Intergovernmental
Oceanographic
Commission

Manuals and guides 11

THE DETERMINATION OF PETROLEUM HYDROCARBONS IN SEDIMENTS

1982 Unesco

PREFACE

This report was prepared by the Intergovernmental Oceanographic Commission (IOC) of Unesco with the collaboration of a consultant, Dr. Karsten H. Palmork of the Institute of Marine Research, Bergen, Norway, at the request of the UNEP Environment Programme, through its Regional Seas Programme Activity Centre's programme, the Co-ordinated Mediterranean Pollution Monitoring and Research Programme (MED POL). The support of UNEP is gratefully acknowledged.

The analytical methods presented and their limitations have been considered and approved by the IOC Group of Experts on Methods, Standards and Intercalibration, subject to the proviso that, as more information becomes available with regard to choice of solvents, extraction efficiency, choice of standards, etc., the proposed methods will be amended accordingly.

The methods presented in this report were produced for the purpose of serving as reference methods for the determination of petroleum in sediments for MED POL. However, since they have much wider applicability than for just one regional area, they are published for the use of interested marine scientists throughout the world.

LIST OF CONTENTS

DETERMINATION OF PETROLEUM HYDROCARBONS IN SEDIMENTS

1. INTRODUCTION

The need to analyze for petroleum hydrocarbons in sediments may arise for different reasons. For example, (i) because of the desire to establish background values before offshore drilling and oil production activities are started, (ii) because the area in question, a fishing ground, has been subjected to an oil spill, and (iii) because the area in question is subjected to continuous pollution from heavy traffic or from a refinery, are but a few. All the situations may have an impact on marine life and fisheries. It is, therefore, of paramount importance to know how widespread is the oil pollution to what extent it has affected marine life and for how long the effect of the oil pollution will last.

Analysis of sea water samples from an area will reveal whether or not the pollution still affects the sea water. Analysis of samples of fat fish or liver from lean fish will provide information on the degree to which the oil pollution has affected fish. Analysis of sediment samples can provide the results of atmospheric fallout over time, baseline values before activities leading to the discharge of pollutants starts, results of an oil spill, a chronic seepage, chronic pollution from a refinery and simply the result of hydrocarbons associated with sinking particles, like faecal pellets, dead or living organisms, clay minerals and silicates. These particles are usually responsible for the removal of hydrocarbons from the water column. Additionally, the dissolved petroleum hydrocarbons may be absorbed directly by the sediment according to their content of indigenous matter as for example, humic acids.

What sort of methodology should be used to obtain an estimate of the petroleum hydrocarbons? Numerous analytical methods have been described on how to estimate petroleum hydrocarbons. The methods range from gravimetric (non volatile extractables),

UV-absorption (conjugated polyolefins, aromatics), UV-fluorescence (unsaturated compounds, aromatics, etc. depending on- λ -excitation, λ-emission, extraction and volatility) infrared (IR) (C-H_2 stretching frequency, 2930 cm^{-1}) gas chromatography using packed columns (total hydrocarbons, individual n-alkanes, n-paraffin/isoprenoid ratios), gas chromatography using fused silica caplillary columns (individual n-alkanes and polycyclic aromatic hydrocarbons after preseparation on silica or silica topped up with alumina or alumina only), to gas chromatography/mass spectrometry coupled to a data system (individual components, preselected components) using the system in the selected ion monitoring mode (SIM).

This reference paper on methods will deal with those methods which have been used to date (i.e. UV-fluorescence, gas chromatography using packed or fused silica capillary columns and gas chromatography/mass spectrometry, all of which have been endorsed by various groups, the most recent being the IOC's GIPME Group of Experts on Methods, Standards and Intercalibration at their Fourth Session in Curaçao, Netherlands Antilles, 25-31 March 1982.

All three methods require the same careful treatment, sampling, extraction and work-up procedure (e.g. see sections 7.3, 7.4 and 8.1, 8.2, 8.3, 8.4 and 8.5). Differences occur at the analytical step, the use of instrumentation and the expression of the result. In the UV-fluorescence method (described in section 8.7) the result is presented as a number of chrysene-units, an expression of the "oil" present in the sample. In the gas chromatographic method using a packed column, the result is expressed as total hydrocarbons (THC). In the gas chromatography- and gas chromatography/mass spectrometry using capillary columns, the results can be expressed as individual components either of n-alkanes, polycyclic aromatic hydrocarbons and/or other preselected components using GC/MS in the SIM-mode.

The types of oil that might end up in the marine environment may differ significantly; that is from crude oil to different distillates like bunker oil, petrol or atmospheric fallout. All

these "types" of oil have something in common, they contain components that are dissolved in sea water and components that are degraded by bacteria and by UV light. It is, therefore, no point trying to analyse for "oil" as such, because it does not any longer exist as the original oil when it has reached the sea. As a result, the UV-fluorescence method can be used in this case as a screening method to detect "hot spots".

The philosophy in petroleum hydrocarbon analysis should be to ask the following questions: What sort of components are most harmful? Which components originating from petroleum end up in the sediment? When these questions have been answered, then the time has come to analyze the individual components.

2. SCOPE AND FIELD OF APPLICATION

The reference methods described are a UV-fluorescence screening method, a gas chromatographic (FID) and gas chromatographic/-mass-spectrometric method for the determination of total and individual petroleum hydrocarbons in sediment samples. For chemical analysis of the samples, the hydrocarbons are extracted with pentane after the sediment has been saponified under reflux for 1.5 hours with methanolic KOH. The extract is separated into an alkane fraction and an aromatic fraction on an alumina column (section 8.5 for UV-fluorescence and capillary gas chromatography analyses) and purified on a silica gel column (section 8.6 for packed column gas chromatography and GC/MS analyses). The detection limit for "total" petroleum hydrocarbons is approximately 1 mg/kg sediment; for individual hydrocarbons 100 ng/kg.

3. REFERENCES AND LITERATURE FOR CONSULTATION

Anon, 1980. Guidelines for Data Aquisition and Data Quality Evaluation in Environmental Chemistry. Analyt. Chem. 52 (1980) 2242-2249.

Farrington, J.W., 1980. An Overview of the Biogeochemistry of Fossile Fuel Hydrocarbons in the Marine Environ-

ment, in Petroleum in the Marine Environment, Advances in Chemistry Series 185, Leonid Petrakis and Fred T. Weiss eds., American Chemical Society, Washington D.C. 1980.

