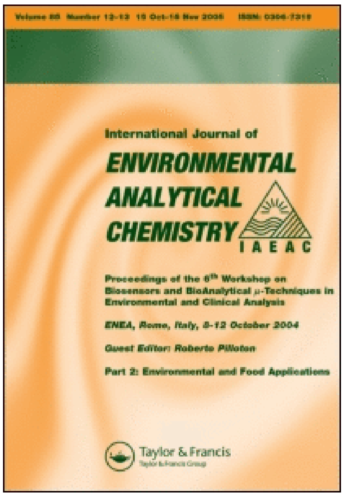

# International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

# Improved Accuracy of GC-MS Quantification of Aliphatic and Aromatic Hydrocarbons in Marine Sediments and Petroleums. Validation on Reference Matrices and Application to the Erika Oil Spill

Laurent Mazeas[a]; Hélène Budzinski[a]
[a] Laboratoire de Physico-et Toxico-Chimie des Systèmes Naturels (UMR 5472 CNRS), Université, Bordeaux I, Talence Cedex, France

**To cite this Article** Mazeas, Laurent and Budzinski, Hélène(2002) 'Improved Accuracy of GC-MS Quantification of Aliphatic and Aromatic Hydrocarbons in Marine Sediments and Petroleums. Validation on Reference Matrices and Application to the Erika Oil Spill', International Journal of Environmental Analytical Chemistry, 82: 3, 157 — 173

**To link to this Article: DOI:** 10.1080/713714609

**URL:** http://dx.doi.org/10.1080/713714609

*Intern. J. Environ. Anal. Chem.*, Vol. 82, No. 3, pp. 157–173

Taylor & Francis
Taylor & Francis Group

# IMPROVED ACCURACY OF GC-MS QUANTIFICATION OF ALIPHATIC AND AROMATIC HYDROCARBONS IN MARINE SEDIMENTS AND PETROLEUMS. VALIDATION ON REFERENCE MATRICES AND APPLICATION TO THE ERIKA OIL SPILL

LAURENT MAZEAS and HÉLÈNE BUDZINSKI*

*Laboratoire de Physico-et Toxico-Chimie des Systèmes Naturels (UMR 5472 CNRS), Université, Bordeaux I, 351 cours de la libération, 33405 Talence Cedex, France*

*(Received 1 March 2001; In final form 11 October 2001)*

A rapid and simple analytical procedure allowing accurate quantification of aliphatic and aromatic hydrocarbons in sediments and petroleum has been developed. Sediments were Soxhlet extracted using methylene chloride for 48 h. Oil maltene fractions were isolated by asphaltene precipitation in pentane. Sediment and oil extracts were first purified using alumina micro-columns. Saturated fractions were then separated from aromatic ones by fractionation on silica gel. Alkanes and polycyclic aromatic hydrocarbons (PAHs) were analyzed separately by gas chromatography coupled to mass spectrometry (GC-MS). PAHs and alkanes were quantified relative to perdeuterated PAHs and alkanes introduced prior to extraction. For accurate quantification, it is important to use several perdeuterated alkanes that cover the volatility range of alkanes of interest and at least one perdeuterated PAH per class of aromaticity. This analytical procedure has been validated on a reference material sediment (SRM 1941a) and a reference material crude oil (SRM 1582). This analytical procedure was then applied to the study of the contamination of marine sediments collected along the Atlantic coast after the Erika oil spill (December 1999).

*Keywords:* Alkanes; PAHs; GC-MS; Internal standard; Quantification; Purification; Crude oil; Sediment; Environmental samples

## INTRODUCTION

Aliphatic and polycyclic aromatic hydrocarbons (PAHs) are widespread organic compounds in the environment. They have natural and anthropogenic sources, like petrogenic (oil spill and natural leakage) and diagenetic (transformation products of biogenic precursors). Polycyclic aromatic hydrocarbons can also be introduced into the environment via incomplete organic matter combustion (pyrolytic origin). Alkane and PAH

---

*Corresponding author. Fax: +33-556-846998; E-mail: h.budzinski@lptc.u-bordeaux.fr

ISSN 0306-7319 print: ISSN 0000-000X online/01/010000-00
DOI: 0000-0000.00.000000

studies can help to distinguish between those different sources[1, 4] and between different types of organic matter (e.g. marine and terrestrial). Moreover, PAH source and behavior elucidation presents an ecotoxicological interest as PAHs have been shown to have mutagenic and carcinogenic effects[5]. The quantification of those two types of compounds allows a better understanding of their behaviors. For example, the level of contamination of a site and its remediation after an oil spill can be quantitatively followed[6].

The quantification of PAHs and alkanes in complex matrices, such as environmental samples and petroleums, presents some difficulties as a lot of compounds can interfere with them during the analysis. The use of a selective detector, such as a mass spectrometer, is useful but it does not solve all analytical problems. In the present study, a simple sample preparation procedure, allowing accurate gas chromatography-mass spectrometry (GC-MS) quantification of aliphatic and aromatic hydrocarbons in sediments and petroleum, is presented. The efficiency of the purification step prior to GC-MS analysis is pointed out. The importance of using several and adequate surrogate internal standards for alkane and PAH quantification is demonstrated. This analytical procedure was then applied to two reference materials, a sediment (SRM 1941a) and a crude oil (SRM 1582), in order to validate the protocol. The sample preparation procedure developed was then applied to assess the contamination of two sediments collected along the Atlantic shoreline after the Erika oil spill.

## EXPERIMENTAL

### Standards, Solvents and Reagents

#### *Alkanes*

Standard alkanes ($n$-$C_{10}$, $n$-$C_{12}$, $n$-$C_{13}$, $n$-$C_{14}$, $n$-$C_{15}$, $n$-$C_{16}$, $n$-$C_{17}$, pristane, $n$-$C_{18}$, phytane, $n$-$C_{19}$, $n$-$C_{20}$, $n$-$C_{22}$, $n$-$C_{24}$, $n$-$C_{26}$, $n$-$C_{28}$, $n$-$C_{30}$) used for response factors calculation were purchased from Aldrich (Saint Quentin Fallavier, France). Perdeuterated $n$-alkanes used as internal standards ($n$-$C_{12}$-$d_{26}$, $n$-$C_{14}$-$d_{30}$, $n$-$C_{15}$-$d_{32}$, $n$-$C_{16}$-$d_{34}$, $n$-$C_{20}$-$d_{42}$, $n$-$C_{24}$-$d_{50}$, $n$-$C_{30}$-$d_{62}$) were bought from Cambridge Isotope Laboratories (CIL, Andover, USA).

#### *Polycyclic Aromatic Hydrocarbons*

The Standard Reference Material, SRM 2260, containing 24 aromatic hydrocarbons (23 are certified) in toluene (nominal concentration 60 μg/ml) used for response factors calculation was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Perdeuterated PAHs were used as internal standards. Phenanthrene-$d_{10}$, benzo[a]pyrene-$d_{12}$ and benzo[g,h,i]perylene-$d_{12}$ were purchased from Cambridge Isotope Laboratories (CIL, Andover, USA), dibenzothiophene-$d_8$, fluoranthene-$d_{12}$, chrysene-$d_{12}$, and pyrene-$d_{12}$ from MSD isotopes (Division of Merck Frost Canada Inc., Montreal, CND). Dibenzothiophene response factor was determined by injecting a solution containing a known amount of dibenzothiophene (MSD, Division of Merck Frost Canada Inc., Montreal, CND) and dibenzothiophene-$d_8$.

### *Solvents*

Mallinckrodt nanograde methylene chloride and *n*-pentane were used (Atlantic Labo, Floirac, France). *n*-Pentane was distilled prior to its use for sample preparation. HPLC grade quality iso-octane and acetone were from Scharlau (ICS, St. Médard en Jalles, France).

