than 0.5‰. This analytical procedure has then been successfully applied to confirm the contamination of a sediment by the petroleum product spilled by the Erika tanker after its wreck on 12 December 1999 close to the Atlantic Coast of France.

Keywords: Isotope ratio mass spectrometry; Mass spectrometry; Oils; Petroleum; Marine sediments; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a widespread class of organic contaminants in recent sediments due to their stability and the multiplicity of their sources [1,2]. Indeed, PAHs can be present in sediment as transformation products of natural precursors (diagenetic origin). They can also be formed during incomplete combustion processes (pyrolytic origin) of organic matter (e.g., coal, oil, wood). PAHs, that are then present in vehicular, industrial and fire emissions, end up in marine sediments via atmospheric deposition and urban run off. Finally, PAHs which are major constituents of crude oil as they can represent between 25 and 40% of the mass of a petroleum [3] are introduced in the marine environment during natural and anthropogenic release of petroleum (petrogenic origin). As PAHs

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exhibit some mutagenic and carcinogenic properties [4] the study of their sources presents an ecotoxicological interest.

PAHs show different molecular distributions depending on their origin [5,6]. Source assessment studies based on these compounds rely then mostly on molecular fingerprint examination [6–9]. Nevertheless, the different processes affecting petroleum products in the marine environment (evaporation, dissolution, photo-oxidation, biodegradation) can lead to some alteration of the initial molecular fingerprint due to compound-specific degradations [10–14]. Source identification of PAHs in recent environments, using molecular considerations, can then be in some cases inconclusive and ambiguous.

In this context, the use of independent and discriminant additional properties are needed to improve PAH origin and fate studies. Molecular stable carbon isotopic composition of organic compounds, which depends on their origin and their fate, has been shown to be of particular interest in elucidation studies of hydrocarbons origin in ancient depositional environment [15,16]. The use of the molecular $^{13}C/^{12}C$ ratio for source apportionment of hydrocarbons in modern environment receives increasing attention. For example, PAHs have been recently source apportioned using their molecular isotopic composition in sediments, in soils and in aerosols [17–20]. Moreover, it has been shown that the different processes affecting PAHs in the environment do not induce significant isotopic fractionation [21–23]. The molecular stable carbon isotopic composition appears then to be a powerful tool to be used in conjunction with molecular considerations in environmental studies dealing with the origin and the fate of hydrocarbons in the environment.

The measurement of molecular isotopic composition of compounds present in a complex mixture such as a petroleum or a sedimentary organic extract is now possible using a gas chromatograph interfaced to an isotopic ratio mass spectrometer via a combustion furnace [24,25]. In this technique, individual compounds eluting from a gas chromatograph are converted to CO_2 and water in a combustion furnace heated at 940°C containing CuO. Water is trapped via a hygroscopic membrane (naphion). Purified CO_2 is then directly introduced in a magnetic mass spectrometer continuously monitoring ions having 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$), 46 ($^{12}C^{18}C^{16}O$) as m/z ratio. The isotopic composition is then calculated using the ratio m/z 44/45 and m/z 44/46 (for the correction of the ^{17}O contribution to the m/z 45 signal). Due to the fact that compounds are converted to CO_2 before detection, the molecular isotopic composition of a compound can be measured accurately only if this compound does not suffer from any coelution. It is then necessary to perform some purification steps in order to isolate the compounds of interest.

The aim of this paper is then to describe a simple and rapid analytical procedure allowing the measurement of molecular isotopic composition of PAHs in petroleum and in sediments. Possible isotopic composition fractionations introduced by the analytical procedure have been first investigated on standard methylphenanthrenes. The analytical procedure has been performed on a reference material petroleum (SRM 1582) and a reference material marine sediment (SRM 1944). The protocol developed has then been applied to a sediment suspected to have been contaminated by the product spilled by the Erika tanker after its wreck on 12 December 1999 close to the French Atlantic Coast.

2. Experimental

2.1. Solvents, reagents, materials

2.1.1. Solvents

Mallinckrodt Nanograde methylene chloride and *n*-pentane were used (Atlantique Labo, Floirac, France). HPLC-grade quality isooctane and acetone were from Scharlau (ICS, St. Médard en Jalles, France).

2.1.2. Reagents

Copper (40 mesh, 99.5% purity) (Aldrich, Saint Quentin Fallavier, France) was activated with 7 *M* hydrochloric acid. Copper was washed with water until neutral pH was obtained, with acetone (three times) in order to remove water and finally with methylene chloride (three times) in order to remove acetone. It was then stored in a closed vial in methylene chloride.

Alumina (150 basic, type T, particle size, 0.063–0.2 mm) and silica (silica gel 60, 0.063–0.2 mm) (Merck, Darmstadt, Germany) were both washed several times with methylene chloride, then placed under a hood during 12 h in order to remove methylene chloride. Phases were activated at 150°C and then stored in an oven maintained at 150°C in order to avoid water adsorption that could affect phase retention behaviour.

2.1.3. Standard reference material

Both standard reference materials (SRMs) were from the American National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Standard reference material, SRM 1582, is a crude oil that provides a typical specimen of an oil matrix for use in developing analytical methods.

Standard reference material, SRM 1944, is a mixture of marine sediment collected near urban areas in New York and New Jersey, USA.

2.1.4. Erika oil sample

The Erika oil sample comes from the refinery of the Flandres in Dunkerque (France) and was provided by the CEDRE (French Water Pollution Institute, Brest, France).