Friedrich, H., 1973 . Marine Biology. An introduction to its problems and results. Sidgwick & Jackson, London.

Futoma, D.J., Ruven Smith, S., Smith, T.E. and J. Tanaka, 1981 . Polycyclic Aromatic Hydrocarbons in Water Systems. CRS Press, Inc., Boca Raton, Florida, 1981.

Hites, R.A., R.E. Laflamme and J.G. Windsor jr., 1980. Polycyclic Aromatic Hydrocarbons in Marine/Aquatic Sediments: Their Ubiquity, in Petroleum in the Marine Environment, Advances in Chemistry Series 185, Leonid Petrakis and Fred T. Weiss eds., American Chemical Society, Washington D.C. 1980.

McAuliffe, C.D., 1980 . The Multiple Gas-Phase Equilibration Method and Its Application to Environmental Studies, in Petroleum in the Marine Environment, Advances in Chemistry Series 185, Leonid Petrakis and Fred T. Weiss eds., American Chemical Society, Washington D.C., 1980.

Moore, H.B. and Neil, R.G., 1930 . An instrument for sampling marine muds. J. mar. biol. Ass. U.K. XVI: 589-594.

Overton, E.B. and J.L Laseter, 1980 . Distribution of Aromatic Hydrocarbons in Sediments from Selected Atlantic, Gulf of Mexico, and Pacific Outer Continental Shelf Areas, in Petroleum in the Marine Environment, Advances in Chemistry Series 185, Leonid Petrakis and Fred T. Weiss eds., American Chemical Society, Washington D.C. 1980.

4. PRINCIPLE

After sampling of the sediment with a van Veen grab or gravity corer, the petroleum hydrocarbons are isolated from the sediment samples by saponification with methanolic KOH for 1.5 hours followed by extraction with pentane. A subsample is used for the determination of the dry weight. In samples with a high degree of pollution, the "total" amount of petroleum hydrocarbons can be estimated using a UV-fluorescence screening method (section 8.7) and also gas chromatography with packed columns and flame ionization detector, integrating both the resolved and unresolved complex mixture (section 8.8). An oil, as similar to the polluting oil as possible, is used as an external standard. Selected nonbiogenic aromatic hydrocarbons (e.g. naphthalenes, phenanthrenes and dibenzothiophenes), which are the dominating aromatics in samples polluted by petroleum hydrocarbons, are quantitatively analysed using a fused silica capillary column, three internal standards (i.e. diphenyl-d_{10}, anthracene-d_{10} and pyrene-d_{10}) and a mass-spectrometer as the detector.

In the case of an anoxic sediment sample, elemental sulphur might disturb the gas chromatographic pattern. This problem can be alleviated by adding a drop or two of metallic mercury during the reflux period or using a Cu-column. However, if the sample is saponified with methanolic KOH, sulphur is completely eliminated.

5. REAGENTS

All the reagents used must be of the best obtainable quality; p.a. or chromatography grade. Solvents used must, if they have high blanks, be redistilled to insure the best quality.

5.1 Chemicals:

5.1.1 Distilled water

5.1.2 Methanol p.a. Merck 6009.

5.1.3 Potassium hydroxide (KOH) p.a. Merck 5033.

5.1.4 Boiling stones, Alundum (R) Approx. 8 to 14 mesh. Cat. No. 1590-D 18, Arthur H. Thomas Company, Philadelphia, Pa. 19105, U.S.A.

5.1.5 Pentane, Uvasol Merck 7179

5.1.6 Hexane, Uvasol Merck 4369

5.1.7 Nitrogen, Grade 3.

5.1.8 Chromic acid containing 4g potassium dichromate ($K_2Cr_2O_7$) in one liter of concentrated sulphuric acid.

5.1.9 Silica gel (Kieselgel 60) particle size 0.063-0.125 mm (120-230 mesh ASTM) Merck 9385.

5.1.10 Aluminum oxide, Woelm acid (anionotropic) activity grade I for chromatography. M. Woelm ESCHWEGE, Germany.

5.2 Internal standards:

5.2.1 Diphenyl-d_{10} MD-208 (99 atom %D) Lot no. B- 1011. Merck & Co., Inc. Rahway, N.J.
or Merck Sharp & Dohme Canada Limited, Montreal, Canada (MSD- ISOTOPES)

5.2.2 anthracene-d_{10} MD-46 (98 atom %D) Lot no. C-570. Merck & Co., Inc. Rahway, N.J.
or Merck Sharp & Dohme Canada Limited, Montreal, Canada (MSD- ISOTOPES)

5.2.3 pyrene-d_{10} MD-363 (98% atom %D) Lot. No. C-569. Merck & Co., Inc. Rahway, N.J.
or Merck Sharps & Dohme Canada Limited, Montreal, Canada (MSD- ISOTOPES)

5.2.4 A mixture containing approximately 0.5 µg of 5.2.1, 5.2.2 and 5.2.3 per mL dissolved in hexane. The mixture can be prepared as follows: transfer 100 mg 5.2.1, 5.2.2 and 5.2.3 into a volumetric flask and dilute to 100 mL with hexane; solution A.

Take 1 mL of solution A and dilute to 100 mL with hexane. 1 mL of this solution (Solution B) contains approx. 10 µg each of the standards per mL.

Take 5 mL of solution B and dilute to 100 mL with hexane. The resulting solution (solution C) contains approx. 0.5 µg/mL of each of the deuterated standards. The weighing must be done to the nearest 0.0001 g and noted so that an exact calculation of the strength of the solution can be made.

6. APPARATUS AND EQUIPMENT

6.1 Plastic thermoisolated boxes, during warm periods refrigerator (+1 to +4°C) or ice for cooling.

6.2 Deep freezer (-18 to -20°C), if sampling trips take more than 48 hours.

6.3 Clean aluminium foil for wrapping sediment samples.

6.4 Clean glass jars for sediment samples.

6.5 Labels or tags for the identification of sediment samples (see fig. 5).

6.6 6x250 mL glass flasks; Quickfit or Jena.

6.7 A balance, E Mettler type B5, no. 14788 (±0.001g) and one top loading type K71 no. 88298 (100-200g) (±0.1g).

6.8 Isomantel, type FSEµ/1L/6, 220/240 volts 6x280 watts, phase S/PH, circuits 1, serial number WB 4649, Isopad Ltd., Bosehamwood, Herts.

