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Determination of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment

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Our cover photo was taken by N. Penny Holliday aboard the RRS "Discovery" in rough seas in the Rockall Trough.

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Abstract

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This document provides advice on the analysis of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in total sediment, sieved fractions, suspended particulate matter, and biota (shellfish). The determination of parent and alkylated PAHs in sediment and biota includes extraction with organic solvents, clean-up, and analysis by gas chromatography (GC) with mass spectrometry (GC-MS). Advice is given on the treatment and storage of samples. Extraction and clean-up methods commonly used are described. GC-MS is the only recommended method for the analysis of both parent and alkylated PAHs and advice is provided on standards and calibration. All steps in the procedure are susceptible to insufficient recovery and/or contamination. Quality-control procedures are recommended to check the performance of the method. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods critically and to improve their procedures and/or the associated quality-control measures, where necessary.

Keywords: polycyclic aromatic hydrocarbons, sediment, mussels, sample pretreatment, storage, extraction, clean-up, calibration, gas chromatography, mass spectrometry.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least two fused rings. PAHs in the marine environment arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are found in oil and oil products, including a wide range of alkylated PAHs formed as a result of diagenetic processes. In contrast, PAHs from combustion sources are mainly parent (non-alkylated) PAHs. PAHs in the marine environment are of concern for two main reasons: first, low-molecular-weight PAHs can taint fish and shellfish and render them unfit for sale (Davis *et al.*, 2002); second, metabolites of some of the high-molecular PAHs are potent animal and human carcinogens, benzo[*a*]pyrene being a prime example. Carcinogenic activity is closely related to structure (Harvey, 1991). Benzo[*e*]pyrene and the four benzofluoranthene isomers all have a molecular weight of 252 Da, but are much less potent carcinogens than benzo[*a*]pyrene. Less is known about the toxicity of alkylated PAHs, although one study has demonstrated that alkylated PAHs may have greater toxicity than the parent compound (Marvanova *et al.*, 2008).

PAHs are readily taken up by marine animals, both across gill surfaces, for lower-molecular-weight PAHs, and through their diet (Baumard *et al.*, 1999). They may bioaccumulate, particularly in shellfish. Filter-feeding organisms, such as bivalve molluscs, can accumulate high concentrations of PAHs, because of exposure to chronic discharges to the sea, for example, of sewage and marine oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet. However, they do not generally accumulate high concentrations of PAHs, because they possess an effective mixed-function oxygenase (MFO) system that allows them to metabolize PAHs and to excrete them in bile (Stagg *et al.*, 1995; Richardson *et al.*, 2001). Other marine vertebrates and marine mammals also metabolize PAHs efficiently. An assessment of the exposure of fish to PAHs therefore requires the determination of PAH metabolite concentrations in bile, because turnover times can be extremely rapid.

There are marked differences in the behaviour in the aquatic environment between the low-molecular-weight PAHs, such as naphthalene (128 Da), and the high-molecular-weight PAHs, such as benzo[*g,h,i*]perylene (276 Da), because of their differing physicochemical properties. The low-molecular-weight compounds are appreciably water soluble, naphthalene for example, and can be bioaccumulated from the dissolved phase by transfer across gill surfaces. The high-molecular-weight compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited in the sea already adsorbed onto atmospheric particulates, such as soot particles. Sediment will act as a sink for PAHs in the marine environment.

The objective of these guidelines is to provide advice on the analysis of parent and alkylated PAHs in total sediment, sieved fractions, suspended particulate matter, and biota. The determination of parent and alkylated PAHs in sediment and biota generally includes extraction with organic solvents, clean-up, and analysis by gas chromatography (GC) with mass-spectrometric (MS) detection. All steps in the procedure are susceptible to insufficient recovery and/or contamination. Quality-control procedures are recommended to check the performance of the method. These

guidelines are intended to encourage and assist analytical chemists to evaluate their methods critically and to improve their procedures and/or the associated quality-control measures, where necessary.

2 Appropriate species for analysis of parent and alkylated PAHs

For the Northeast Atlantic area, guidance on the selection of appropriate species for contaminant monitoring is given in the OSPAR Joint Assessment and Monitoring Programme guidelines (OSPAR, 1999). The HELCOM COMBINE Manual[1] provides advice on appropriate species for the Baltic Sea. All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish, particularly molluscs, generally have a lesser metabolic capacity for PAHs; therefore, they are preferred for monitoring, because PAH concentrations are generally higher in their tissues.

The blue mussel (*Mytilus edulis*) occurs in shallow waters along almost all coasts of the Northeast Atlantic. It is therefore suitable for monitoring in nearshore waters. No distinction is made between *M. edulis* and *M. galloprovincialis*, because the latter species, which can occur along Spanish and Portuguese coasts, fills a similar ecological niche. Samples should be of a similar size, 30–70 mm shell length. In some areas (e.g. the Barents Sea), other species may be considered. Recent monitoring studies have indicated a seasonal cycle in PAH concentrations, particularly for combustion-derived PAHs in mussels, with maximum concentrations in winter before spawning and minimum concentrations in summer (Webster *et al.*, 2006; Hellou *et al.*, 2005). It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year and preferably in the first months of the year, before spawning.

For the purposes of temporal trend monitoring, it is essential that long time-series with either a single species or a limited number of species be obtained. Care should be taken that the sample is representative of the population and that sampling can be repeated annually. There are advantages in the use of molluscs for this purpose, because they are sessile; they therefore reflect contamination in the local area to a greater degree than fish, which are mobile. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies. Associations have been demonstrated between the incidence of some diseases in flatfish (e.g. liver neoplasia) and the concentrations of PAHs in the sediments over which they live and feed (Malins *et al.*, 1988; Vethaak and Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH metabolites in bile and by measuring the induction of mixed-function oxygenase enzymes that catalyse the formation of these metabolites (Richardson *et al.*, 2001).

[1] http://www.helcom.fi/groups/monas/CombineManual/en_GB/main/

3 Pretreatment and storage

3.1 Contamination

Sample contamination can occur during sampling, sample handling, pretreatment, and analysis, owing to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships, there are multiple sources of PAHs, such as the oils used for fuel and lubrication and the exhaust from the ship's engines. It is important that likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination could occur. A ship is a working vessel, and procedures carried out during day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) might affect the sampling process. It is advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of sediment or biota samples may be a source of contamination from the back-streaming of oil vapours from the rotary vacuum pumps. Moreover, drying the samples may result in loss of the more volatile lower-molecular-weight PAHs through evaporation (Law and Biscaya, 1994). Therefore, possible losses and contamination must be checked before freeze-drying samples.

Plastic materials must not be used for sampling and storage because of possible adsorption of the PAHs onto the container material. Sediment samples should be transported in closed containers; a temperature of 25 °C should not be exceeded. Live biota should be transported in closed containers at temperatures between 5 °C and 10 °C. For live animals, it is important that the transport time is short and controlled (e.g. maximum of 24 h).

If the samples are not analysed within 48 h after collection, they must be stored at 4 °C (short-term storage). Storage over several months is only possible for frozen (i.e. below −20 °C) or dried samples (Law and de Boer, 1995).