2.2. Asphaltene precipitation for petroleum

A 20-mg amount of petroleum was dissolved in 10 ml of pentane. After 2 h, maltene fractions (supernatants), were isolated from the asphaltene fraction by recovering the supernatant using a Pasteur pipette. Maltene fractions were reduced to a small volume (500 μl) under a stream of nitrogen.

2.3. Microwave extraction for sediments

A 10-g amount of sediments was extracted using microwave assisted extraction (10 min, 30 W, Maxidigest MX 4350, Prolabo, Fontenay-sous-bois, France). Methylene chloride was the extraction solvent. The sample was filtered and the total organic extract was reduced to a small volume using a rotary evaporator.

2.4. Petroleum maltene fraction and sedimentary organic extract purification

The liquid chromatography purification procedure on open micro-columns was adapted from a protocol developed by Behar et al. [26]. The maltene petroleum fractions and the sedimentary organic extracts were eluted with 10 ml of methylene chloride through micro-columns filled with alumina (1.4 g, length: 8 cm) in order to remove polar compounds and macromolecules. Before this purification, alumina micro-columns were washed and conditioned by eluting 10 ml of methylene chloride. For marine sediment purification, activated copper was added at the top of the alumina micro-columns in order to remove elemental sulfur. Eluates were concentrated under nitrogen stream to 500 μl, the solvent was then exchanged to isooctane (i.e., by adding 500 μl of isooctane and then concentrating the sample to remove methylene chloride).

Eluates were fractionated on micro-columns filled with washed silica gel (0.8 g, length: 8 cm) in order to collect separately saturated and aromatic compounds. Saturated fractions were eluted with 6 ml of pentane, aromatic hydrocarbons were then recovered using 10 ml of a pentane–methylene chloride (65:35, v/v) mixture. Before this purification, silica microcolumns were washed and conditioned by eluting 10 ml of pentane.

2.5. HPLC fractionation

The aromatic fraction was fractionated by high-performance liquid chromatography (HPLC) on aminosilane phase (Dynamax, 10 μm, 25 cm×10 mm I.D., Varian, Walnut Creek, CA, USA) using *n*-pentane as mobile phase [27]. The flow-rate was 4 ml/min for 50 min. This HPLC fractionation, based on ring number, allows one to collect seven different fractions: monoaromatic, diaromatic (naphthalenes and biphenyls), dibenzothiophenes+fluorenes, phenanthrenes, fluoranthenes+pyrenes, chrysenes+benz[*a*]anthracene and penta-+hexaaromatics as illustrated for the SRM 1582 and SRM 1944 in Fig. 1. The integrity of each fraction was controlled by gas chromatographic–mass spectrometric (GC–MS) analysis. This HPLC purification step also allows one to remove the aromatic unresolved complex