6.9 Isopad F.E.R. Control, type No. 7422 Bx.

6.10 6x250 mL glass separatory funnels with teflon stopcocks; Borosilicate glass BS 2021

6.11 A Rotavapor; Büchi Rotavapor, EL, Glasapparatefabrik, Flawil, Switzerland with temperature bath and controller.

6.12 100 mL round bottomed glass flasks for the Rotavapor.

6.13 Packets of vials (2mL) 5080-8712, vial-4330-0525 P, Hewlett Packard.

6.14 Packets of seals, 5080-8713, seal-1540-0132P, Hewlett Packard.

6.15 A pair of pliers for the sealing of vials; Wheaton 224301, Millville, N.J.

6.16 Syringe (100µL) for solvent transfer (Hamilton).

6.17 Syringes (10 and 1µL) for injection of sample into GC and GC/MS, Hamilton Syringe 7001, Supelco, 1 mc. Bellefonte, Pensylvania 16823.

6.18 Gas chromatograph for packed and fused silica capillary columns.

6.19 Gas chromatograph/mass-spectrometer/data system fitted for glass capillary or fused silica columns.

6.20 Glass or fused silica capillary columns approximately 25 m x 0.21 mm 1.D. coated with SE-52, SE-54 or SP-2100.

6.21 Drying cabinet or oven (100° to 300°C).

6.22 UV-fluorescence spectrophotometer

NOTE: Glassware that is used for the first time in the procedure (section 8) should be thoroughly cleaned, using the following procedure:

- wash with soap and water
- rinse in clean water, preferably running water and finally rinse with distilled water and let drain.
- leave the glassware in chromic acid (5.1.8), preferably overnight.
- rinse with water and finally several times with distilled water and let drain.

7. SAMPLING PROCEDURE

The sampling scheme is the basis of the work on petroleum hydrocarbon pollution studies, and therefore very important. The different methods of sampling sediments for chemical analysis can be subdivided into 4 categories: (1) planning, (2) selection of sampling sites and the numbers of samples necessary to cover the area in question, (3) the sample collection (van Veen grab or gravity corer) and storage techniques, and (4) sample type.

7.1 Planning

A sampling plan should be designed in accordance with the nature of the situation to be investigated.

Sediments are unlikely to contain an appreciable amount of dissolved hydrocarbons, since the oil would have passed through the water column on its way to the sediments. Most probably the soluble hydrocarbons have then been removed before the droplets have reached the sediments (McAuliffe, 1980).

Before a survey or monitoring is started, a baseline should be performed: The sedimentation-rate is normally slow and sampling once a year or every two years should be sufficient for a trend monitoring.

7.2 Selection of sample sites

Sample sites are normally chosen on a broad grid network which covers the geographical area that has been affected by an oilspill, which is chosen as a reference area, or where drilling activities are going to take place in the future (Fig. 1).

Because of the analysis of petroleum hydrocarbon samples is expensive, care should be taken in selection of both the number of samples collected and the sample sites. Samples must be selected in a manner that will allow achievment of goals; this usually means over-sampling where only a selected number of samples will be chosen for analysis at a later stage.

Replicate samples should be collected whenever possible at each grid location. Analysis of replicates will allow for the variance in the measured parameters to be determined. This variance can then be used to indicate the number of sample replicates needed to detect a statistical change in the measured values within specified confidence limits. The number of replicates calculated for statistical purposes assumes that the values are "normally distributed" (i.e. Gaussian, Fig. 2).

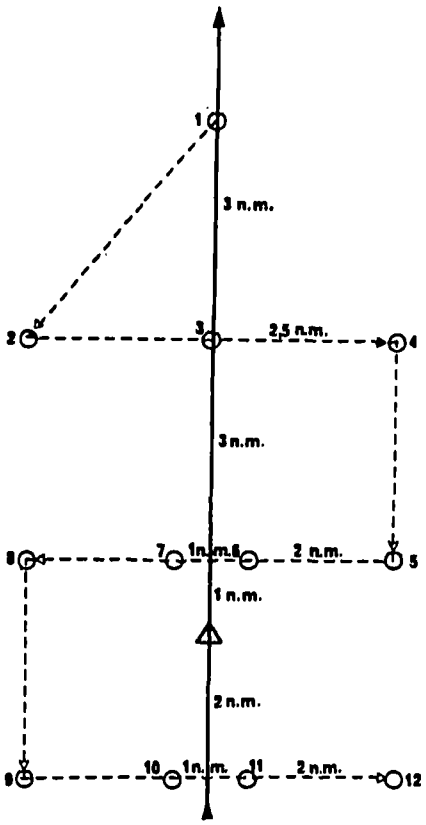


Fig. 1. Grid system for the sampling of sediments. O-sampling stations, Δ-drilling position and the arrow is pointing in the direction of the residual current.

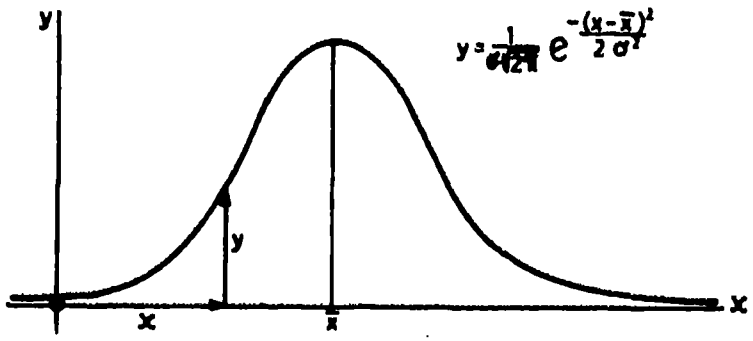


Fig. 2. Normal distribution, Gaussian-curve.

This distribution is usually found if the petroleum hydrocarbons present in the sediment reflect the atmospheric fallout over time. If the components present originated from a recent oilspill with uneven distribution of oil in the sediments, then the hydrocarbon concentrations in a group of replicates may not be normally distributed and therefore, Student's t-tests do not

apply. In such cases these samples should be classified according to the situation causing the pollution.

7.3 Sample collection and storage

Sediment samples can be collected by a van Veen grab sampler.