### *Reagents*

Copper (40 mesh, 99.5% purity) (Aldrich, Saint Quentin Fallavier, France) was activated with 7 N HCl. Copper was washed with water until neutral pH was obtained, with acetone (3 times) in order to remove water, and finally with methylene chloride (3 times) in order to remove acetone. It was then stored in a closed vial in methylene chloride.

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

## Standard Reference Materials

Standard Reference Material, SRM 1941a[7], was provided by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). It is a marine sediment collected from Chesapeake Bay at the mouth of Baltimore Harbor. Indicative concentrations are given for 17 alkanes. Concentrations of 23 PAHs are certified.

Standard Reference Material, SRM 1582[8], is a crude oil. Concentrations of 6 PAHs are certified.

## Addition of the Internal Standards Solutions

The addition of alkane and PAH surrogate internal standard solutions at the beginning of the sample preparation has to be done with accuracy. Therefore, those additions were performed gravimetrically. The introduced weight of each solution is used for concentration calculation.

## Soxhlet Extraction of Sediments

Samples of SRM 1941a (2 g), spiked with internal standards (perdeuterated alkanes and PAHs), were Soxhlet extracted for 48 h using methylene chloride (400 ml). A blank experiment was simultaneously performed in order to correct concentrations for possible contamination during the sample preparation procedure. Extracts were filtered, rotary evaporatored and finally reduced to a small volume (500 µl) under nitrogen stream.

## Asphaltene Precipitation from Oil

Samples of SRM 1582 (100 mg), spiked with internal standards (perdeuterated alkanes and PAHs), were dissolved in pentane (10 ml). The samples were treated for 5 min by sonication. After two hours, the maltene fractions (supernatants), containing alkanes and PAHs, were isolated from asphaltene fractions by recovering the supernatant using a Pasteur pipette. A blank experiment was simultaneously performed in order to correct concentrations for possible contaminations during the sample preparation procedure. Maltene fractions were reduced to a small volume (500 µl) under nitrogen stream.

### Oil and Sediment Extracts Purification Procedure

The liquid chromatography purification procedure on open micro-columns was adapted from a protocol developed by Behar *et al.*[9]. Micro-columns were placed in a modified vacuum manifold (lichrolut vacuum manifold, Merck) allowing faster solvent elution. 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 sulphur that could affect GC-MS analyses. Eluates were concentrated under nitrogen stream to 500 µl, the solvent was then changed 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 micro-columns were washed and conditioned by eluting 10 ml of pentane. Both fractions were concentrated under nitrogen stream, transferred again into isooctane (i.e., by adding 500 µl of isooctane and then concentrating the sample to remove methylene chloride or pentane) and finally analyzed using GC-MS.

### Gas Chromatography-Mass Spectrometry Analyses

Saturated and aromatic fractions were analyzed separately by GC-MS. The GC was an HP 5890 series II (Hewlett-Packard, Palo Alto, CA, USA) equipped with a split/splitless injector (splitless time: 1 min, flow 60 ml/min). The injector temperature was maintained at 270°C. The interface temperature was 290°C. For PAH analyses, the GC temperature program was: from 50°C (2 min) to 290°C (20 min) at 2°C/min. For alkane analyses, the temperature gradient was: from 50°C (2 min) to 290°C (30 min) at 3°C/min. The carrier gas was helium at a constant flow of 1 ml/min. The capillary column used was a CP-SIL8 CB (Chrompack, Middelburg, The Netherlands): 60 m × 0.25 mm ID × 0.10 µm film thickness. The GC was coupled to an HP 5972 Mass Selective Detector (MSD) (Electron Impact: 70 eV, voltage: 2000 V). PAH quantification was performed using the single ion monitoring (SIM) mode with the molecular ions of each compound at 1.4 cycles/s. Alkane analyses were also performed with the SIM mode using the ion $m/z$ 57 for alkanes and $m/z$ 66 for perdeuterated alkanes at 1.7 cycles/s. For full scan analyses, the signal was recorded from $m/z$ 50 to $m/z$ 550 at 1.5 scan/s.

Some coelutions have to be noted between structural isomers: chrysene (Chrys) coelutes with triphenylene (Triph); benzo[b]fluoranthene (B[b]F) coelutes with

TABLE I Target compounds and surrogate internal standards (IS) used for quantification

| *PAHs* | | | | | *Alkanes* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *m/z* | *Compounds* | *IS* | *m/z* | | *m/z* | *Compounds* | *IS* | *m/z* |
| Phen | 178 | Phenanthrene | P-$d_{10}$ | 188 | $n$-C$_{12}$ | 57 | Dodecane | C$_{12}$-$d_{26}$ | 66 |
| Anth | 178 | Anthracene | P-$d_{10}$ | 188 | $n$-C$_{13}$ | 57 | Tridecane | C$_{12}$-$d_{26}$ | 66 |
| DBT | 184 | Dibenzothiophene | Dbt-$d_8$ | 192 | $n$-C$_{14}$ | 57 | Tetradecane | C$_{14}$-$d_{30}$ | 66 |
| Fluo | 202 | Fluoranthene | Fluo-$d_{12}$ | 212 | $n$-C$_{15}$ | 57 | Pentadecane | C$_{15}$-$d_{32}$ | 66 |
| Pyr | 202 | Pyrene | Pyr-$d_{12}$ | 212 | $n$-C$_{16}$ | 57 | Hexadecane | C$_{16}$-$d_{34}$ | 66 |
| BaA | 228 | Benz[a]anthracene | Chrys-$d_{12}$ | 240 | $n$-C$_{17}$ | 57 | Heptadecane | C$_{20}$-$d_{42}$ | 66 |
| Chry | 228 | Chrysene | Chrys-$d_{12}$ | 240 | Prist | 57 | Pristane | C$_{20}$-$d_{42}$ | 66 |
| Trip | 228 | Triphenylene | Chrys-$d_{12}$ | 240 | $n$-C$_{18}$ | 57 | Octadecane | C$_{20}$-$d_{42}$ | 66 |
| BbF | 252 | Benzo[b]fluoranthene | BaP-$d_{12}$ | 264 | Phyt | 57 | Phytane | C$_{20}$-$d_{42}$ | 66 |
| BjF | 252 | Benzo[j]fluoranthene | BaP-$d_{12}$ | 264 | $n$-C$_{19}$ | 57 | Nonadecane | C$_{20}$-$d_{42}$ | 66 |
| BkF | 252 | Benzo[k]fluoranthene | BaP-$d_{12}$ | 264 | $n$-C$_{20}$ | 57 | Eicosane | C$_{20}$-$d_{42}$ | 66 |
| BeP | 252 | Benzo[e]pyrene | BaP-$d_{12}$ | 264 | $n$-C$_{22}$ | 57 | Docosane | C$_{20}$-$d_{42}$ | 66 |
| BaP | 252 | Benzo[a]pyrene | BaP-$d_{12}$ | 264 | $n$-C$_{24}$ | 57 | Tetracosane | C$_{24}$-$d_{50}$ | 66 |
| Per | 252 | Perylene | BaP-$d_{12}$ | 264 | $n$-C$_{26}$ | 57 | Hexacosane | C$_{24}$-$d_{50}$ | 66 |
| IP | 278 | Indeno[1,2,3-cd]pyrene | BP-$d_{12}$ | 288 | $n$-C$_{28}$ | 57 | Octacosane | C$_{30}$-$d_{62}$ | 66 |
| BP | 278 | Benzo[ghi]perylene | BP-$d_{12}$ | 288 | $n$-C$_{30}$ | 57 | Triacontane | C$_{30}$-$d_{62}$ | 66 |
| DA | 276 | Dibenz[a,h]anthracene | BP-$d_{12}$ | 288 | | | | | |

benzo[j]fluoranthene (B[j]F) and benzo[k]fluoranthene (B[k]F); dibenz[a,h]anthracene (D[a,h]A) coelutes with dibenz[a,c]anthracene (D[a,c]A). The concentrations found for these were calculated as follows: Chrys + Trip, BF = B[b]F + B[j]F + B[k]F and DA = D[a,h]A + D[a,c]A.