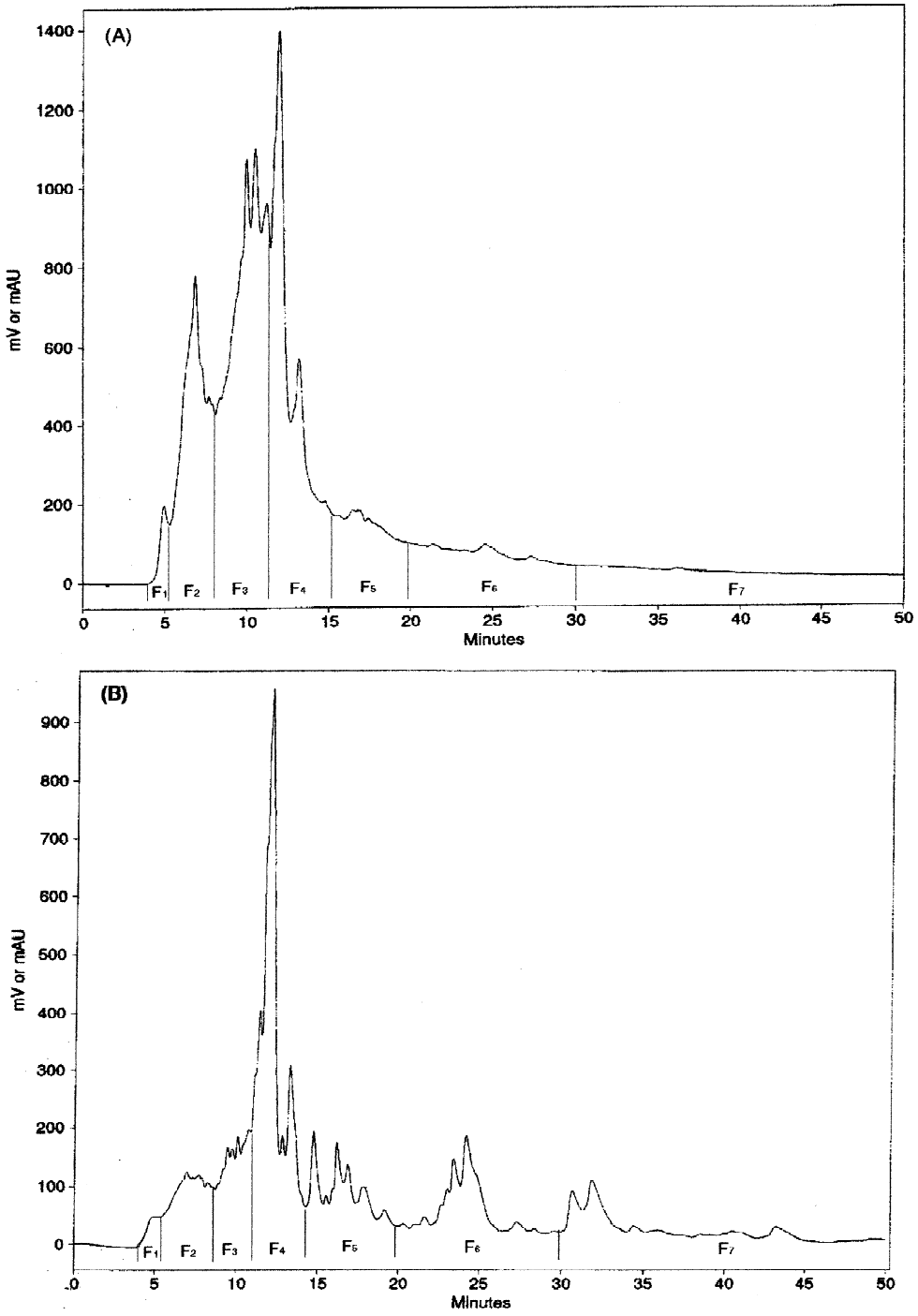

Fig. 1. High-performance liquid chromatograms obtained for the aromatic fraction of SRM 1582 (A) and SRM 1944 (B) using a an aminosilane phase (Dymamax, 10 μm, 25 cm×10 mm I.D.) and pentane as mobile phase. The different fractions are: monoaromatics (F1), diaromatics (naphthalenes and biphenyls) (F2), dibenzothiophenes+fluorenes (F3), phenanthrenes (F4), fluoranthenes+pyrenes (F5), chrysenes+benz[*a*]anthracene (F6) and penta-+hexaaromatics (F7).

mixture (UCM). Gas chromatography–isotope ratio mass spectrometry (GC–IRMS) analyses were then performed on the different fractions.

2.6. Gas chromatography–isotope ratio mass spectrometry

Stable carbon isotopic composition analyses of aromatic fractions were carried out using a HP 5890 Series II Plus gas chromatograph interfaced via a CuO furnace (940°C) and a hygroscopic membrane (nafion) to a Delta Plus isotopic ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Injections were performed in the splitless mode. The injector temperature was maintained at 270°C. The GC temperature program was from 50°C (2 min) to 180°C at 10°C/min, from 180°C to 230°C at 2°C/min and from 230°C to 290°C at 10°C/min. For the other fraction the temperature was from 50°C (2 min) to 290°C (20 min) at 5°C/min. The carrier gas was helium (flow-rate: 1 ml/min). The capillary column used was a SGE BPX5: 60 m×0.22 mm I.D., 0.25 μm film thickness.

For calculation purpose, CO_2 reference gas was automatically introduced into the isotopic ratio mass spectrometer in a series of pulses at the beginning and at the end of each analysis.

3. Results and discussion

3.1. Validation of the analytical sample purification procedure

In order to be sure that the analytical sample purification procedure described in the Experimental section does not introduce some isotopic fractionation, the protocol was applied to a mixture containing standard 3-, 2-, 9- and 1-methylphenanthrenes. This experiment was carried out in triplicate in order to check also the reproducibility of the analytical protocol. The isotopic compositions of the four methylphenanthrene isomers, measured before and after the application of the analytical procedure, are compared in Fig. 2. First it is to note that a good reproducibility between the three assays is obtained as uncertainties are lower than 0.5‰. Moreover, no significant difference was observed between the isotopic compositions of methylphenanthrenes present in the initial standard solution and the ones measured after the application of the analytical

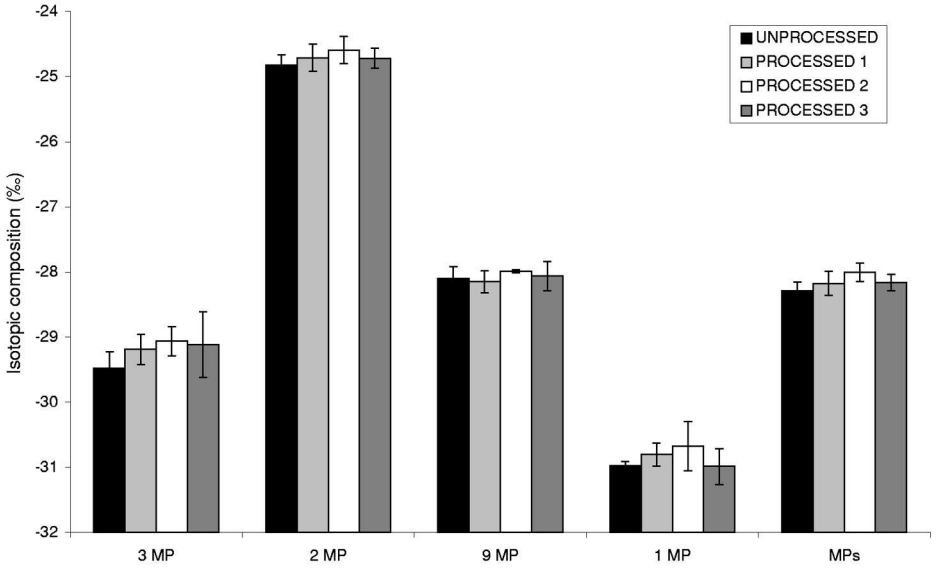

Fig. 2. Validation of the analytical procedure on standard methylphenanthrene compounds. The values represented are the mean of three analyses for each assay.

protocol to this mixture. It can be concluded that no significant isotopic fractionation has occurred during the sample preparation procedure.

3.2. Evaluation of accuracy of PAH isotopic composition measurement

The analytical procedure described in the Experimental section was applied to a reference material crude oil (SRM 1582) and a reference material marine sediment (SRM 1944).