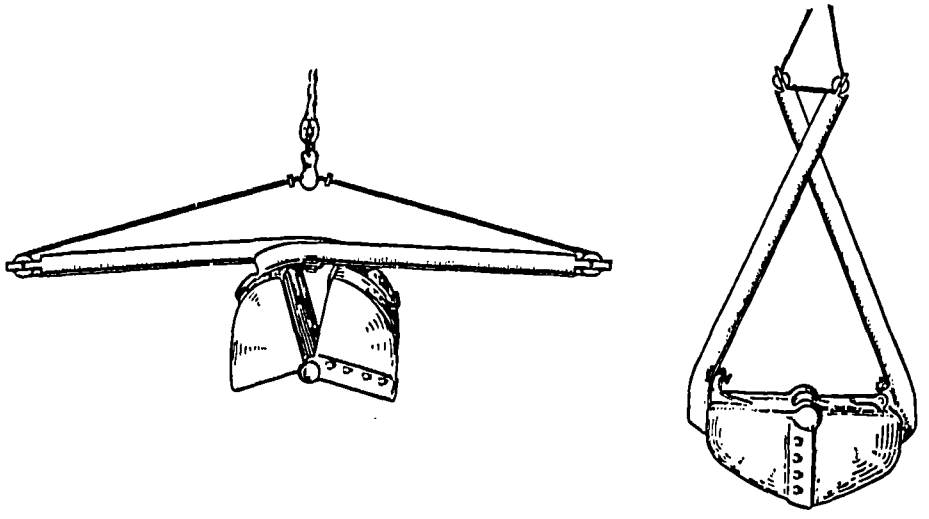


Fig. 3. van Veen grab for sediment sampling (after H. Friedrich).

After retrieval of the sampler, the water should be allowed to drain off, avoiding disturbing the surface layer of the samples. Duplicate subsamples of approximately 500 mL, should be taken carefully from the top 5 cm layer through the hatch in the top of the sampler (Fig. 3). As soon as the subsamples are retrieved, they should be wrapped in aluminium foil or placed in glass jars and immediately frozen at -20°C for analysis at a later time.

The sediment samples can also be collected using a sediment corer equipped with a glass liner (MOORE and NEIL, 1930, Fig.4).

The glass liner containing the sediment sample can be frozen. The sediment can be forced out of the glass liner after thawing with a warm towel and cut to the desired length with a knife.

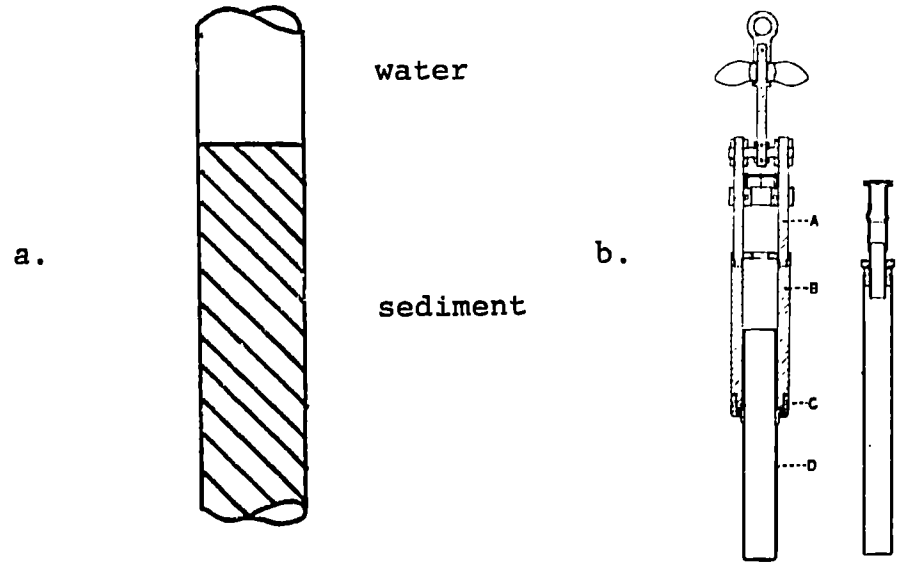


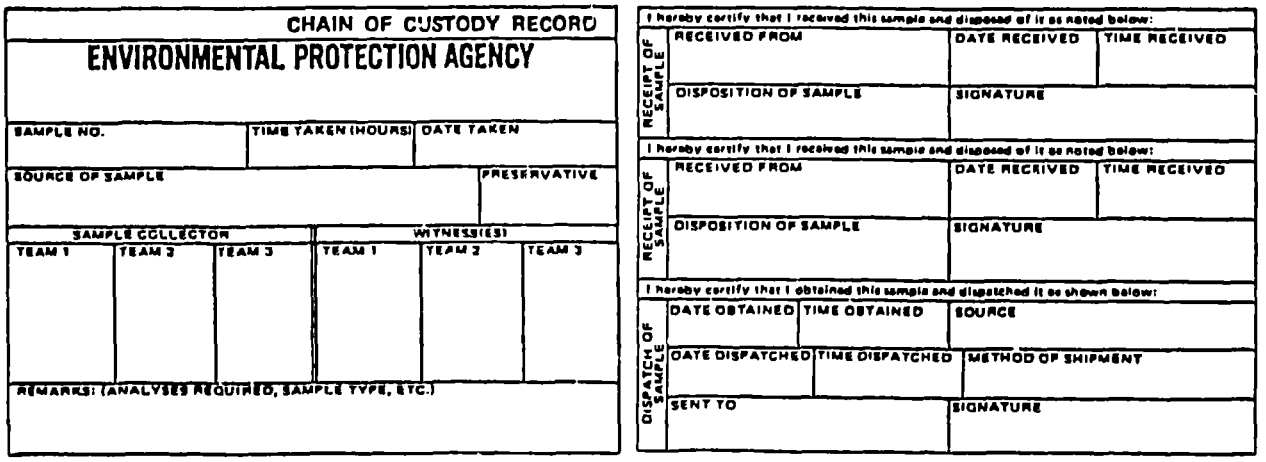
Fig. 4. a. Glass liner from a gravity corer. The sediment from the upper part may be used for trend-analyses. b. Total display of bottom corer.

When the sediment sample is retrieved it is very important that it is stored in a safe place and with a tag showing its identity and all necessary information (Fig. 5).

The sample should at all times be protected against sunlight and be kept cool either in a refrigerator (+1 to+4°C) or in a thermoisolated box cooled with ice until it can be frozen, preferably at -18 to -20°C.

7.4 Sample types

In some cases when sampling sediments (e.g. during oil spills) it is advisable also to collect other types of samples for chemical analysis. To establish the source and identity of the polluting oil, a sample of the oil (1-5 g) should be obtained. In some cases water samples (3-5 L) should be collected to establish whether the oil pollution of the water masses is still present and marine organisms should also be collected for analyses of petroleum hydrocarbons, thus establishing the degree of pollution.