### Calculation of Alkanes and PAH Concentrations

PAHs and alkanes were quantified relative to perdeuterated PAHs and alkanes (Table I) spiked to the samples prior to extraction. PAH response factors were measured by injecting a standard reference material solution (SRM 2260, NIST, Gaithersburg, MD, USA) spiked with the perdeuterated compound solution introduced in the sample at the beginning of the sample preparation procedure. Response factors of each alkane were measured by injecting a homemade alkane solution spiked with the internal standard perdeuterated alkane solution used. Response factors were determined every 10 h as some day-to-day variations of the instrumental response factors of PAHs were observed previously by Baumard and Budzinski[10].

## RESULTS AND DISCUSSION

### Purification Procedure Efficiency

As sedimentary and petroleum organic extracts contain a lot of macromolecules and polar compounds (NSO compounds) that can clog up the gas chromatograph injector and interfere with analytes of interest, it is necessary to purify them prior to GC-MS analyses. Moreover, aliphatic and aromatic fractions have to be analyzed separately as some interferences between alkanes and PAHs can occur during GC-MS analyses[11].

The validity of the sample preparation has been checked from a qualitative point of view. Figure 1 represents the chromatograms of the SRM 1941a aliphatic (A) and aromatic (B) fractions. Figure 2 represents the chromatograms of the SRM 1582 aliphatic (A) and aromatic (B) fractions. The target compounds appear in the adequate fraction, there is no overlapping between aliphatic and aromatic compounds. The purity of each compound was checked by full scan analysis.

Absolute compound losses due to the purification step were evaluated on a standard solution. Alkane and PAH concentrations were compared with the ones

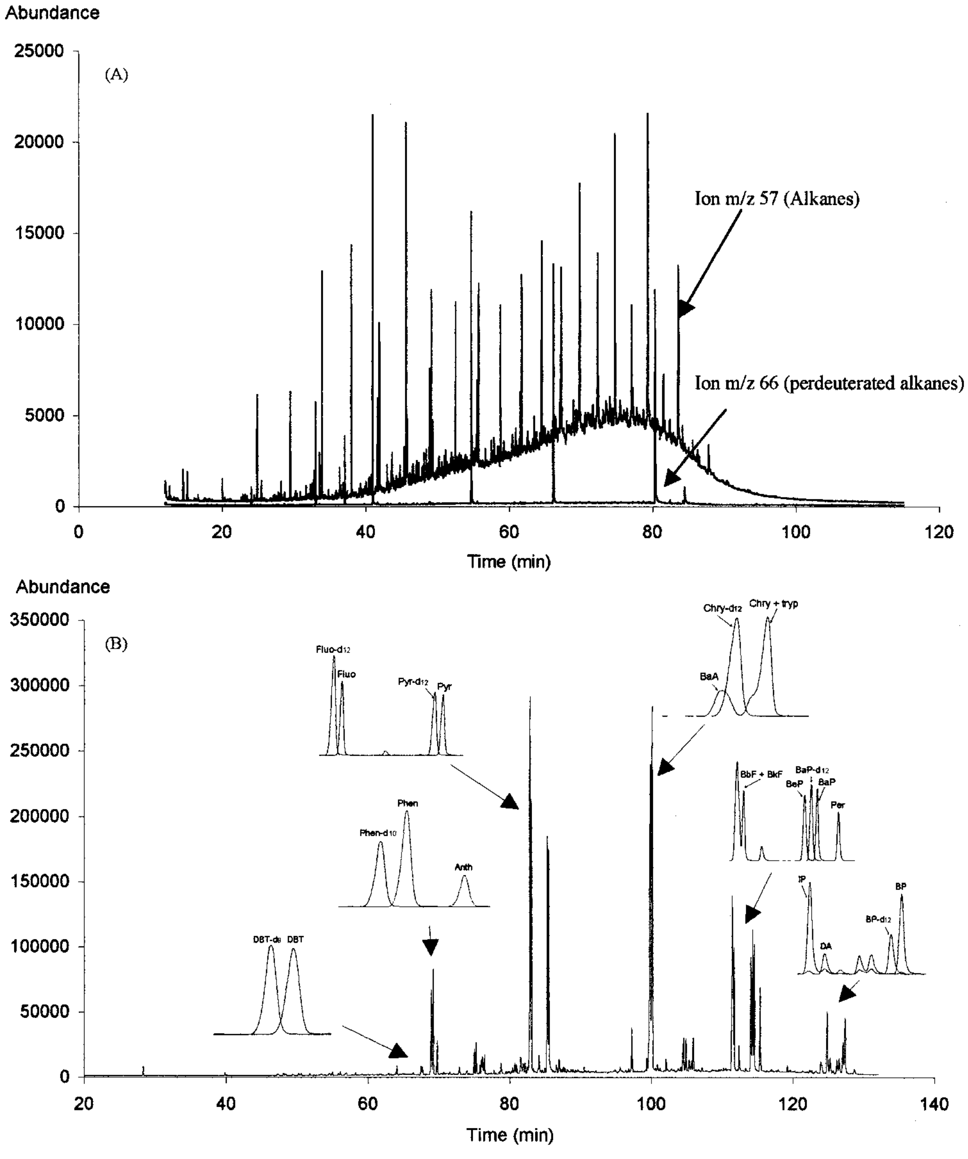

FIGURE 1 GC-MS chromatograms (SIM) of SRM 1941a aliphatic (A) and aromatic (B) fractions.