3.2.1. Determination of PAH isotopic composition in petroleum SRM 1582

Petroleums are featured by the presence of alkylated PAHs, which form a complex mixture in which coelutions between compounds are numerous due to the superposition of the different aromatic families. A HPLC fractionation of petroleum in different aromatic families allows one to limit coelutions. The maltene fraction of SRM 1582 has then been fractionated using HPLC as described in the Experimental section. The only fraction in which enough compounds were present for an accurate determination of molecular $^{13}C/^{12}C$ ratio is the phenanthrenic fraction. Fig. 3 shows the GC–IRMS chromatographic trace (m/z 44) obtained. Three independent assays were performed in order to evaluate the reproducibility of the analytical procedure. Each of those assays was analysed three times. Fig. 4 represents the isotopic composition measured in the three assays. It is first to note that for alkylated PAHs, uncertainties were lower than 0.5‰. For phenanthrene uncertainties higher than 0.5‰ were observed for an unknown reason. No significant isotopic composition differences were observed between the three assays showing that the analytical procedure allows one to generate reproducible measurement.

3.2.2. Determination of PAH isotopic composition in marine sediment SRM 1944

For the determination of PAH isotopic composition in marine sediment the necessity of the HPLC purification depends on the origin of the contamination. Indeed, for sediments showing a petrogenic

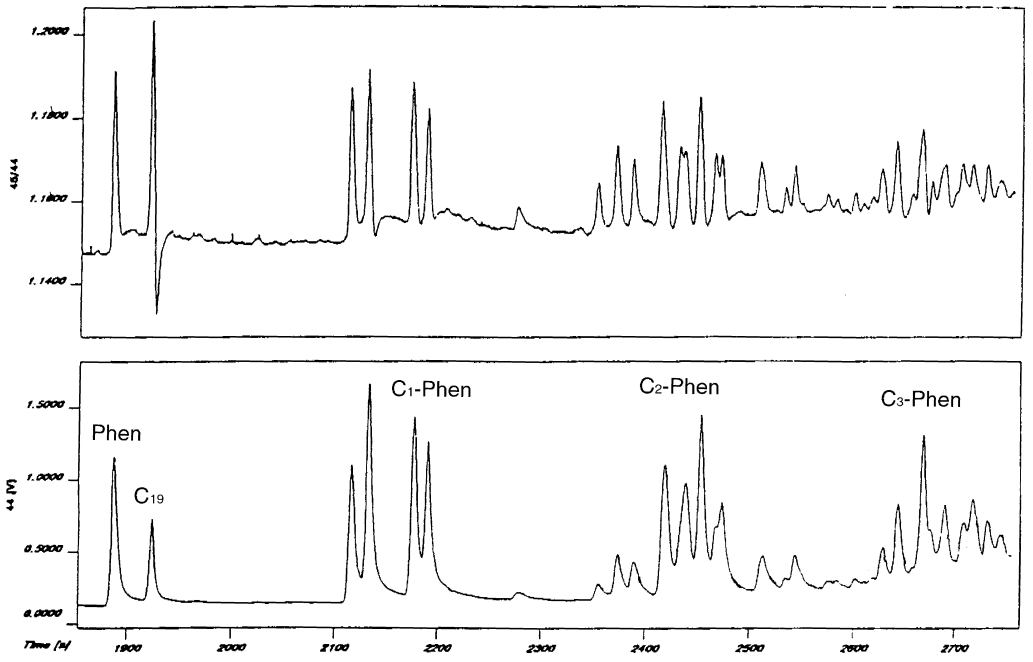

Fig. 3. GC–IRMS chromatographic trace (m/z 44) of the phenanthrenic fraction of SRM 1582 obtained after the performance of the sample preparation protocol described in the Experimental section.

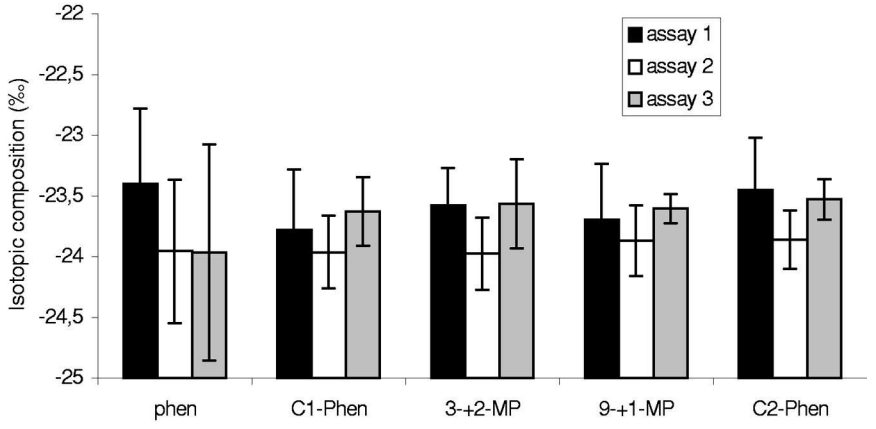

Fig. 4. Stable carbon isotopic composition of phenanthrenic compounds in a crude oil reference material (SRM 1582) determined using the analytical procedure described in the Experimental section. Mean of three analyses for each assay. Phen=Phenanthrene, C_1-Phen=Σ methylphenanthrenes, MP=methylphenanthrene, C_2-Phen=Σ dimethylphenanthrenes.

contamination, HPLC fractionation is required for the same reason as for petroleum. When the contamination is from pyrolytic origin as alkylated PAHs are much less abundant than parent ones, the requirement of the HPLC fractionation appears not so obvious. For SRM 1944 which shows a dominant pyrolytic contamination, PAH isotopic composition was then measured without any HPLC purification, after a simple HPLC fractionation (collection of all the aromatics after the naphthalene fraction) and after complete fractionation in the different aromatic families in order to study the effect of the HPLC purification on PAH isotopic composition measurement for this type of sediment. Fig. 5 shows the GC–IRMS chromatograms obtained after those three different purification protocols. Note that without the performance of the HPLC fractionation an important background signal is observed due to the presence of the UCM. This UCM appears to have been partly removed by the simple HPLC fractionation. The fractions obtained when each aromatic family was isolated appear clean and the background signal is mainly due to the bleeding of the gas chromatographic column.