Because PAHs are sensitive to photodegradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples, as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994).

3.2 Shellfish

3.2.1 Depuration

Depuration of shellfish may be required to void the gut contents and any associated contaminants before freezing or sample preparation. Shellfish collected close to point sources are usually depurated. The gut contents of these shellfish may contain significant quantities of PAHs associated with food and sediment particles not assimilated into the tissues of the mussels. Depuration should be done under controlled conditions in clean seawater; depuration for a period of 24 h is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

3.2.2 Dissection and storage

Mussels should be shucked live and opened with minimal tissue damage, by detaching the adductor muscles from the interior of at least one valve. The soft tissues should be removed, homogenized as soon as possible, and frozen in solvent-washed glass jars or aluminium cans at −20 °C until analysis.

When samples are processed, both at sea and onshore, the dissection must be undertaken on a clean bench by trained personnel wearing clean gloves and using PAH-free stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment, such as homogenizers, should be cleaned by wiping with tissue and rinsing with solvent.

3.3 Sediment

Before taking a subsample for analysis, the samples should be thoroughly mixed using a metal spatula. The mass of sample extracted depends on the expected concentrations. For the marine environment, as a rule of thumb, the mass of sample taken for analysis can be equal to an amount representing 50–100 mg organic carbon. PAHs can be extracted from wet or dried samples. However, storage, homogenization, and extraction are much easier when the samples are dry. Care must be taken if freeze-drying samples for the reasons described in Section 3.1. Possible losses and contamination have to be checked. Contamination can be checked by exposing 1–2 g C18-bonded silica to drying conditions and analysing it as a sample (clean-up can be omitted; Smedes and de Boer, 1997). Contamination during freeze-drying can be reduced by placing a lid, with a hole of ca. 3 mm in diameter, on the sample container. This minimizes exposure of the sample to possible contaminants, although not hindering the evaporation of the water.

4 Lipid determination

Although PAH data in shellfish are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterizing the samples. The lipid content should be determined on a separate subsample of the tissue homogenate, because some of the extraction techniques used routinely for PAH determination (e.g. pressurized liquid extraction (PLE) with fat retainers, alkaline saponification) destroy or remove lipid materials. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method, such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been demonstrated that if the lipid content is high (>5%), the extractable lipid will be comparable with the total lipid.

5 Extraction

Exposure to light must be kept to a minimum during extraction and further handling of the extracts (Law and Biscaya, 1994). Photodegradation occurs more rapidly in the absence of a sample matrix, the most photosensitive PAH is benzo[*a*]pyrene followed by anthracene.

5.1 Wet sediments

Wet sediments should be extracted by mixing with organic solvents, using a stepwise procedure. Extraction is enhanced by shaking, Ultra Turrax-mixing, ball-mill tumbling, or ultrasonic treatment. Water-miscible solvents, such as acetone, methanol, or acetonitrile, are used in the first step. The extraction efficiency of the first step will be low, because there is a considerable amount of water in the liquid phase. For sufficient extraction, at least two subsequent extractions are required. Ultrasonication is frequently used for extraction of sediment samples. However, the efficiency of the extraction may decrease, owing to ageing of the sonication probe or bath (Woodhead *et al.*, 1999). The contact time with the solvent should be sufficient to complete desorption of the PAHs from the sediment pores. Heating by microwave or refluxing will accelerate this process.

When using Soxhlet extraction, wet sediments should be extracted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment, the solvent flask is replaced, and the extraction continued with a less polar solvent or solvent mixture (e.g. acetone/hexane). Thereafter, the extracts must be combined. Water must be added to the combined extracts, and the PAHs must be extracted to a non-polar solvent.

Extraction of wet sediments by pressurized liquid extraction (PLE) is a more recent method, requiring less solvent and time. Wet sediment is dried by mixing with sufficient anhydrous sodium sulfate to form a free-flowing mixture that is then packed into stainless steel tubes for extraction. Extractions are done at elevated temperatures and pressures. Various extracting solvents (dichloromethane (DCM), acetone, methanol, acetonitrile, hexane, DCM:acetone (1:1), hexane:acetone (1:1)) were investigated by Saim *et al.* (1998). As long as the solvent was more polar than hexane, no significant differences were noted. Extraction temperatures can be manipulated to suit the analytical requirements.

5.2 Dry sediments

Although all of the methods mentioned above can also be used for dried sediments, Soxhlet extraction is the most frequently applied technique to extract PAHs from dried sediments. Medium-polar solvents, such as dichloromethane or toluene, or mixtures of polar and non-polar solvents can be used. When using dichloromethane, losses of PAHs have occasionally been observed. Although toluene is not favoured, because of its high boiling point, it should be chosen as solvent when it is expected that sediment samples might contain soot particles. For routine marine samples, the use of a mixture of a polar and a non-polar solvent (e.g. acetone/hexane (1:3, v/v)) is recommended.

The extraction can be done with a regular or a hot Soxhlet (Smedes and de Boer, 1997). A sufficient number of extraction cycles must be done; approximately 8 h for the hot Soxhlet and 12–24 h for normal Soxhlet. The extraction efficiency has to be checked for different types of sediment by a second extraction step. These extracts should be analysed separately.

PLE can also be used for the extraction of freeze-dried sediments. Instead of adding anhydrous sodium sulfate, a clean sand or diatomaceous earth is mixed with the sample to increase the surface area of the sample. The same solvent mixtures noted above for wet sediment extraction can be used for dry sediments.

Supercritical fluid extraction (SFE) has also been used for the extraction of organic compounds from environmental samples. The optimum conditions may vary for specific sediments (Barnabas *et al.*, 1995; Dean *et al.*, 1995; Reimer and Suarez, 1995; Librando *et al.*, 2004). The sample size that can be extracted using SFE is small (<5 g sediment) and, as a result, detection limits will be higher. Therefore, SFE is not recommended for the extraction of PAHs from environmental samples.

5.3 Shellfish

PAHs are lipophilic and therefore are concentrated in the lipids of an organism. A number of methods have been described for PAH extraction (Ehrhardt *et al.*, 1991). These methods generally use either Soxhlet extraction or alkaline digestion, followed by liquid–liquid extraction with an organic solvent. In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical drying agent (e.g. anhydrous sodium sulfate), in which case, a period of several hours is required between mixing and extraction, to allow complete binding of the water in the sample. Samples are spiked with recovery standard and should be left overnight to equilibrate. Alkaline digestion is conducted on wet tissue samples, making the drying procedure unnecessary.

Apolar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction. Mixtures such as hexane/dichloromethane may be effective in place of solvents, such as benzene and toluene, which have been used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons, and its use is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10 % water, and combines disruption of the cellular matrix, lipid extraction, and saponification in a single procedure, thereby reducing sample handling and treatment. Solvents used for liquid–liquid extraction of the homogenate are usually apolar, such as pentane or hexane, and they will effectively extract all PAHs.