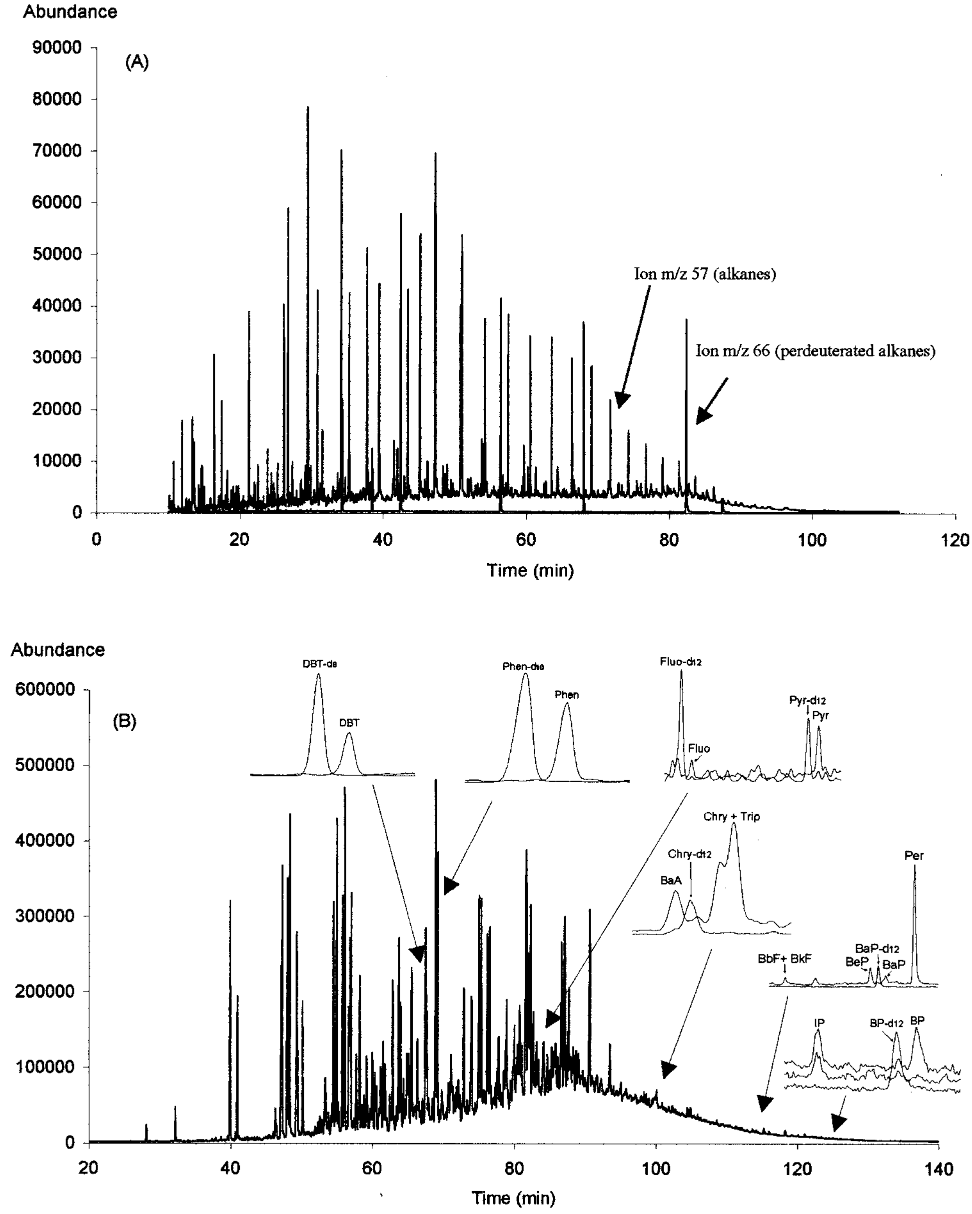

FIGURE 2 GC-MS chromatograms (SIM) of SRM 1582 aliphatic (A) and aromatic (B) fractions.

determined when internal standards were added after the purification step. Alkane absolute losses are presented in Fig. 3. Absolute losses for alkanes comprised between decane and hexadecane were around 50%, between heptadecane and eicosane around 15% and, over docosane, lower than 10%. The important losses observed for low molecular weight alkanes might be due to the evaporation step performed between alumina and silica gel purification. Elution volumes used on alumina and on silica

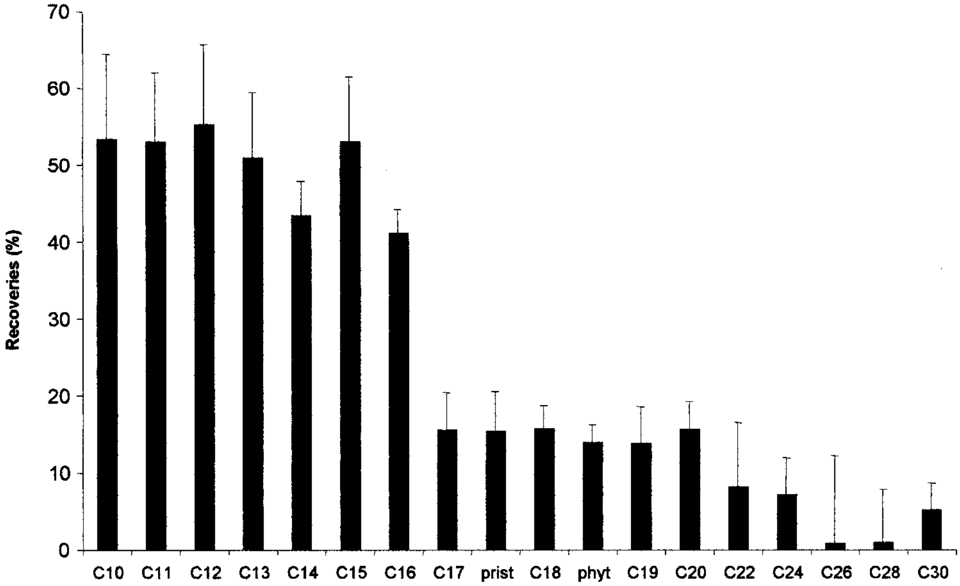

FIGURE 3 Alkane losses (%) due to the purification step.

gel micro-columns are sufficient to quantitatively recover alkanes until triacontane as absolute loss is lower than 5% for this compound. Alkane absolute losses were reproducible as uncertainties are lower than 10% for each compound. PAH absolute losses are presented in Fig. 4. Phenanthrene and anthracene losses were around 30%. For fluoranthene and pyrene losses around 15% were observed. For the other PAHs, absolute losses were approximately 5%. Three and four aromatic ring PAHs might be partly lost during reconcentration between alumina and silica purification. The 5% losses observed for the other PAHs might happen during transfers between the different vials used. Elution volumes used both on alumina and on silica gel micro-columns are sufficient to quantitatively recover PAHs of interest. PAH absolute losses were reproducible as uncertainties were lower than 10% for each compound.

Those results point out the fact that internal standards have to be added at the beginning of the sample preparation procedure in order to compensate for compound absolute losses and then to perform accurate quantification.

## Importance of Using Several and Adequate Surrogate Internal Standards

As some sample preparation steps can introduce selective alkane and PAH losses, it is necessary to use internal standards that will behave in the same way as target compounds during the entire protocol. Ideal standards are isotope-labeled versions of the target analytes, with sufficient isotope labels in each molecule that the natural abundance of this species in the samples is negligible[12]. Perdeuterated internal standards are adequate as they are absent from natural samples, they have similar physical and chemical properties to target analytes and they give a signal without interference with compounds of interest during GC-MS analysis.

For PAH analysis, Baumard and Budzinski[10] have already shown that it is necessary to use at least one internal standard per class of aromaticity in order to determine accu-

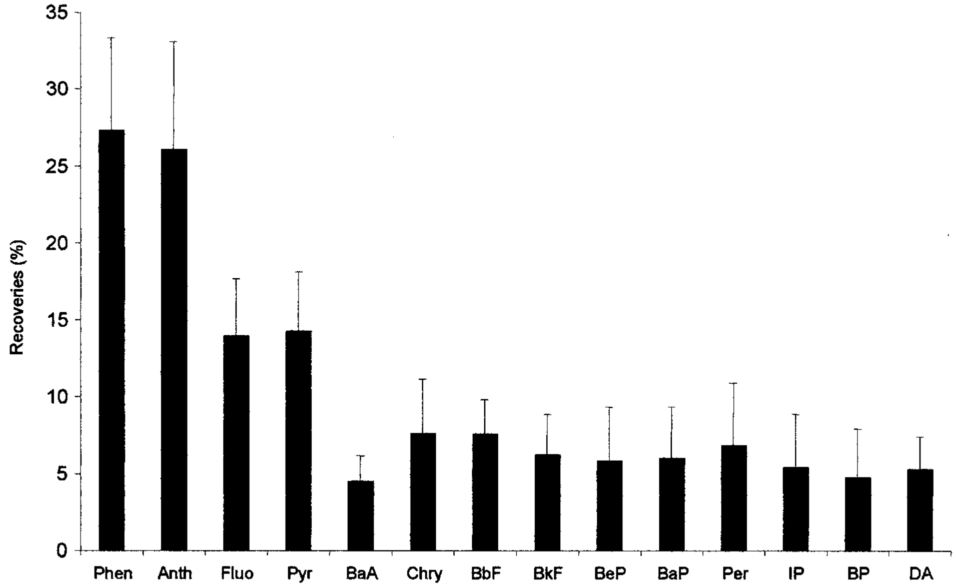

FIGURE 4 PAH absolute losses (%) due to the purification step.

rate concentrations for all PAHs. The necessity of using several perdeuterated internal standards for alkane quantification was then investigated in this study.