The PAH isotopic compositions determined under the three different analytical conditions are compared in Fig. 6. No important isotopic composition difference are observed showing that for this type of sample the application of the HPLC fractionation is not so determinant. It must still be noted that the isotopic composition of the fluoranthene shows a significant ^{13}C-enrichment when measured after the HPLC fractionation in the different aromatic family compare to the values determined under the two other conditions. This can be explained by coelution with some alkylated phenanthrenes or dibenzothiophenes which elute in the same range than fluoranthene and that are removed by the complete HPLC fractionation. Moreover, uncertainties were lower than 0.3‰ for all PAHs when isotopic composition were measured after the complete fractionation in different aromatic fractions when in the two other cases some uncertainties higher than 0.5‰ are observed for some PAHs probably due to the presence of a high background signal.

3.2.3. Methyphenanthrene molecular isotopic composition measurements

Individual isotopic compositions of methylphenanthrenes are not reported as they are not measured with accuracy due to their incomplete separation. During gas chromatographic separation, an isotopic fractionation occurs. Molecules containing ^{13}C elute earlier due to an inverse isotope effect associated with the interaction of the eluent with the GC column stationary phase [28]. Isotope ratios cannot then be determined from partial examination of a GC peak. When two compounds partially co-elute, as it is the case for 3-MP and 2-MP and for 9-MP and 1-MP, an artificially ^{13}C-enriched isotopic composi-