Alternatively, extraction of wet or dry samples of biota may be done using PLE. The wet biota sample is dried by mixing with sufficient anhydrous sodium sulfate to form a free-flowing mixture. The mixture is packed into stainless steel extraction cells containing a glassfibre filter and sodium sulfate or glass powder to fill the cell. To ensure a better recovery, samples may be extracted twice and extractions may have to be done at elevated temperatures and pressure.

6 Clean-up procedures

Similar clean-up procedures can be used for extracts from biota and sediment. If PLE with fat retainers or saponification is used for extraction, the number of clean-up steps required is reduced, because the lipid has been removed or destroyed. Otherwise, the crude extract requires a clean-up to remove lipid from biota samples and the many other compounds that are co-extracted (Wise *et al.*, 1995). Because of chlorophyll-like compounds extracted from sediment, the raw extract will be coloured. The sediment extracts will also contain sulphur and sulphur-containing compounds, oil, and many other natural and anthropogenic compounds. Before the clean-up, the sample must be concentrated, and any polar solvents used in the extraction step should be removed (see Section 7).

If Soxhlet extraction is used for biota, a much greater quantity of residual lipid must be removed, before the analytical determination can be made, than with alkaline digestion. An additional clean-up stage may therefore be necessary. The most

commonly used clean-up methods involve fractionation, using deactivated alumina or silica adsorption chromatography. When applying fractionation, the elution pattern has to be checked frequently. This should be done in the presence of sample matrix, because that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high-performance liquid chromatography (HPLC)-based methods are also used (Nondek *et al.*, 1993; Nyman *et al.*, 1993; Webster *et al.*, 2001). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility. Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster *et al.*, 2001). A metal-free silica column is used for the clean-up/fractionation, because dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples.

For GC-MS analysis, sulphur should be removed from sediment extracts to protect the detector. This can be achieved by the addition of copper powder, wire, or gauze during or after Soxhlet extraction. Sulphur can also be removed by GPC. Copper can also be added to the PLE cell; however, this is not always sufficient, and further treatment with copper may be required following extraction. Ultrasonic treatment may improve the removal of sulphur. There are other methods that can be used for the removal of sulphur (Smedes and de Boer, 1997).

7 Preconcentration

All of the methods suggested above result in an extract dominated by non-polar solvents. This sample extract will usually have to be concentrated before clean-up. Extracts can be concentrated down to as little as 0.5 ml, especially when labelled internal standards are used. Evaporation can be done using either a rotary-film evaporator or parallel evaporating systems, such as Buchi Syncore.

Evaporation of solvents using a rotary-film evaporator should be done at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions to prevent loss of the more volatile PAHs, such as naphthalenes. For the same reason, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas.

Syncore parallel evaporators can be used, with careful optimization of the evaporation parameters. The Buchi Syncore Analyst is a sealed system, thereby avoiding contamination from the laboratory air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles, and if the flush-back module is fitted, the sides of the tubes are rinsed automatically, thus reducing the loss of the heavier components.

Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, isohexane, and isooctane.

8 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward. There are differences in the range of PAH compounds resulting from combustion processes and from crude oil and oil products. In addition, the aims of specific monitoring programmes can

require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (non-alkylated) compounds, whereas crude oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination. Both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes. However, in areas of significant oil contamination and following oil spills, only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures that can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table 1. This list differs both from the list previously developed within ICES specifically for intercomparison purposes, and from the historical list of Borneff (1987). In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs, because of analytical limitations. This approach completely neglects the determination of alkylated PAHs that allows the interpretation of PAH accumulation from multiple sources, including those caused by oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list, depending on the specific aims of the monitoring programme to be undertaken.

Table 1. Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics, standards are not available for any isomers in the group.

Compound	MW	Compound	MW
Naphthalene	128	Benzo[*b*]naptho[2,3-d]thiophene	234
C1-Naphthalenes	142	C1-Benzo[*b*]naptho[2,3-d] thiophene	248
C2-Naphthalenes	156	Benz[*a*]anthracene	228
C3-Naphthalenes	170	Chrysene	228
C4-Naphthalenes	184	Benz[*b*]anthracene	228
Acenaphthylene	152	C1- Benz[*a*]anthracene/ Chrysene	242
Acenaphthene	154	C2- Benz[*a*]anthracene/ Chrysene	256
Fluorene	166	C3- Benz[*a*]anthracene/ Chrysene	270
C1-Fluorenes	180	Benzo[*a*]fluoranthene	252
C2-Fluorenes	194	Benzo[*b*]fluoranthene	252
C3-Fluorenes	208	Benzo[*j*]fluoranthene	252
Dibenzothiophene	184	Benzo[*k*]fluoranthene	252
C1-Dibenzothiophenes	198	Benzo[*e*]pyrene	252
C2-Dibenzothiophenes	212	Benzo[*a*]pyrene	252
C3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	C1-Benzofluoranthene/ Benzopyrene	266
Anthracene	178	Indeno[1,2,3-cd]pyrene	276
C1-Phenanthrenes/Anthracenes	192	Benzo[*g,h,i*]perylene	276
C2-Phenanthrenes/Anthracenes	206	Dibenz[*a,h*]anthracene	278
C3-Phenanthrenes/Anthracenes	220	Cyclopenta[*c,d*]pyrene	226
Fluoranthene	202	Naphtho[2,1-a]pyrene	302
Pyrene	202	Dibenz[*a,e*]pyrene	302
C1-Fluoranthenes/Pyrenes	216	Dibenz[*a,l*]pyrene	302
C2-Fluoranthenes/Pyrenes	230	Dibenz[*a,l*]pyrene	302
Benzo[*b*]naptho[2,1-d]thiophene	234	Dibenz[*a,h*]pyrene	302
Benzo[*b*]naptho[1,2-d]thiophene	234		

9 Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by either combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS; de Boer and Law, 2003). However, for the analysis of parent and alkylated PAHs, GC-MS is the method of choice. For flexibility, GC-MS is the most capable technique, because in principle it does not limit the selection of determinands in any way. HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionization detection, but neither can be recommended for alkylated PAHs, because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrumentation, and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05–0.5 μg kg^{-1} wet weight in biota, or dry weight in sediments, for individual PAH compounds should be achievable by GC-MS. However, this limit can be lowered in routine analysis.

9.1 Gas chromatography–mass spectrometry (GC-MS)

Three injection modes are commonly used for GC-MS; splitless, on-column, and programmed temperature vaporizer (PTV). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important, if adsorption effects and discrimination are to be avoided. In addition, the analytical column should not contain active sites onto which PAHs can be adsorbed. Helium is the preferred carrier gas and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15–0.3 mm. Film thicknesses of 0.2–1 μm are generally used; this choice has little effect on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is required. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% poly(diphenylsiloxane) + 95% poly(dimethylsiloxane; DB-5 or equivalent). However, this will not resolve critical isomers, such as benzo[*b*], [*j*], and [*k*]fluoranthenes, or chrysene from triphenylene. If necessary, chrysene and triphenylene can be separated on other columns of differing stationary phase or length, for example, on a 60 m non-polar column, such as DB-55 MS.