An alkane solution spiked with seven internal standards (perdeuterated $n$-$C_{12}$-$d_{26}$, $n$-$C_{14}$-$d_{30}$, $n$-$C_{15}$-$d_{32}$, $n$-$C_{16}$-$d_{34}$, $n$-$C_{20}$-$d_{42}$, $n$-$C_{24}$-$d_{50}$, $n$-$C_{30}$-$d_{62}$) was analyzed by GC-MS to study the importance of using several internal standards for the quantification step. Alkane concentrations were calculated relatively to $n$-$C_{14}$-$d_{30}$, to $n$-$C_{24}$-$d_{50}$ and using the seven internal standards as mentioned in Table I. The mean recoveries were 101%, 95% and 100% when $n$-$C_{14}$-$d_{30}$, $n$-$C_{24}$-$d_{50}$ and the seven internal standards were respectively used. Figure 5 represents the discrepancies between the nominal $n$-alkane concentrations present in the solution analyzed and the $n$-alkane concentrations determined using each quantification procedure. Individual recoveries were all comprised between 95% and 105% when seven internal standards were used. Recoveries under or overestimation higher than 5% were observed for several compounds ($n$-$C_{12}$, $n$-$C_{13}$, $n$-$C_{24}$, $n$-$C_{28}$, $n$-$C_{30}$) when the two other procedures were used. This shows that the use of several adequate internal standards is required for accurate GC-MS quantification of $n$-alkanes.

SRM 1941a was analyzed following the sample preparation procedure described in this paper. Alkane concentrations were calculated relatively to $n$-$C_{14}$-$d_{30}$, to $n$-$C_{24}$-$d_{50}$ and using the seven internal standards as mentioned in Table I. Figure 6 shows the discrepancies between the NIST $n$-alkane concentrations and the $n$-alkane concentrations determined in SRM 1941a using each quantification procedure. When a unique standard was used, some quantifications were 50% under or overestimated for several compounds. Indeed, when $n$-$C_{14}$-$d_{30}$ was used as unique standard, an underestimation of lower molecular weight compounds and an overestimation of higher molecular weight compounds were observed. This phenomenon is due to differential evaporation of compounds compared to the internal standard ($n$-$C_{14}$-$d_{30}$) during the

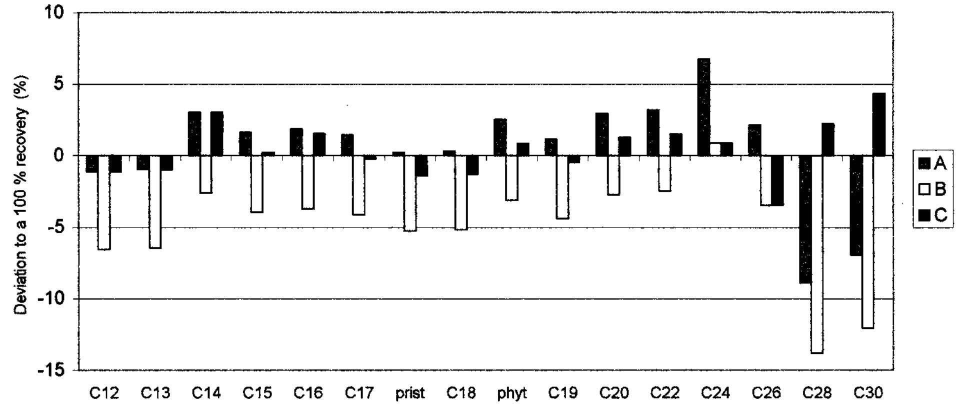

FIGURE 5 Discrepancies between nominal alkane concentrations and concentrations determined using the developed protocol of a standard alkane solution analyzed by GC-MS: A: quantification relatively to $n$-$C_{14}$-$d_{30}$; B: quantification relatively to $n$-$C_{24}$-$d_{50}$; C: quantification relatively to seven internal standards.

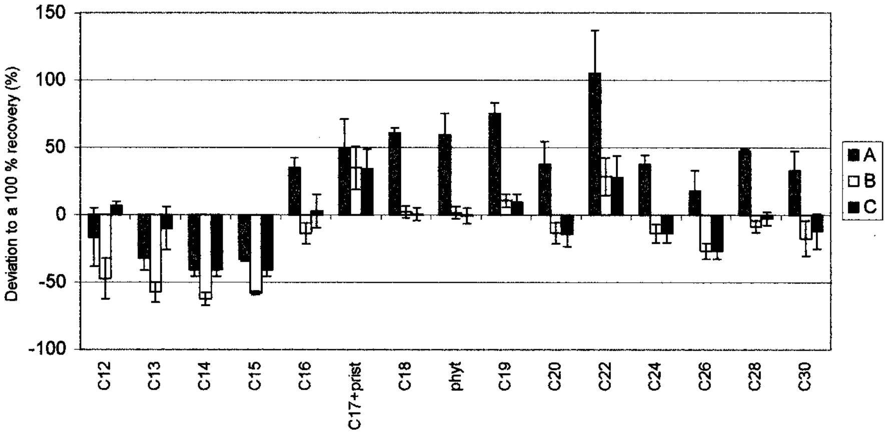

FIGURE 6 Discrepancies between the NIST alkane concentrations and the concentrations determined using the sample preparation procedure developed in SRM 1941a sediment analyzed by GC-MS: A: quantification relatively to $n$-$C_{14}$-$d_{30}$; B: quantification relatively to $n$-$C_{24}$-$d_{50}$; C: quantification relatively to seven internal standards.

analytical procedure. When $n$-$C_{24}$-$d_{50}$ was used, an underestimation of low molecular weight compounds, lost during concentration steps, was observed. This illustrates the fact that when compounds are not quantified relative to an internal standard that has similar physico-chemical properties, some important quantification errors can occur early. When the seven surrogate internal standards were used, results coincided well with the indicative values given by the NIST.

## Analytical Procedure Validation on Reference Materials

The sample preparation procedure using micro-column purification was applied to a sediment (SRM 1941a) and a crude oil (SRM 1582) reference material.

### *Validation on a Marine Sediment Reference Material (SRM 1941a)*

Alkane concentrations determined in SRM 1941a sediment are compared to the NIST indicative values on Fig. 7. PAH concentrations determined in SRM 1941a sediment are compared to the NIST-certified concentrations in Fig. 8.

The average recovery of the sum of alkanes compared to indicative concentrations is 89 ± 9%. This result is in agreement with the NIST concentrations as the indicative value of the sum of alkanes is given with a 16% standard deviation. Individual recoveries

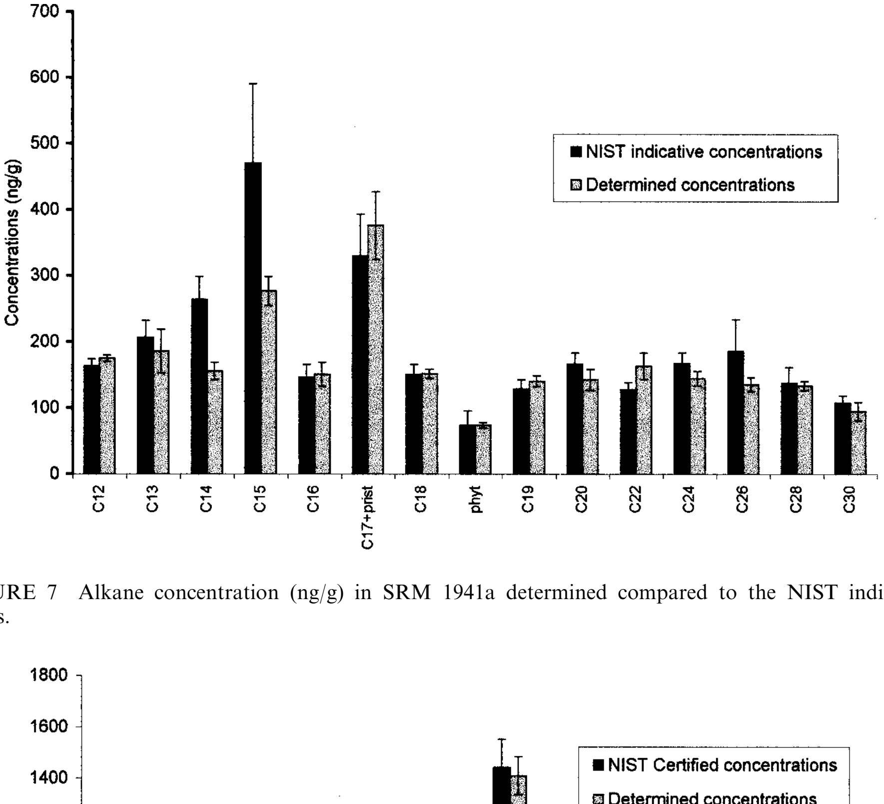

FIGURE 7 Alkane concentration (ng/g) in SRM 1941a determined compared to the NIST indicative values.