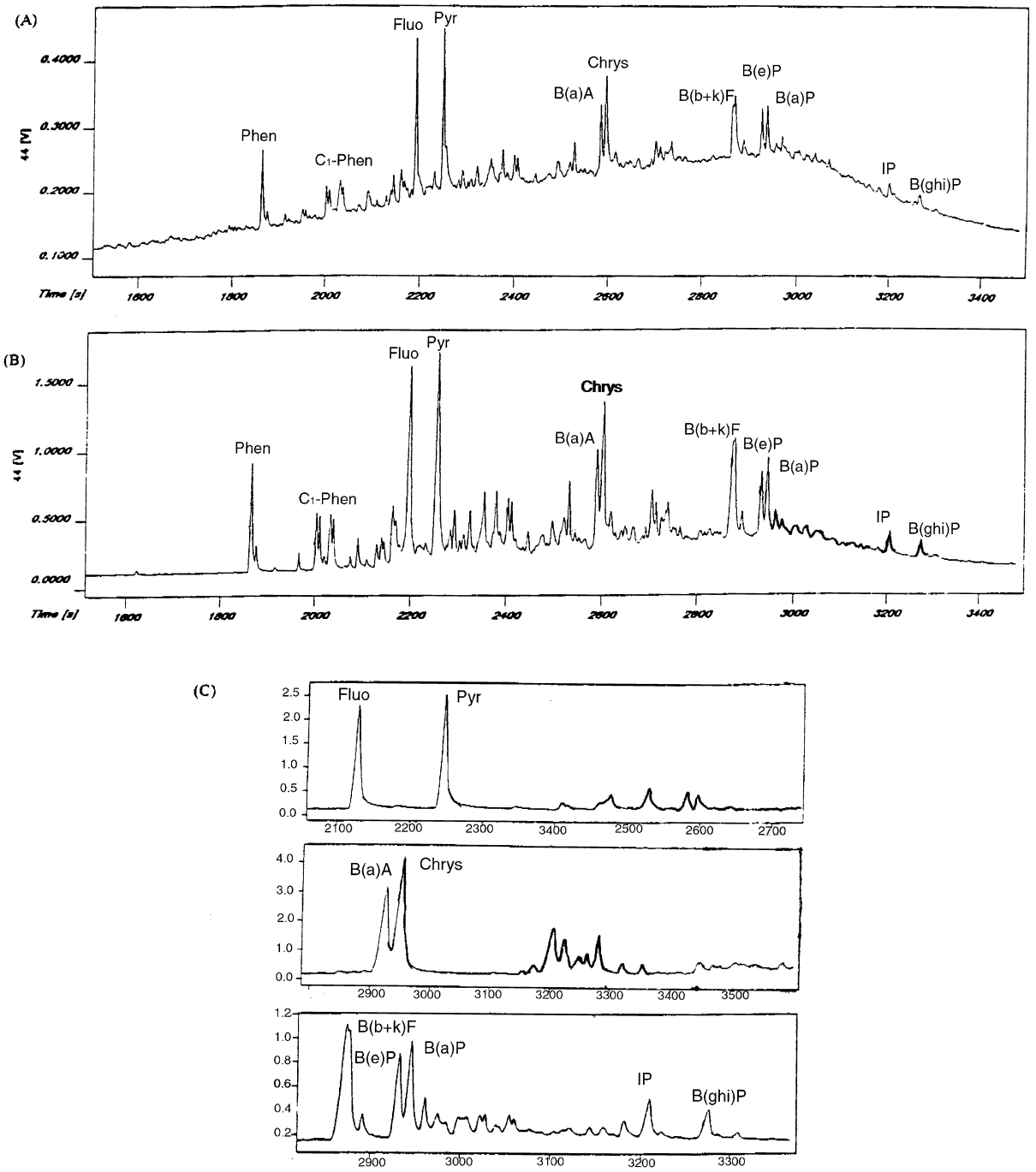

Fig. 5. GC–IRMS chromatograms (m/z 44) of PAHs in SRM 1944: (A) without the HPLC fractionation step; (B) after a simple HPLC fractionation (collection of a unique fraction after the naphthalenic compounds); (C) after complete HPLC fractionation in the different aromatic families. Phen=Phenanthrene; C1-Phen=methylphenanthrene; Fluo=fluoranthene; Pyr=pyrene; B(a)A=benz[*a*]anthracene, Chrys=chrysene; B(b+k)F=benzo[*b*]fluoranthene+benzo[*k*]fluoranthene; B(e)P=benzo[*e*]pyrene; B(a)P=benzo[*a*]pyrene; Per=perylene; IP=indeno[1,2,3-*cd*]pyrene; B(ghi)P=benzo[*ghi*]perylene.

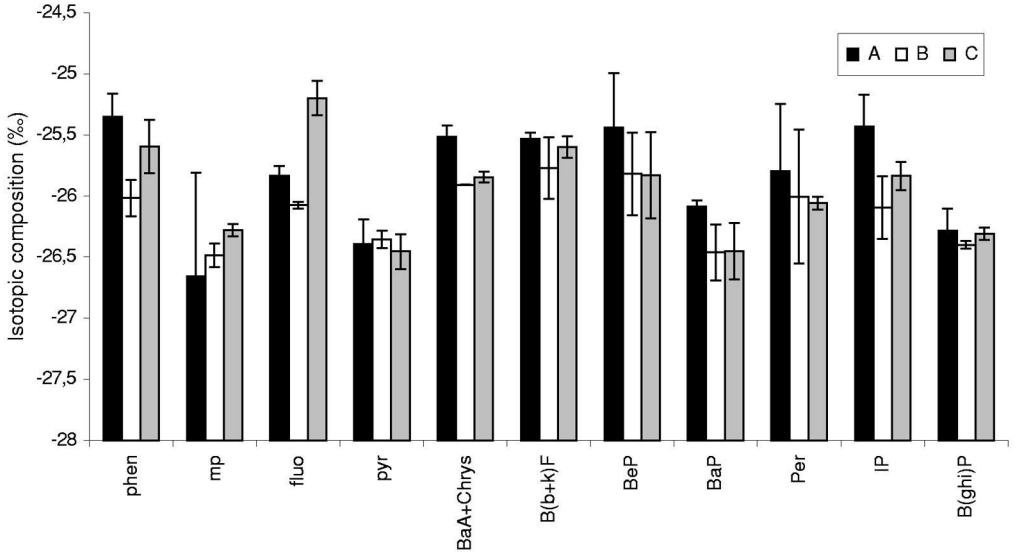

Fig. 6. Isotopic composition (mean of three assays) of PAHs in the marine sediment reference material SRM 1944 determined: (A) without the HPLC fractionation step; (B) after a simple HPLC fractionation (collection of a unique fraction after the naphthalenic compounds); (C) after complete HPLC fractionation in the different aromatic families. Phen=Phenanthrene; MP=methylphenanthrene; Fluo=fluoranthene; Pyr=pyrene; BaA+Chrys=benz[*a*]anthracene+chrysene; B(b+k)F=benzo[*b*]fluoranthene+benzo[*k*]fluoranthene; B(e)P=benzo[*e*]pyrene; B(a)P=benzo[*a*]pyrene; Per=perylene; IP=indeno[1,2,3-*cd*]pyrene; B(ghi)P=benzo[*ghi*]perylene.

tion is measured for the leading compound. Indeed, a contamination of the first component by the ^{13}C-enriched leading edge of the second and a loss of a portion of the ^{13}C-depleted tail of the first peak occurs. Conversely, and for complementary reasons, an artificial ^{13}C-depletion is observed for the trailing peak. Fig. 7 shows, respectively the isotopic composition of methylphenanthrenes determined for SRM 1582 and SRM 1944. The analytical bias, resulting from the isotopic fractionation during the gas chromatographic separation, is illustrated by the ''zigzag'' pattern exhibited by the methylphenanthrene isotopic composition in those two samples. The individual methylphenanthrene isotopic compositions are nevertheless measured with good reproducibility as no significant differences are observed between the three assays. The isotopic composition of the sum of 3-MP+2-MP isomers and the sum of 9-MP+1-MP isomers, obtained by integrating the peaks of the two isomers together, is not subject to this analytical bias as those doublets are well resolved. They do not show significant isotopic differences. The isotopic composition of the sum of methylphenanthrenes (integration of the four peaks together) is also determined with accuracy and good reproducibility and is not different from the ones measured for the sums of the 3-MP+2-MP and of the 9-MP+1-MP.