For PAHs, there is no sensitivity gain from the use of chemical ionization (either positive or negative ion); therefore, analyses are usually conducted in electron-impact mode at 70 eV. Quadrupole and magnetic sector instruments are used in single-ion monitoring mode (SIM) to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM, the molecular ion is used for quantification. Qualifier ions can be used to confirm identification, but they are limited for PAHs. Triple quadrupole mass spectrometry can also be used and will give greater sensitivity. Some instruments,

such as ion-trap and time-of-flight mass spectrometers, exhibit the same sensitivity in both modes; therefore, full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table 2. The ions are grouped and screened within GC time-windows. In general, the number of ions within a group should not be greater than 20. The dwell time is an important parameter and should be similar for each ion. For GC capillary column analysis, a dwell time should not be shorter than 20 ms, whereas the sum of dwell times in each retention time-window should not be greater than 500 ms.

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex, but distinct distribution profiles when analysed by GC-MS (Figure 1; see page). Integration of each isomer separately is difficult for most alkylated PAHs. Well-resolved peaks can be obtained for methyl PAHs with two or three aromatic rings (Figure 1). For example, methyl phenanthrenes/anthracenes give five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene, and 1-methyl phenanthrene (Figure 1b). These may be integrated as a group or as separate isomers. For all other alkylated PAHs, the area for all isomers may be summed and quantified against a single representative isomer. However, this method will result in an overestimation of the concentration, because the sum might include non-target compounds.

Table 2. Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

Group number	Retention time (min)	Dwell time (ms)	Ions in group (Da)
1	8.00	100	128 136 142
2	21.00	100	152 156
3	23.70	100	154 164 170
4	26.80	80	166 180 184
5	31.60	80	178 184 188 194 198
6	35.30	100	192 198
7	36.60	100	206 212
8	39.40	80	202 206 212 216 220 226
9	44.65	100	216 220
10	45.30	100	226 228 230 234 240
11	48.58	90	242 248
12	52.00	100	252 256 264 266
13	59.00	100	266 276 278 288

10 Calibration and quantification

10.1 Standards

The availability of pure PAH compounds is limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast, and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl groups listed in Table 1. Preferably, PAHs should be quantified against an isomer from the same groups; however, if this is impossible, an isomer of lower alkylation may be used for quantification. A range of deuterated PAHs (usually 5–7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully deuterated parent PAHs is available for use as standards in PAH analysis. Suitable

standards could range from d8-naphthalene to d14-dibenz[*a,h*]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier, as for certified reference materials, it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10 μg. Alternatively, certified calibration standards can be purchased that contain a range of PAHs. Calibration standards should be stored in the dark, because some PAHs are photosensitive, and ideally, solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or gas-tight flasks, to avoid evaporation of the solvent during storage.

10.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to define the calibration curve adequately. In general, GC-MS calibration is linear over a considerable concentration range, but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be used, using a range of deuterated PAHs as internal standards.

10.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the higher-molecular-weight compounds. Deuterated standards should be used as internal standards, added to the sample before extraction. A second group of standards may be added before GC-MS injection. This allows the recovery to be calculated.

11 Analytical quality control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination that they consider acceptable. Further information on analytical quality-control procedures for PAHs can be found elsewhere (Law and de Boer, 1995).

11.1 Procedural blanks

A procedural blank should be measured with each sample batch, and should be prepared simultaneously, using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural detection limit is often determined by the blank value. To keep the blank value as low as possible, PAHs or other interfering compounds should be removed from all glassware, solvents, chemicals, adsorption materials, etc., that are used in the analysis. The following procedures should be used:

- Glassware should be thoroughly washed with detergents and rinsed with an organic solvent before use. Further cleaning of the glassware, other than calibrated instruments, can be done by heating at temperatures >250 °C.
- All solvents should be checked for impurities by concentrating the amount usually used to 10% of the normal end volume. This concentrate can then

be analysed by GC and should not contain significant amounts of PAHs or other interfering compounds.

- All chemicals and adsorption materials should be checked for impurities and purified (e.g. by heating or extraction), if necessary. Soxhlet thimbles should be pre-extracted. Glassfibre thimbles are preferred over paper thimbles. Alternatively, full glass Soxhlet thimbles with a G1 glass filter at the bottom can be used. The storage of these cleaned materials for a long period is not recommended, because laboratory air can contain PAHs that will be absorbed by these materials.
- Glassfibre filters used for PLE (pressurized liquid extraction) should be heated at 450 °C overnight.
- Blank values occurring despite all the above-mentioned precautions may be the result of contamination from the air. The most volatile compounds will usually show the highest blanks.

11.2 Reference materials

A laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well characterized for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g. sediment, liver, muscle, mussel tissue) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically to check the method bias. A marine sediment CRM, NIST 1941b, is available with certified values for 24 PAHs and a further 44 as reference, non-certified, values. The availability of biota CRMs for PAHs is very limited, and in all cases, the number of PAHs for which certified values are provided is small. Currently, only NIST 1974a, a frozen wet-mussel tissue, and NIST 2974, a freeze-dried mussel tissue, are available. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise where samples are circulated without knowledge of the determinand concentrations, to provide an independent check on performance.

12 Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been demonstrated in intercomparison studies for PAHs. Control procedures should be established to ensure that data are correct and to obviate transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

Acknowledgements

These guidelines were prepared by ICES Marine Chemistry Working Group. The authors acknowledge the assistance of the chair, Evin McGovern, members of the working group, and members of the ICES Working Group on Marine Sediments in Relation to Pollution for their input.