CHAIN OF CUSTODY RECORD

ENVIRONMENTAL PROTECTION AGENCY

SAMPLE NO.	TIME TAKEN (HOURS)	DATE TAKEN

SOURCE OF SAMPLE	PRESERVATIVE

SAMPLE COLLECTOR			WITNESS(ES)		
TEAM 1	TEAM 2	TEAM 3	TEAM 1	TEAM 2	TEAM 3

REMARKS: (ANALYSES REQUIRED, SAMPLE TYPE, ETC.)

I hereby certify that I received this sample and disposed of it as noted below:

RECEIPT OF SAMPLE	RECEIVED FROM	DATE RECEIVED	TIME RECEIVED
	DISPOSITION OF SAMPLE	SIGNATURE	

I hereby certify that I received this sample and disposed of it as noted below:

RECEIPT OF SAMPLE	RECEIVED FROM	DATE RECEIVED	TIME RECEIVED
	DISPOSITION OF SAMPLE	SIGNATURE	

I hereby certify that I obtained this sample and dispatched it as shown below:

DISPATCH OF SAMPLE	DATE OBTAINED	TIME OBTAINED	SOURCE
	DATE DISPATCHED	TIME DISPATCHED	METHOD OF SHIPMENT
	SENT TO	SIGNATURE	

Fig. 5. Tag for the identification of the collected samples (used by U.S. Environmental Protection Agency).

8. ANALYTICAL PROCEDURE

8.1 Sample preparation

Weigh a round-bottomed clean and dry flask with the ground stopper removed and note the weight. Weigh in approximately 150 g to the nearest 0.1 g of partly thawed sediment and note the exact weight and calculate the wet weight (WW).

8.2 Determination of dry weight.

A clean weighing bottle, with the ground stopper removed, is put into the drying cabinet or drying oven (100^{o}C, 2 hours), using a pair of clean pincers. It is important to use the pincers every time the glass is touched, to avoid leaving finger prints and particles of dirt on the weighing bottle. The stopper and the bottle are put into a dessicator to cool.

The empty bottle and stopper are then carefully weighed on an analytical balance. The weight obtained is the weight of the dried, empty weighing bottle and stopper. Note the weight. Remove the stopper and place about 25-50 g of sediment material in the weighing bottle and replace the stopper.

Determine carefully the weight of the weighing bottle stopper and sediment. The weight obtained is the wet weight of the sediment plus the weight of the bottle and stopper.

Place the bottle in the drying cabinet or oven (100°C), removing the stopper and placing it also in the oven.

After 24 hours replace the stopper in the bottle, remove the bottle with the stopper off from the drying oven and place both in a dessicator to cool. Weigh the stoppered bottle and note the weight. Repeat the drying cycle until the difference between subsequent weighings is less than 5 per cent of the total weight; record wet weight (WW) and dry weight (DW) and calculate the WW/DW ratio.

Note: When analyzing for petroleum hydrocarbons in sediments, less alteration occurs if the sediment sample is weighed as wet weight instead of drying at 100°C. Nevertheless, if both wet weight and dry weight are recorded the values can be compared to values reported previously.

8.3 Mineralization of sediment matrix.

Equipment required for this step is shown in Figure 6. To 80-100 g of wet sediment sample in a round bottomed flask add 100 mL redistilled methanol, 3 g KOH and boiling stones. Add 1 mL of a mixture of internal standard containing 0.5 µg/mL each of deuterated-biphenyl, -anthracene and -pyrene dissolved in hexane. Reflux the mixture for 1 hour 30 minutes (Fig.7).

8.4 Extractior of petroleum hydrocarbons.

Cool the methanol extract to room temperature, transfer to a separatory funnel (Fig. 8) and extract twice with 25 mL pentane (Uvasol). Separate the methanol phase from the pentane phase, and transfer the latter to an evaporating flask and reduce the volume to 0.5 mL using a Rotavapor (Fig 9). Quantitatively transfer the reduced volume to a glass vial using pentane (Fig. 10). Concentrate this extract carefully to approximately

Fig. 6. Overview of equipment necessary for the work up of the sediment samples, A = N_2 (5.1.7), B = vial (6.13), C = KOH 5.1.3), D = methanol (5.1.2), E = boiling stones (5.1.4), F = I.S. (5.2), G = pentane (5.1.5), H = sediment sample, I = separatory funnel (6.10) and J = setup for reflux of samples (6.8).

0.2 mL using dry N_2-gas before cleanup on an alumina (5.1.10) column.

8.5 Separation of alkanes and aromatics using alumina columns (to be used before UV-fluorescence and capillary gas chromatographic analysis).

A Pasteur pipette fitted with a glass wool plug is filled to about 5 cm (1.15 gr) with aluminium oxide (5.1.10). The column is rinsed 3 times with 2 mL pentane (5.1.5) and the sample extract added to the top of the column. The elution is performed as shown in Table 1. The different eluates are reduced to dryness using a stream of N_2-gas. For the UV-fluorescence method dissolve the residues containing the aromatics (i.e. fractions 3 and 4) in aromatic-free hexane and transfer quantitatively to a 1 or 5 mL volumetric flask and add hexane to the mark. For the gas chromatographic analysis dissolve the

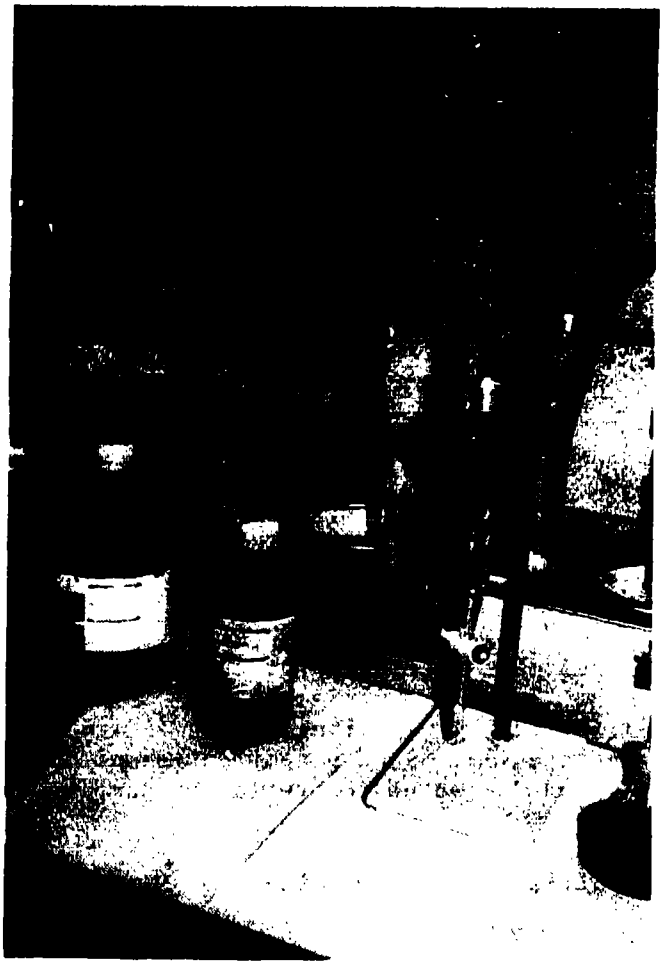


Fig. 7. The flask containing the sediment, methanol, KOH and boiling chips is ready to be refluxed.