3.3. Application to a contaminated sediment

The analytical procedure developed was applied to a sediment collected in the ''Traict du Croizic'' (France, North Atlantic Coast) that was suspected to have been contaminated by the petroleum product spilled by the Erika tanker. The isotopic compositions of a few PAHs were then measured in this contaminated sediment, in a sediment collected in a protected area in the same zone (control sediment) and in the heavy fuel oil transported by the Erika tanker (Fig. 8). The isotopic composition of all PAHs measured in the contaminated sediment are not significantly different from the ones measured for the Erika oil except for fluoranthene. PAHs in the control sediment exhibit ^{13}C-enriched isotopic compositions compared to the Erika oil and the polluted sediment. The fact that the fluoranthene found in the contaminated sediment shows the same isotopic composition

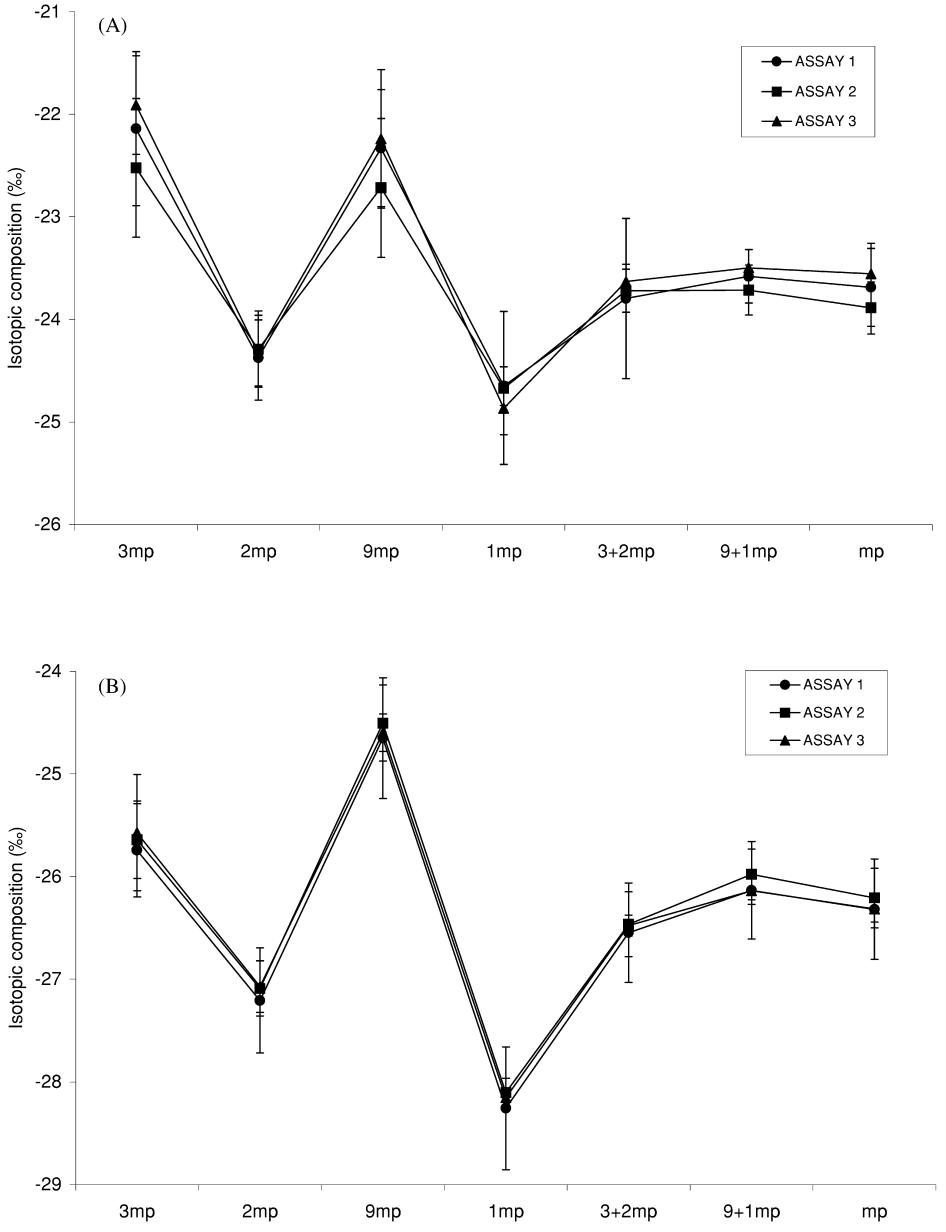

Fig. 7. Isotopic composition of methylphenanthrenes determined in SRM 1582 (A) and SRM 1944 (B). Isotopic compositions are the mean value of three analyses for each assay. MP=Methylphenanthrene.

than in the control sediment is due to the fact that fluoranthene is much less abundant in the Erika oil than the other PAHs. Those isotopic results represent a proof of the contamination of this sediment by the petroleum product spilled by the Erika tanker.

4. Conclusion

The sample preparation protocol described in this paper allows one both to obtain PAHs that do not suffer from coelutions and to reduce the importance

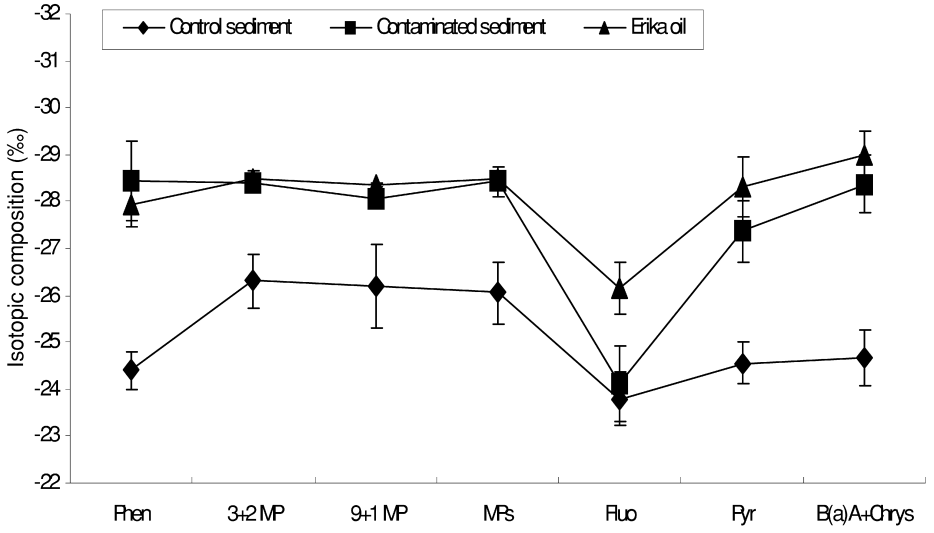

Fig. 8. Stable carbon isotopic composition of PAHs in the Erika oil, in a sediment suspected to have been contaminated by the Erika oil and in a control sediment collected in the same area. Isotopic compositions are the mean value of three analyses.