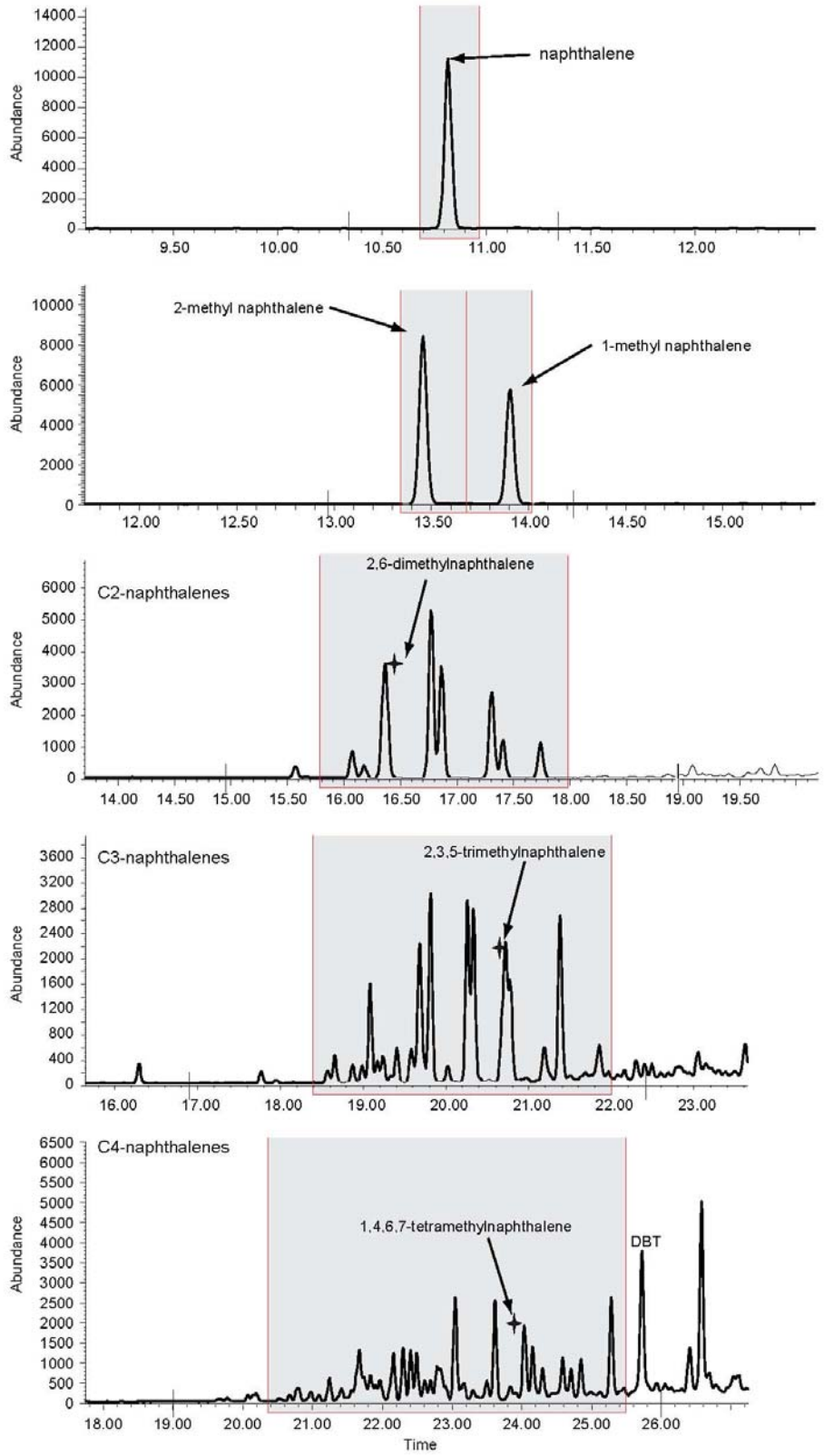

Figure 1a. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (a) parent and alkylated naphthalenes.

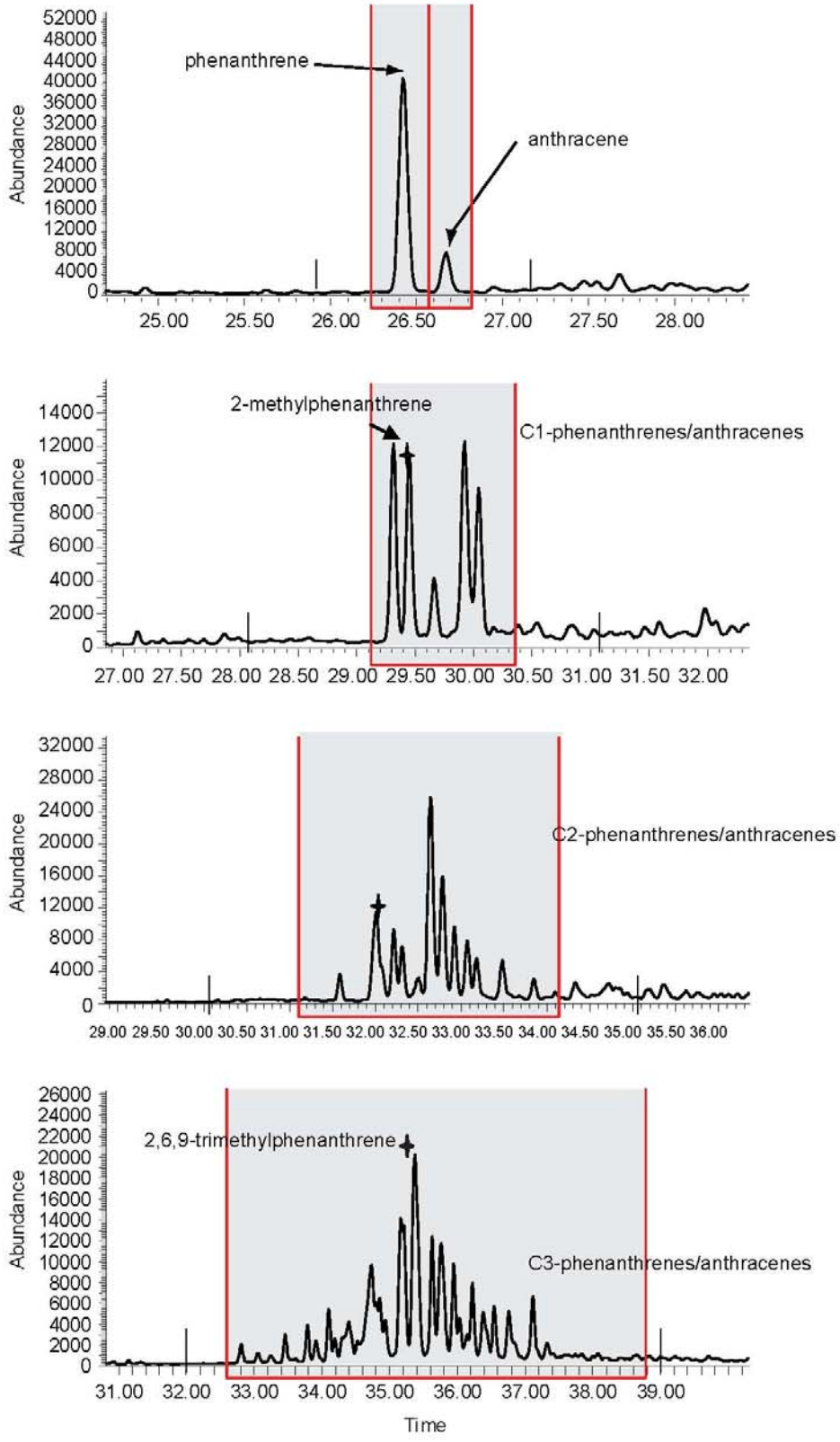

Figure 1b. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (b) parent and alkylated phenanthrenes/anthracenes.