residues in a known amount of hexane (between 40 µL and 1 mL depending on the concentration of hydrocarbons).

8.6 Cleanup of extracts using silica columns.

A Pasteur pipette fitted with a glass wool plug is filled with 0.5 gr silica (5.1.9). Rinse the column 3 times with pentane (5.1.5), then place the sample on top of the column and rinse the vial 3 times with 1.0 mL of pentane. Elute the petroleum components totally with 12 mL of pentane in all. Reduce the pentane extract to dryness using a stream of N_2-gas.

Table 1. The elution of the different fractions in the separation of alkanes and aromatics (from a sediment extract).

Eluate no.	mL eluate	elution solvent	solvent ratio	resulting fractions
1	4	pentane		alkanes
2	4	pentane		solvent only
3	4	pentane : dichloromethane	7:3	aromatics[a]
4	4	dichloromethane		higher aromatics[b]

(see Fig. 13 page 21 for illustration of separations).

a) see Fig. 14a for equivalent to eluate 3

b) see Fig. 14b for equivalent to eluate 4

For the gas chromatographic analyses using packed column and also GC/MS in the SIM-mode, dissolve the residue in a known amount of hexane (between 40 µL and 1 mL depending on the concentration of hydrocarbons).

Fig. 8. Transfering the methanol extract to a separatory funnel (6.10).

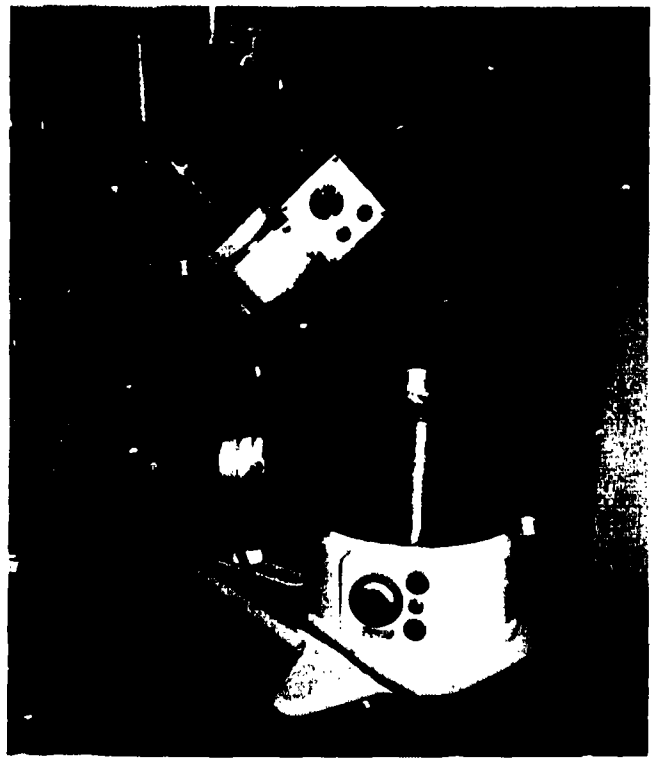


Fig. 9. Reduction of the pentane extract under reduced pressure using the Rotavapor (6.11).

8.7 The UV-fluorescence screening method

The methodology pertaining the analysis of petroleum hydrocarbons using UV-fluorescence is presented below. When conducting these analyses, the points mentioned in Appendix I should be continously kept in mind.

A sample of the dissolved extract in n-hexane should be placed in a capped 1-cm silica cell. Measure the intensity of fluorescence at 360 nm (excitation at 310 nm). If possible, both the excitation and fluorescence spectra for each sample should be scanned. The mixture of fluorescing substances (i.e. primarily substituted benzenes, naphthalenes and polynuclear aromatic compounds) present in crude and residual fuel oils is excited most strongly at 310 nm and fluoresces most intensely in the neighbourhood of 360 nm.

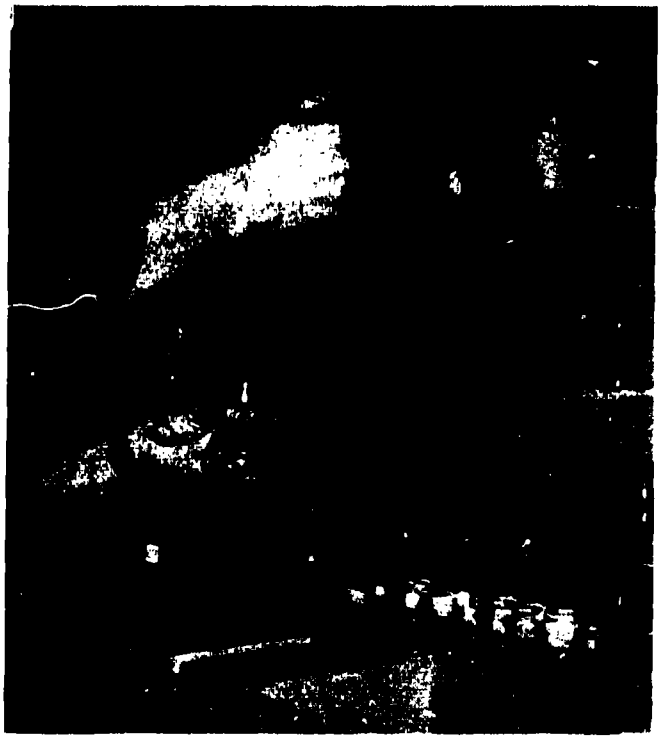


Fig. 10. Transfer of the reduced volume of the pentane extract to a vial (6.13) using a Pasteur pipette.