of the unresolved complex mixture which otherwise contributes to the background signal during GC–IRMS analysis. This analytical procedure allows one then to measure the stable carbon isotopic composition of PAHs in petroleum and sediment with a good reproducibility. Indeed uncertainties between the three assays performed for SRM 1582 and SRM 1944 were lower than 0.5‰.

The isotopic composition of individual methylphenanthrenes are not measured with accuracy due to the fact that they are not completely resolved with the gas chromatographic column used in this study.

The application of this analytical procedure to a sediment collected on the Atlantic Coast France has allowed to confirm its contamination by the petroleum product spilled by the Erika tanker in the Atlantic Ocean on 12 December 1999.

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References

[1] J.M. Neff, in: Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, Applied Science Publishers, London, 1979, p. 7, Chapter 2.
[2] A.E. McElroy, J.W. Farrington, J.M. Teal, in: U. Varanasi (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, CRC Press, Boca Raton, FL, 1989, p. 41.
[3] B. Tissot, D.H. Welte, in: Petroleum Formation and Occurrence, Springer, Berlin, 1984, p. 41, Chapter 1.
[4] K.L. White, Environ. Carcino. Rev. C4 (1986) 163.
[5] R.E. Laflamme, R.A. Hites, Geochim. Cosmochim. Acta 42 (1978) 289.
[6] Z. Wang, M. Fingas, D.S. Page, J. Chromatogr. A 843 (1999) 369.
[7] J.L. Lake, C. Norwood, C. Dimock, R. Bowen, Geochim. Cosmochim. Acta 43 (1979) 1847.
[8] S. Sportsøl, N. Gjos, R.G. Lichtentaler, K.O. Gustovsen, K. Urdal, F. Oreld, J. Skel, Environ. Sci. Technol. 17 (1983) 282.
[9] P. Garrigues, M.L. Angelin, R. de Sury, M. Ewald, C.R. Acad. Sci. Paris 15 (1985) 747.
[10] J.M. Bayona, J. Albaiges, A.M. Solanas, R. Pares, P. Garrigues, M. Ewald, Int. J. Environ. Anal. Chem. 23 (1986) 289.
[11] F.D. Hostettler, K.A. Kvenvolden, Org. Geochem. 21 (1994) 927.
[12] G.S. Douglas, A.E. Bence, R.C. Prince, S. McMillen, E.L. Butler, Environ. Sci. Technol. 30 (1996) 2332.
[13] Z. Wang, M. Fingas, J. Chromatogr. A 712 (1995) 321.

[14] H. Budzinski, N. Raymond, T. Nadalig, M. Gilewicz, P. Garrigues, J.C. Bertrand, P. Caumette, Org. Geochem. 28 (1998) 337.
[15] J.M. Hayes, K.H. Freeman, B.N. Popp, C.H. Hoham, Org. Geochem. 16 (1990) 1115.
[16] K.H. Freeman, J.M. Hayes, J.M. Trendel, P. Albrecht, Nature 343 (1990) 254.
[17] D.C. Ballentine, S.A. Macko, V.C. Turekian, W.P. Gilhooly, B. Martincigh, Org. Geochem. 25 (1996) 97.
[18] C. Mc Rae, G.D. Love, I.P. Murray, C.E. Snape, A.E. Fallick, Anal. Commun. 33 (1996) 331.
[19] V.P. O'Malley, T.A. Abrajano, J. Hellou, Environ. Sci. Technol. 30 (1996) 63.
[20] E. Lichtfouse, H. Budzinski, P. Garrigues, T.I. Eglinton, Org. Geochem. 26 (1997) 353.
[21] V.P. O'Malley, T.A. Abrajano, J. Hellou, Org. Geochem. 21 (1994) 809.
[22] B.A. Trust, R.B. Coffin, L.A. Cifuentes, J.G. Mueller, in: R.E. Hinchee, G.S. Douglas, S.K. Ong (Eds.), Monitoring and Verification of Bioremediation, Battelle Press, Colombus, OH, 1995, p. 233.
[23] L. Mazéas, H. Budzinski, N. Raymond, Org. Geochem., submitted for publication.
[24] M. Sano, Y. Yatsui, J. Abe, S. Sasaki, Biomed. Mass Spectrom. 3 (1976) 1.
[25] D.E. Matthews, J.M. Hayes, Anal. Chem. 50 (1978) 1465.
[26] F. Behar, C. Leblond, C. Saint-Paul, Rev. Inst. Fr. Pétrole 44 (1989) 387.
[27] H. Budzinski, P. Garrigues, J. Connan, J. Bellocq, Quim. Anal. 12 (1993) 69.
[28] W.A. Van Hook, in: R.F. Gould (Ed.), Isotope Effects in Chemical Processes, Advances in Chemistry Series, No. 89, American Chemical Society, Washington, DC, 1969, p. 99.