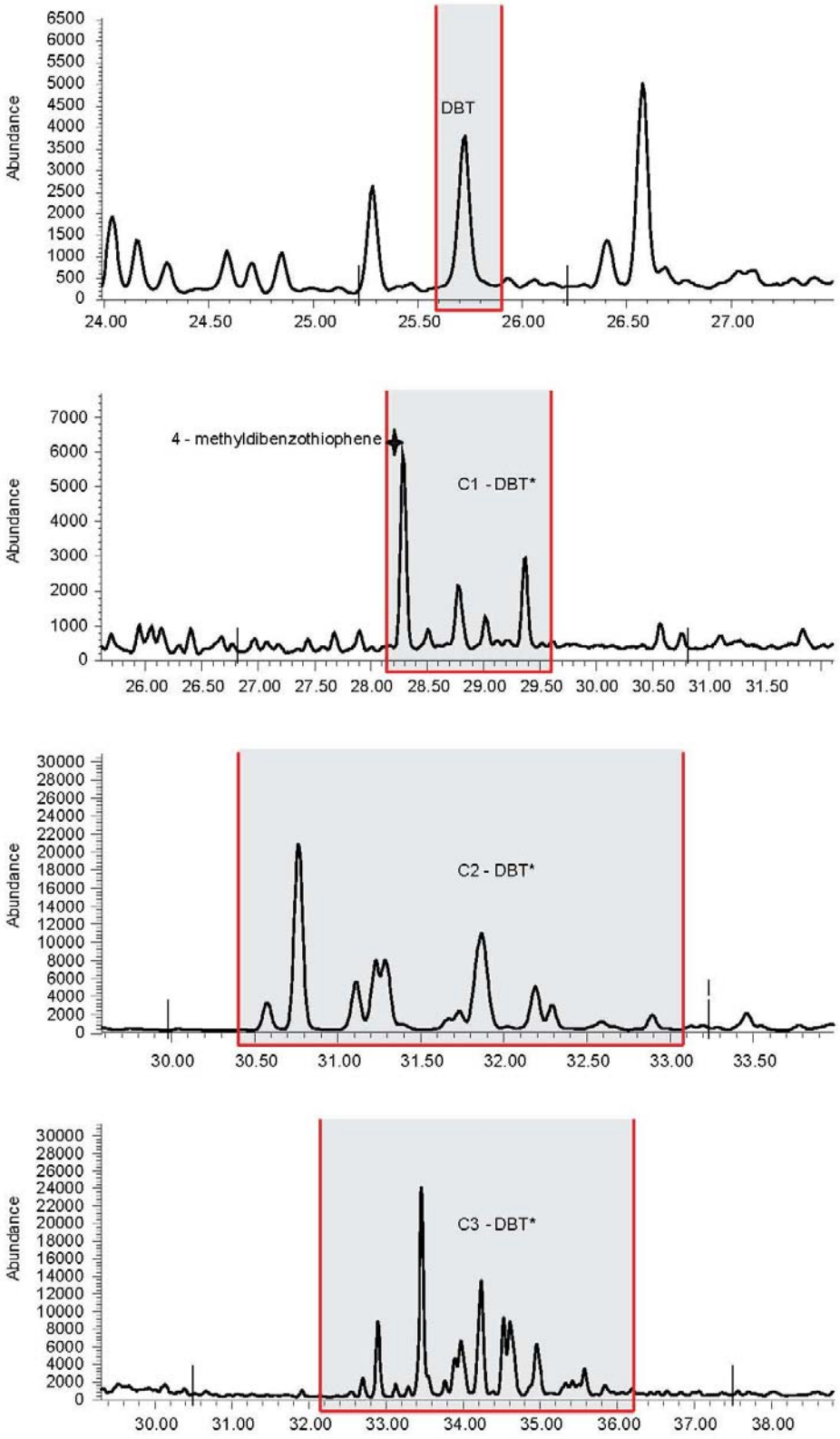

Figure 1c. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (c) Parent and alkylated dibenzothiophene (DBT).

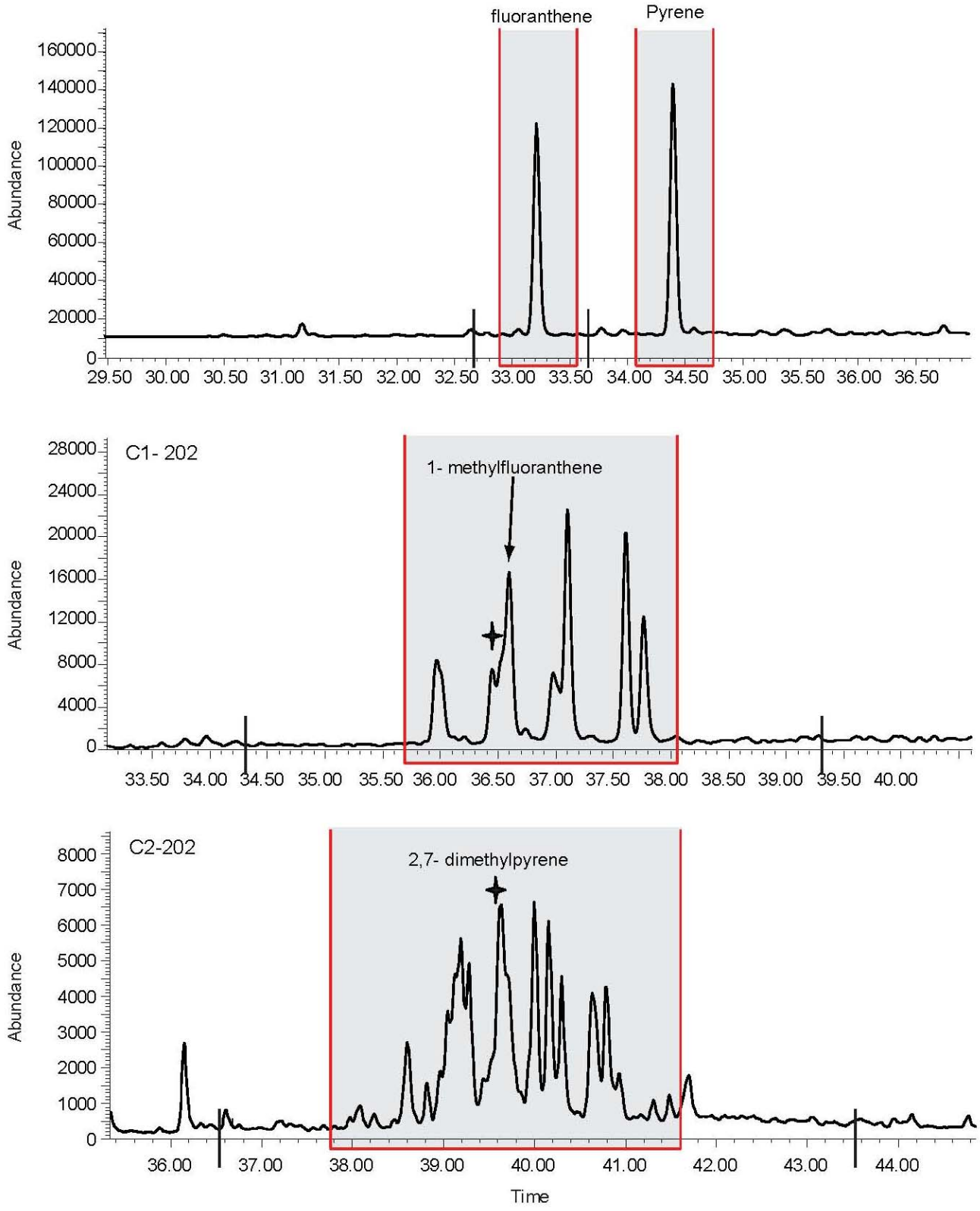

Figure 1d. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (d) Parent and alkylated fluoranthene/pyrene.