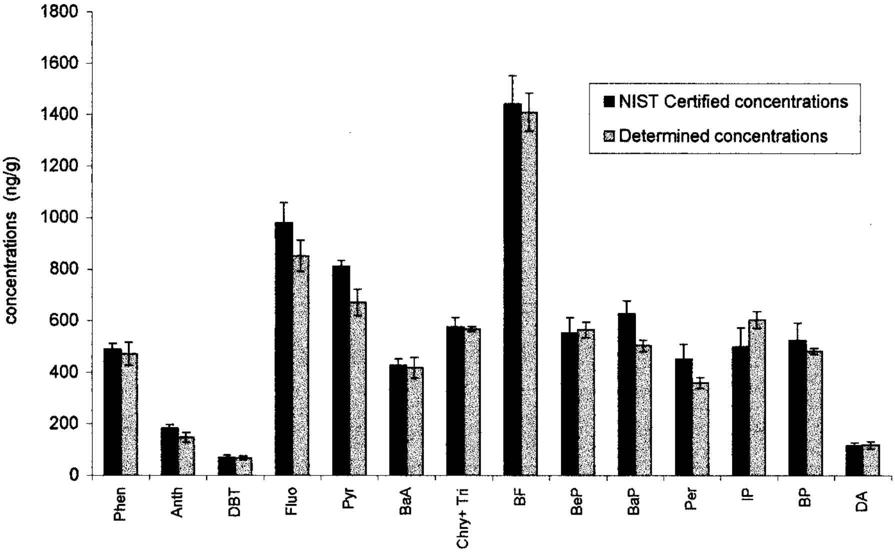

FIGURE 8 PAH concentrations (ng/g) in SRM 1941a determined compared to the NIST certified values.

are in good agreement with indicative concentrations except for $n$-$C_{14}$ and $n$-$C_{15}$ for which recoveries are around 60%. The reason for the discrepancy between the indicative concentrations and the concentrations determined using the analytical procedure for $n$-$C_{14}$ and $n$-$C_{15}$ is unknown. These two compounds are quantified relative to their perdeuterated homologues ($n$-$C_{14}$-$d_{30}$ and $n$-$C_{15}$-$d_{32}$) that do not suffer from coelution during GC-MS analysis as checked by full scan analysis. As $n$-$C_{14}$-$d_{30}$ and $n$-$C_{15}$-$d_{32}$ are used, volatilization cannot account for those low values. Standard deviations obtained for alkanes were below 10% except for $n$-$C_{13}$ (18%), $n$-$C_{17}$ + prist (14%) and $n$-$C_{26}$ (15%).

The average recovery of the sum of PAHs compared to certified concentration is 96 ± 6%. Individual recoveries are for the less important ones around 80% (anthracene, fluoranthene, pyrene, benzo[a]pyrene and perylene). For the other PAHs, recoveries are around 100% except for indeno[l,2,3-cd]pyrene for which recovery is around 115%. PAH concentrations were determined with good reproducibility as standard deviations obtained for all PAH concentrations were below 10%.

### *Validation on a Crude Oil Reference Material (SRM 1582)*

Alkane concentrations determined in SRM 1582 crude oil are given in Table II. PAH concentrations measured for SRM 1582 crude oil are compared to the NIST certified values in Table III.

The accuracy of alkane concentrations determined in SRM 1582 cannot be evaluated as reference values were not provided by the NIST. Nevertheless, it is to be noted that alkane concentrations were determined with good reproducibility as uncertainties are lower than 10%, except for $C_{12}$ (11%) and $C_{30}$ (13%).

In SRM 1582, as in most petroleums, low molecular weight PAHs (naphthalene, dibenzothiophene and phenanthrene) are much more abundant than higher molecular weight compounds. It was then first necessary to dilute the aromatic fractions prior to GC-MS analysis for phenanthrene and dibenzothiophene quantification in order not to have a chromatographic column saturation for those compounds. Aromatic fractions were then concentrated and reanalyzed for quantification of higher molecular weight PAHs. PAH concentrations are in relatively good agreement with certified

TABLE II Alkane concentrations (μg/g) in SRM (Conc = concentration; CV = coefficient of variation)

| *Compounds* | *Conc (μg/g)* | *CV%* |
|---|---|---|
| $C_{12}$ | 691 ± 74 | 11 |
| $C_{13}$ | 844 ± 56 | 7 |
| $C_{14}$ | 736 ± 14 | 2 |
| $C_{15}$ | 815 ± 16 | 2 |
| $C_{16}$ | 719 ± 27 | 4 |
| $C_{17}$ + pristane | 2250 ± 247 | 11 |
| $C_{18}$ | 697 ± 54 | 8 |
| Phytane | 1289 ± 28 | 2 |
| $C_{19}$ | 612 ± 34 | 5 |
| $C_{20}$ | 631 ± 21 | 3 |
| $C_{22}$ | 609 ± 27 | 4 |
| $C_{24}$ | 503 ± 55 | 1 |
| $C_{26}$ | 324 ± 20 | 6 |
| $C_{28}$ | 184 ± 4 | 2 |
| $C_{30}$ | 175 ± 23 | 13 |

TABLE III PAH concentrations (μg/g) in SRM 1582 determined compared to the NIST certified values (conc = concentration; CV = coefficient of variation)

| | *NIST concentrations* | | *Experimental concentrations* | | *Recovery %* |
|---|---|---|---|---|---|
| | *conc* (μg/g) | *CV%* | *conc* (μg/g) | *CV%* | |
| Phenanthrene | 101[a] ± 5 | 4.9 | 110 ± 2 | 1.6 | 109 |
| Dibenzothiophene | 33[a] ± 2 | 6 | 40 ± 0.4 | 1 | 121 |
| Fluoranthene | 2.5[a] ± 0.3 | 12 | 2.7 ± 0.5 | 18.3 | 109 |
| Pyrene | 7[b] | | 8.6 ± 0.5 | 6.2 | 124 |
| Benz[a]anthracene | 3[a] ± 0.3 | 10 | 2.9 ± 0.1 | 4.4 | 97 |
| Benzo[a]pyrene | 1.1[a] ± 0.3 | 27.3 | 1.3 ± 0.05 | 3.5 | 121 |
| Benzo[e]pyrene | 3.5[b] | | 3.3 ± 0.1 | 4.3 | 94 |
| Perylene | 31[a] ± 3 | 9.7 | 29.3 ± 1.6 | 5.6 | 95 |
| Benzo[ghi]perylene | 1.7[b] | | 1.6 ± 0.1 | 7.9 | 94 |
| Indeno[1,2,3-cd]pyrene | 0.17[b] | | nd | | |

[a] certified concentration.

[b] noncertified concentration.

values given by the NIST as recoveries were comprised between 94% and 124%. Indeno[1,2,3-cd]pyrene was not quantified because this compound was in too small an amount (0.17 μg/g) in this crude oil. If more accurate concentrations of high molecular weight PAHs are needed, an HPLC fractionation[13] can be performed in order to collect specifically, PAHs with more than three aromatic rings.