Fig. 11. Using a pair of pliers (6.15) to seal the vial containing the extract to be injected into the gas chromatograph.

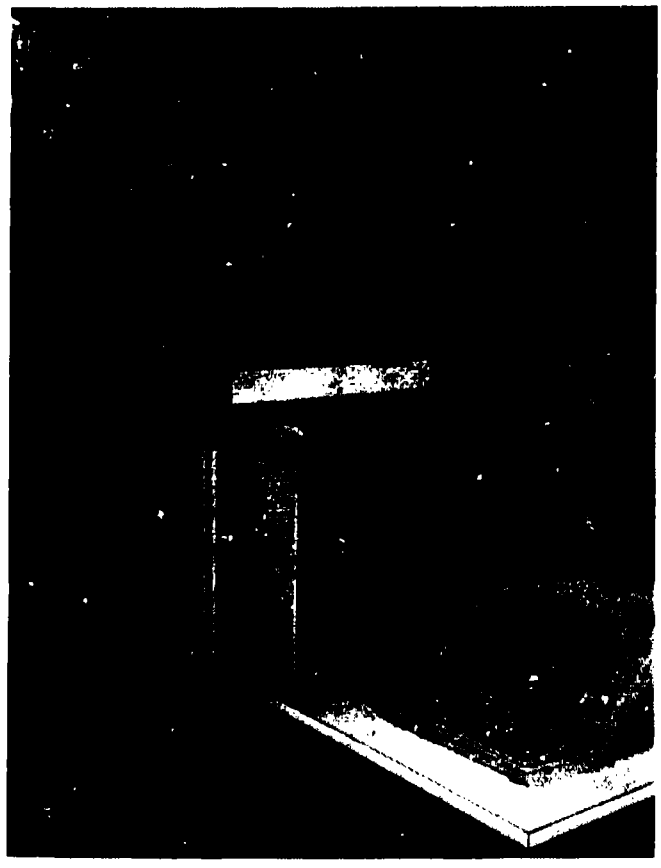


Fig. 12. Injection of sample into the gas chromatograph, HP 5880, (6.18) equipped with a fused silica capillary column (6.20).

8.7.1 Calibration

The fluorescence intensity of the hexane solution is compared with the fluorescence of a reference solution. Chrysene should be used as the standard reference material until a more suitable reference material is obtainable. The chrysene solution should have almost the same emission intensity as the unknown extract. Calibrations should be run at least once a day under identical instrumental conditions.

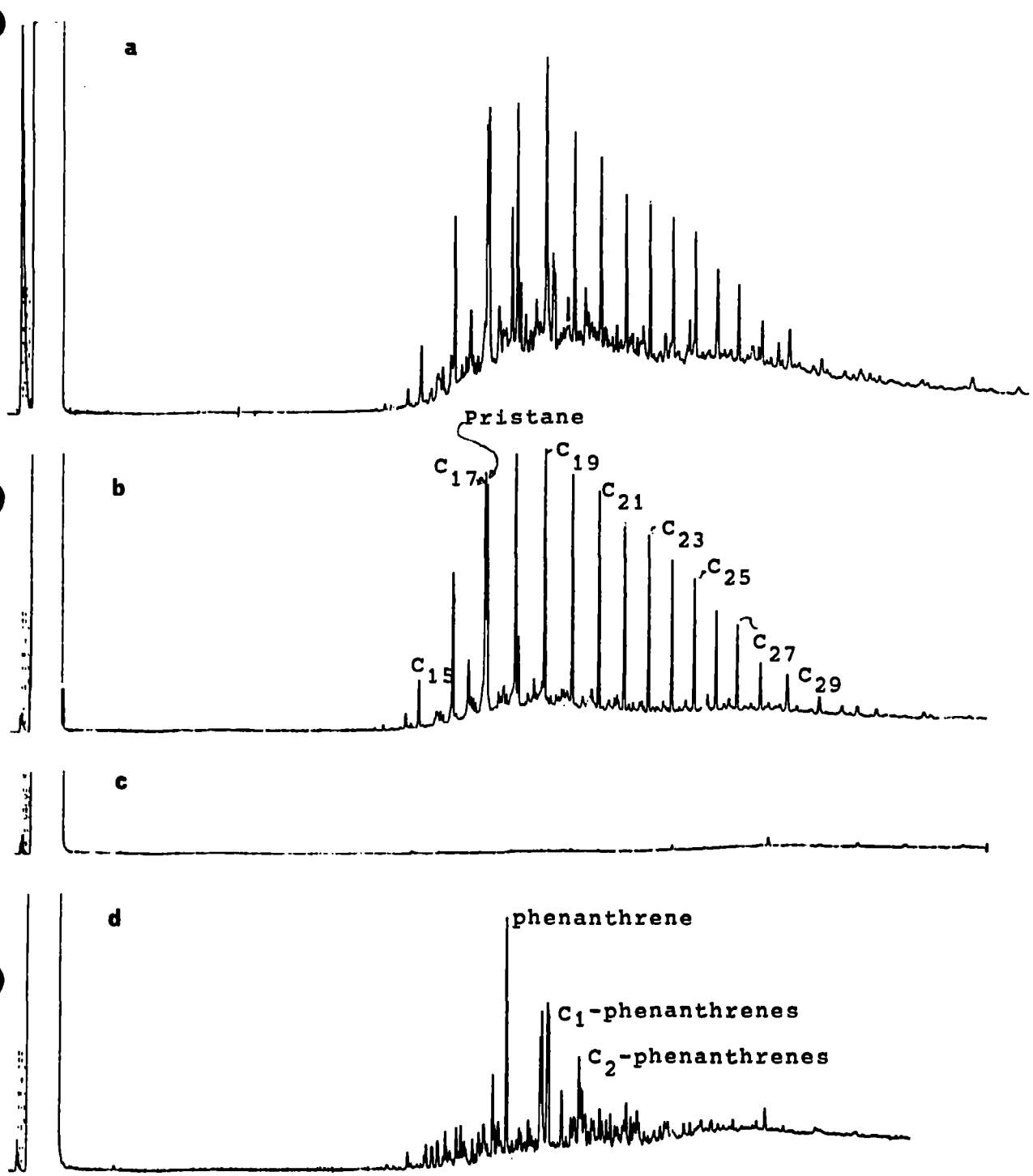


Fig. 13. Separation of sediment sample extract from Spitsbergen (Oilspill at the Svea Coalmine 1979)

a) Before separation on acid alumina
b) 1. eluate, 4 mL pentane; alkane fraction
c) 2. eluate, 4 mL pentane
d) 3. eluate, 4 mL pentane; CH_2Cl_2 (7:3): aromatic fraction