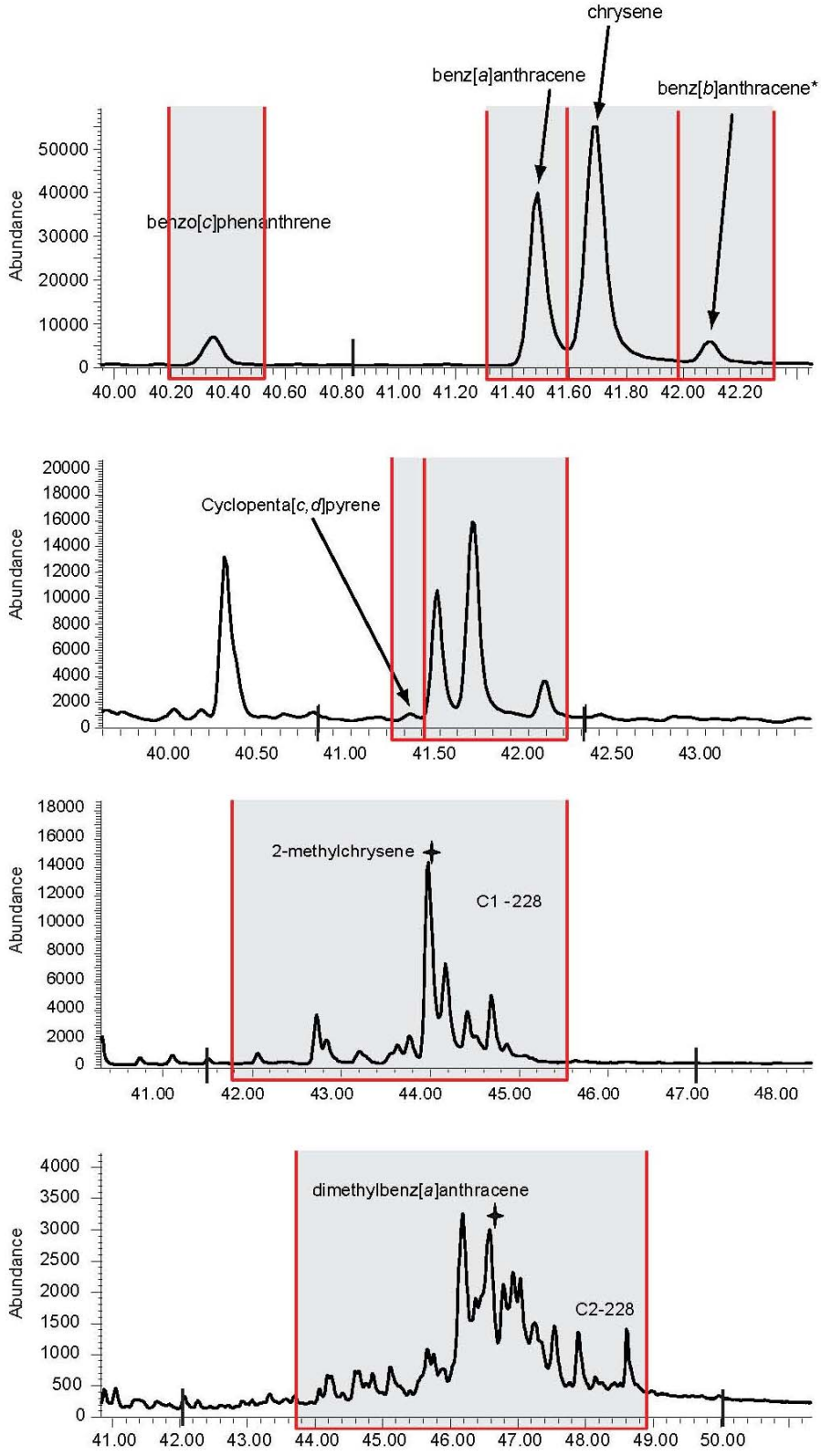

Figure 1e. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (e) Parent and alkylated benz[*c*]phenanthrene, benz[*a*]anthracene, chrysene, and benz[*b*]anthracene. Note: Triphenylene coelutes with chrysene.

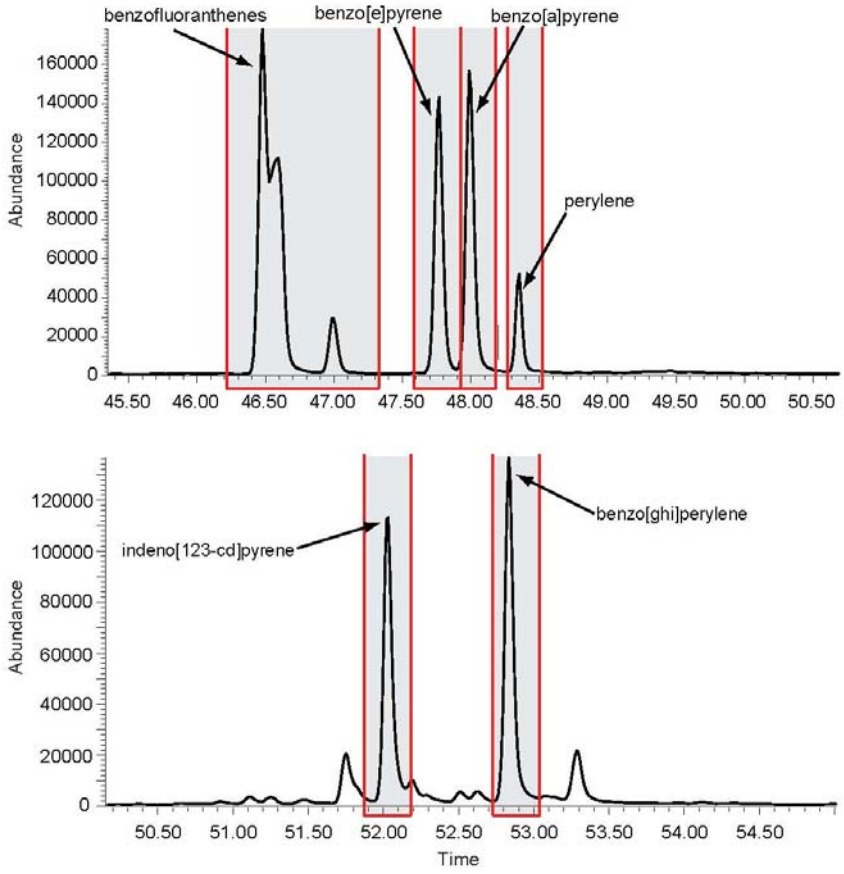

Figure 1f. Integration of parent and alkylated PAHs analysed by gas chromatography–mass spectrometry (GC-MS) using an HP-5 column (30 m). The standards used for the calibration are marked with an asterisk. (f) Parent benzofluoranthenes, benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, and indenopyrene and benzoperylene.