### Application to the Pollution Source Assessment of Sediment after the Erika Oil Spill

The Maltese tanker Erika broke in two, close to the Atlantic coast of Brittany, in France, in the early morning on the 12th of December 1999. The tanker was traveling from Dunkerque (France) to Livorno (Italy) with a cargo of approximately 30,000 tons of Heavy Fuel Oil. About 10,000 tons of oil are believed to have spilled into the sea[14]. Oil from the tanker Erika came ashore on the 25th of December 1999. It was then necessary to quantify PAHs contained in the Erika oil in order to get an idea of the potential toxicity of this product. Moreover, alkane and PAH quantifications are helpful for correlation with samples collected on the beaches of the Atlantic coast of France.

The sample preparation procedure described in this paper was applied to a sample of the oil carried by the Erika tanker. Concentrations of aliphatic hydrocarbons and PAHs are presented in Tables IV and V respectively. Methyl and dimethyl-dibenzothiophenes were detected using ions $m/z$ 198 and $m/z$ 212 respectively. For methyl and dimethyl-phenanthrenes detection, ions $m/z$ 192 and $m/z$ 206 respectively were used. Alkylated dibenzothiophenes and phenanthrenes were quantified using dibenzothiophene $d$-8 and phenanthrene $d$-10 respectively as surrogate internal standards.

Aliphatic and aromatic hydrocarbons were quantified with good repeatability in this heavy oil product as uncertainties were lower than 15% in most of the cases.

The saturated fraction is featured by a homologous series of $n$-alkanes ranging from $n$-$C_{12}$ to $n$-$C_{30}$ with a maximum around $n$-$C_{24}$ to $n$-$C_{26}$. Isoprenoids are also present, with pristane and phytane being the most abundant. The evolution of $n$-$C_{17}$/pristane and $n$-$C_{18}$/phytane ratio values can then provide information on the effect of microbial biodegradation on the losses of hydrocarbons in the impacted area as $n$-alkanes are more rapidly biodegraded than isoprenoids.

TABLE IV Alkane concentrations (μg/g) in SRM 1582 determined in the oil spilled in the Atlantic ocean after the Erika oil spill (Conc = concentration; CV = coefficient of variation)

| *Compounds* | *Conc* (μg/g) | *CV%* |
|---|---|---|
| $C_{12}$ | 254 ± 10 | 4 |
| $C_{13}$ | 345 ± 25 | 7 |
| $C_{14}$ | 308 ± 23 | 7 |
| $C_{15}$ | 325 ± 18 | 5 |
| $C_{16}$ | 341 ± 17 | 5 |
| $C_{17}$ | 443 ± 36 | 8 |
| Pristane | 143 ± 11 | 8 |
| $C_{18}$ | 484 ± 50 | 10 |
| Phytane | 166 ± 21 | 13 |
| $C_{19}$ | 594 ± 36 | 6 |
| $C_{20}$ | 775 ± 49 | 6 |
| $C_{21}$ | 1020 ± 48 | 5 |
| $C_{22}$ | 1561 ± 75 | 5 |
| $C_{23}$ | 1957 ± 74 | 4 |
| $C_{24}$ | 2344 ± 155 | 7 |
| $C_{25}$ | 2406 ± 72 | 3 |
| $C_{26}$ | 2463 ± 192 | 8 |
| $C_{27}$ | 1981 ± 331 | 17 |
| $C_{28}$ | 1447 ± 306 | 21 |
| $C_{29}$ | 1184 ± 121 | 10 |
| $C_{30}$ | 1016 ± 46 | 5 |

TABLE V PAH concentrations (μg/g) determined in the oil spilled in the Atlantic Ocean after the Erika oil spill (Conc = concentration; CV = coefficient of variation)

| *Compounds* | *Conc* (μg/g) | *CV%* |
|---|---|---|
| Phen | 614 ± 34 | 5 |
| $C_1$-Phen | 2743 ± 212 | 8 |
| $C_2$-Phen | 3172 ± 314 | 10 |
| $C_3$-Phen | 2748 ± 356 | 13 |
| DBT | 226 ± 14 | 6 |
| $C_1$-DBT | 617 ± 50 | 8 |
| $C_2$-DBT | 1226 ± 128 | 10 |
| $C_3$-DBT | 1251 ± 166 | 13 |
| Anth | 90 ± 11 | 12 |
| Fluo | 46 ± 5 | 12 |
| Pyr | 218 ± 33 | 15 |
| BaA | 152 ± 11 | 7 |
| Chrys+TriP | 324 ± 19 | 6 |
| B(b+k)F | 69 ± 9 | 13 |
| BeP | 101 ± 10 | 10 |
| BaP | 96 ± 13 | 14 |
| Per | 46 ± 6 | 13 |
| IP | 15 ± 2 | 12 |
| BP | 40 ± 2 | 5 |
| DA | 23 ± 1 | 5 |

The aromatic fraction is characterized by high concentrations of phenanthrene and dibenzothiophene compounds. Pyrene is much more abundant than fluoranthene. The ratio between the concentrations of these two isomers (Fluo/Pyr = 0.2) can be used as a source diagnostic ratio. Erika oil presents a high content of high molecular weight PAHs such as benzo[a]pyrene which is one of the most toxic PAHs.

The concentrations of saturated and aromatic hydrocarbons were then determined in sediments collected in a part of the Atlantic coast suspected to have been impacted by the Erika oil.

The saturated hydrocarbon concentrations measured in two of those sediments are compared with those of the Erika oil (Fig. 9) in order to illustrate the usefulness of quantitative data for oil pollution source assessment. In those two sediments,

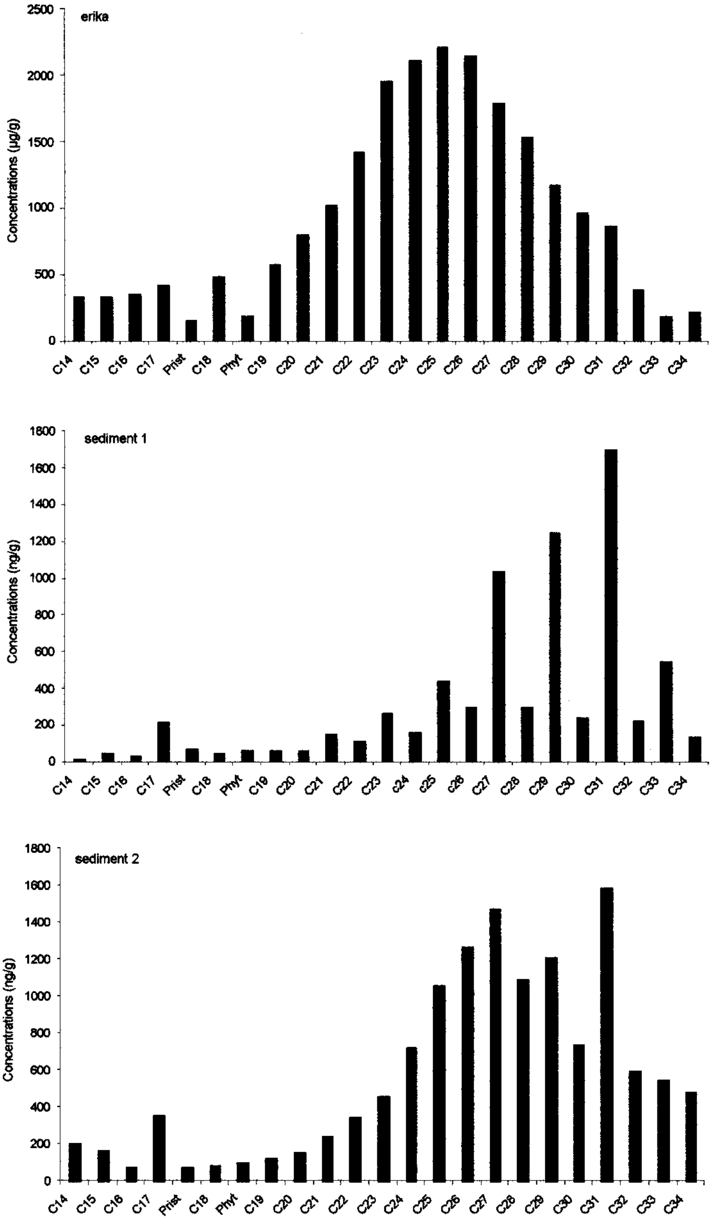

FIGURE 9 Comparison between the concentrations of saturated hydrocarbons measured in the Erika oil and in two sediments collected in a part of the Atlantic coast suspected to have been impacted by the Erika oil.

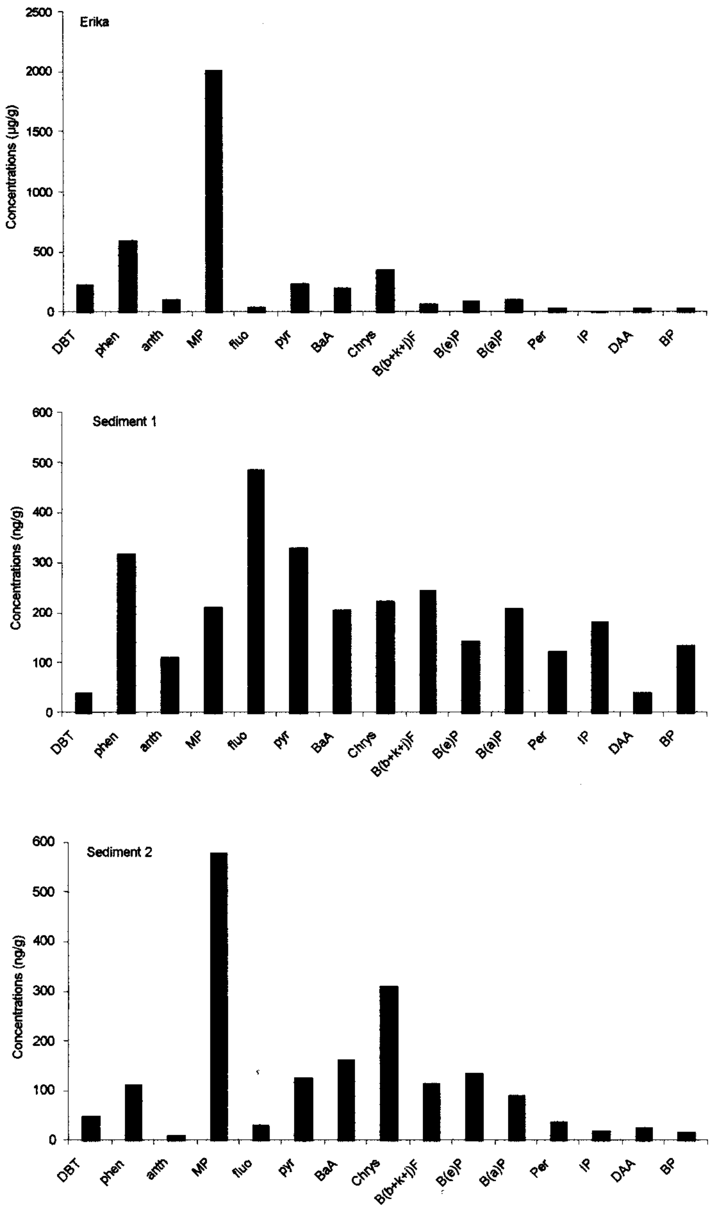

FIGURE 10 Comparison between the concentrations of aromatic hydrocarbons measured in the Erika oil and in two sediments collected in a part of the Atlantic coast suspected to have been impacted by the Erika oil.

saturated hydrocarbon concentrations are in the same order of magnitude. Sediment 1 shows a molecular profile featured by the predominance of alkanes containing an odd number of carbon atoms between the $n$-$C_{21}$ and the $n$-$C_{33}$. This profile is typical of biogenic saturated hydrocarbons. Sediment 2 shows a molecular profile closer to the Erika oil with a homologous series of alkanes with a maximum around $n$-$C_{27}$ with still a predominance of the $n$-$C_{27}$, $n$-$C_{29}$ and $n$-$C_{31}$. This molecular profile reflects the presence of both petrogenic and biogenic hydrocarbons. Those results provide evidence for the contamination of this sediment by a heavy petroleum product.

The examination of PAH concentrations should be helpful in pointing out if this sediment has been impacted by the heavy fuel oil released by the Erika tanker. The concentrations of PAH measured in those two sediments are compared with the ones measured for the Erika oil in Fig. 10. First it is to be noted that the PAH content in those two sediments are in the same order of magnitude. Nevertheless, they do not show the same molecular profiles. Indeed, in Sediment 1 low and high molecular weight PAHs are present more or less at the same concentration whereas in Sediment 2, high molecular weight compounds are much less. The molecular profile observed in Sediment 2 is quite close to the Erika oil one except that high molecular weight PAH are more abundant compared to the three aromatic PAH. This difference can be due to the preferential losses of the low molecular weight compounds during the transfer of the Erika oil to the environment. If we compare some Erika oil characteristic ratios between the concentration of different PAHs such as Phen/MPs, FIuo/Pyr and B(a)A/Chrys with the ones determined for the sediments (Table VI), it appears clearly that Sediment 2 has been impacted by the Erika oil. Indeed, ratios measured for this Sediment are very close to the ones measured for Erika oil when for respectively Sediment 1 they are significantly different.

## *Acknowledgements*

This work was done in the framework of HYCAR Project ("Biogeochemical cycle of natural and anthropic hydrocarbons in marine system"). Elf Aquitaine and CNRS are acknowledged for financial support. The CEDRE is thanked for the gift of the Erika oil and oil slick samples.

## *References*

[1] J.M. Neff, *Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates and biological effects*, Applied Science Publishers Ltd., London. 262 pp. (1979).

[2] J.L. Lake, C. Norwood, C. Dimock and R. Bowen, *Geochim. Cosmochim. Acta*, **43**, 1847–1854 (1979).

[3] S. Sportsol, N. Gjos, R.G. Lichtenthaler, K.O. Gustovsen, K. Urdal, F. Oreld and J. Skel, *Environ. Sci. Technol.*, **17**, 282–286 (1983).

[4] Z. Wang, M. Fingas and D.S. Page, *J. Chromatog*. A, **843**, 383–395 (1999).

[5] K.L. White, *Carcino. Revs.*, **C4**(2), 163–202 (1986).

[6] A.E. Bence, K.A. Kvenvolden and M.C. Kennicutt, *Org. Geochem.*, **24**, 7–42 (1996).

[7] National Institute of Standards and Technology, USA, Certificate of analysis for Standard Reference Material SRM 1941a, Organic in Marine Sediment (1994).

[8] National Institute of Standards and Technology, USA, Certificate of analysis for Standard Reference Material SRM 1582, Petroleum Crude Oil (1984).

[9] F. Behar, C. Leblond and C. Saint-Pau, *Rev Institut Français du Pétrole*, **44**, 387–411 (1989).

[10] P. Baumard and H. Budzinski, *Analusis*, **25**, 246–252 (1997).

[11] C.K. Huynh and T. Vu Duc, *J. High Res. Chromatogr. Chromatogr. Commun.*, **7**, 270–273 (1984).

[12] R.K. Boyd, *Rapid Commun. Mass Spectrom.*, **7**, 257–271 (1993).

[13] H. Budzinski, P. Garrigues, J. Connan and J. Bellocq, *Quimica Analitica*, **12**, 69–74 (1993).

[14] International Tanker Owners Pollution Federation (ITOPF).