13 References

Barnabas, I. J., Dean, J. R., Tomlinson, W. R., and Owen, S. P. 1995. Experimental design approach for the extraction of polycyclic aromatic hydrocarbons from soil using supercritical carbon dioxide. Analytical Chemistry, 67: 2064–2069. doi: 10.1021/ac00109a027.

de Boer, J., and Law, R. J. 2003. Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons. Journal of Chromatography A, 1000: 2232–2251.

Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J. F., Burgeot, T., Michel, X., and Belloccq, J. 1999. Polycyclic aromatic hydrocarbon burden of mussels (*Mytilus* sp.) in different marine environments in relation with sediment and PAH contamination and bioavailability. Marine Environment Research, 47: 415–439.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911–917.

Borneff, J. 1987. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7. International Agency for Research on Cancer.

Davis, H. K., Moffat, C. F., and Shepherd, N. J. 2002. Experimental tainting of marine fish by three chemical dispersed petroleum products, with comparisons to the Braer oil spill. Spill Science and Technology Bulletin, 7: 257–278.

Dean, J. R., Barnabas, I. J., and Fowlis, I. A. 1995. Extraction of polyaromatic hydrocarbons from highly contaminated soils: A comparison between Soxhlet, microwave, and supercritical fluid extraction techniques. Analytical Proceedings, 32: 305–308.

Ehrhardt, M., Klungsøyr, J., and Law, R. J. 1991. Hydrocarbons: review of methods for analysis in sea water, biota, and sediments. ICES Techniques in Marine Environmental Sciences No. 12: 19–22.

Hanson, S. W. F., and Olley, J. 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochemical Journal, 89: 101–102.

Harvey, R. G. 1991. Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity, Cambridge Monographs on Cancer Research, Cambridge University Press, Cambridge, UK.

Hellou, J., Stellar, S., Leonard, J., Langille, M. A., and Tremblay, D. 2005. Partitioning of polycyclic aromatic hydrocarbons between water and particles compared to bioaccumulation in mussels: a harbour case. Marine Environment Research, 59: 1011–1017.

Law, R. J., and Biscaya, J. L. 1994. Polycyclic aromatic hydrocarbons (PAHs)—problems and progress in sampling, analysis and interpretation. Marine Pollution Bulletin, 29: 235–241.

Law, R. J., and de Boer, J. 1995. Quality assurance of analysis of organic compounds in marine matrices: application to analysis of chlorobiphenyls and polycyclic aromatic hydrocarbons. *In* Quality assurance in environmental monitoring. Ed. by P. Quevauviller. VCH Weinheim, New York.

Librando, V., Hutzinger, O., Tringali, G., and Aresta, M. 2004. Supercritical fluid extraction of polycyclic aromatic hydrocarbons from marine sediments and soil samples. Chemosphere, 54: 1189–1197.

Malins, D. C., McCain, B. B., Landahl, J. T., Myers, M. S., Krahn, M. M., Brown, D. W., Chan, S. L., and Roubal, W. T. 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquatic Toxicology, 11: 43–67.

Marvanova, S., Vondracek, J., Pencikova, K., Trilecova, L., Krcmar, P., Topinka, J., Novakova, Z., *et al*. 2008. Toxic effects of methylated benz[*a*]anthracenes in liver cells. Chemical Research in Toxicology, 21: 503–512.

Nondek, L., Kuzilek, M., and Krupicka, S. 1993. LC clean-up and GC-MS analysis of polycyclic aromatic hydrocarbons in river sediment. Chromatographia, 37: 381–391.

Nyman, P. J., Perfetti, G. A., Joe, F. L., and Diachenko, G. W. 1993. Comparison of two clean-up methodologies for the gas chromatographic-mass spectrometric detection of low nanogram per gram levels of polynuclear aromatic hydrocarbons in seafood. Food Additives and Contaminants, 10: 489–501.

OSPAR. 1999. JAMP Guidelines for Monitoring Contaminants in Biota. Oslo and Paris Commissions Joint Assessment and Monitoring Programme. Agreement 1999-02.

Reimer, G., and Suarez, A. 1995. Comparison of supercritical fluid extraction and Soxhlet extraction for the analysis of native polycyclic aromatic hydrocarbons in soil. Journal of Chromatography A, 699: 253–263.

Richardson, D. M., Davies, I. M., Moffat, C. F., Pollard, P., and Stagg, R. M. 2001. Biliary PAH metabolites and EROD activity in flounder (*Platichthys flesus*) from a contaminated estuarine environment. Journal of Environmental Monitoring, 3: 6106–6115.

Saim, N., Dean, J. R., Abdullah, P., and Zakaria, Z. 1998. An experimental design approach for the determination of polycyclic aromatic hydrocarbons from highly contaminated soil using accelerated solvent extraction. Analytical Chemistry, 70: 4204–4224.

Smedes, F. 1999. Determination of total lipid using non-chlorinated solvents. Analyst, 124: 17111–17718.

Smedes, F., and de Boer, J. 1997. Chlorobiphenyls in marine sediments: guidelines for determination. ICES Techniques in Marine Environmental Sciences No. 21.

Stagg, R. M., McIntosh, A. M., and Mackie, P. 1995. The induction of hepatic mono-oxygenase activity in dab (*Limanda limanda*) in relation to environmental contamination with petroleum hydrocarbons in the North Sea. Aquatic Toxicology, 33: 2542–2564.

Vethaak, A. D., and Rheinallt, T. 1992. Fish disease as a monitor for marine pollution: the case of the North Sea. Review of Fish Biology and Fisheries, 2: 1–32.

Webster, L., Fryer, R. J., Megginson, C., Dalgarno, E. J., and Moffat, C. F. 2001. The polycyclic aromatic hydrocarbon and geochemical biomarker composition of sediments from voes and coastal areas in Shetland and Orkney Islands. Journal of Environmental Monitoring, 3: 5916–6001.

Webster, L., Russell, M., Packer, G., and Moffat, C. F. 2006. Long-term monitoring of polycyclic aromatic hydrocarbons (PAHs) in blue mussels (*Mytilus edulis*) from a remote Scottish location. Polycyclic Aromatic Compounds, 26: 283–298.

Wise, S. A., Schantz, M. M., Benner, B. A., Hays, M. J., and Schiller, S. B. 1995. Certification of polycyclic aromatic hydrocarbons in a marine sediment standard reference material. Analytical Chemistry, 67: 1171–1178.

Woodhead, R. J., Law, R. J., and Matthiessen, P. 1999. Polycyclic aromatic hydrocarbons in surface sediments around England and Wales and their possible biological significance. Marine Pollution Bulletin, 38: 776–790.

14 Abbreviations and technical terminology

CRM	certified reference material
DBT	dibenzothiophene
DCM	dichloromethane
GC	gas chromatography
GPC	gel-permeation chromatography
HPLC	high performance liquid chromatography
LRM	laboratory reference material
MFO	mixed-function oxygenase
MS	mass spectrometry
PAH	polycyclic aromatic hydrocarbon
PLE	pressurized liquid extraction
PTV	programmed temperature vaporizer
SFE	supercritical fluid extraction
SIM	single-ion monitoring

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