(see page 17, Table 1, section 8.5)

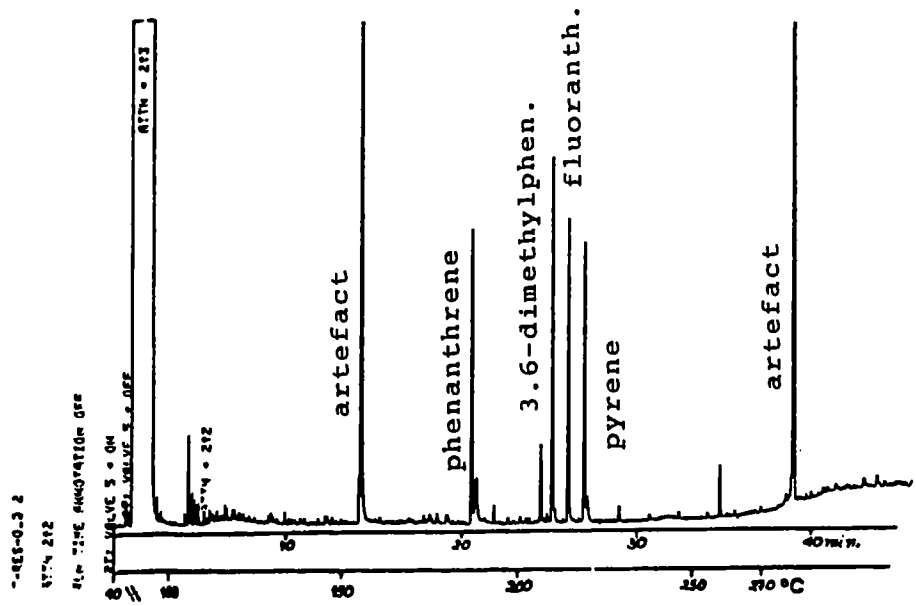


Fig. 14a. A mixture of polycyclic aromatic hydrocarbons (PAH) chromatographed on acid alumina (8.5), 3. eluate (4 mL pentane : CH_2Cl_2; 7 : 3).

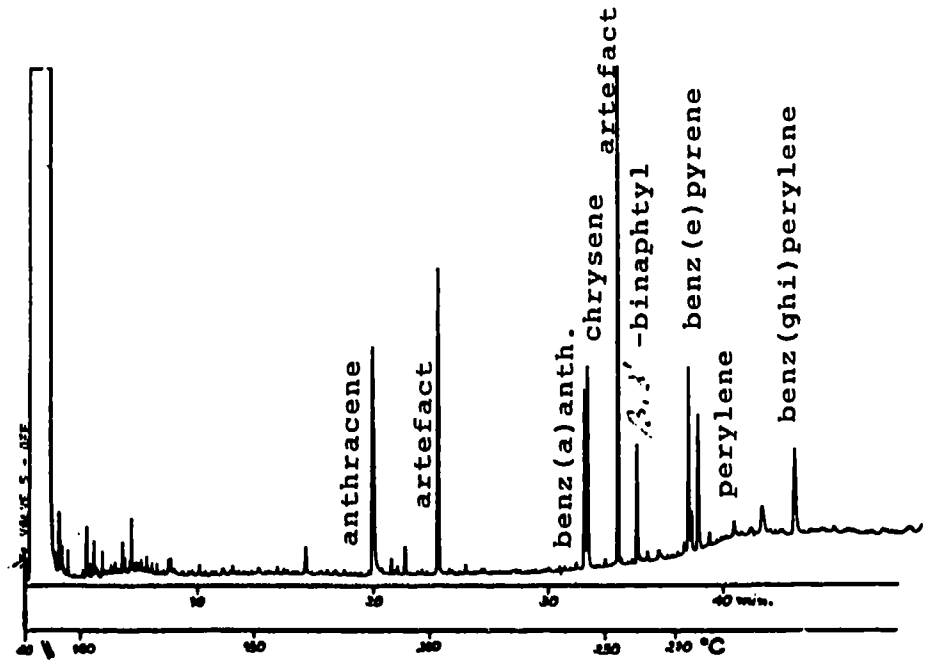


Fig. 14b. A mixture of polycyclic aromatic hydrocarbons (PAH) chromatographed on acid alumina (8.5), 4. eluate (4 mL CH_2Cl_2).

8.7.2 Blanks

Throughout the procedure, great care must be taken to ensure that samples are not being contaminated; for example, avoid unnecessarily exposing the sediment sample, the n. hexane or the final extract to the atmosphere or other potential sources of contamination. Solvents and equipment should be frequently checked for contamination, by analyzing blanks. This is best done by running through the whole procedure omitting the sediment sample. When blanks fluoresce high enough to indicate contamination (i.e., fluorescence is greater than that for 1 microgramme per litre chrysene), sources of contamination should be eliminated rather than correcting the data obtained for the blank value.

8.7.3 Calculation of Original Sample Concentration

The concentration of hydrocarbons in the sample extract, in chrysene equivalents, is read from the chrysene calibration curve and corrected for any blank fluorescence. The original sample concentration in chrysene equivalents is then calculated according to the following:

$$A = \frac{B \times C}{D}$$

A = Conc. of hydrocarbons in original samples in µg/g
B = Conc. in sample extract in µg/mL
C = Volume of extract in mL
D = Weight of original sample in grams

The result reported is the chrysene equivalent concentration in µg/grams.

8.7.4 Testing for Fluorescence Quenching

Instances may occur where high concentrations of compounds, other than hydrocarbons, may be present in the sample and could quench or inhibit the fluorescence of the hydrocarbons in the

sample. An experiment to test whether this is the case can be performed as follows:

(i) the fluorescence of the sample extract is measured;

(ii) the fluorescence of the chrysene standard is measured;

(iii) equal volumes of the sample extract and chrysene standard are combined and the fluorescence of the mixture measured;

The fluorescence of the mixture should be equal to:

$$\text{Fluorescence Mixture} = \frac{\text{fluorescence chrysene std.}}{2} + \frac{\text{fluorescence sample extract}}{2}$$

If the fluorescence is less than the predicted amount by 20% or more, significant quenching is occurring and a sample clean-up should be attempted (8.6).

8.8 Gas chromatography using packed columns

For the estimation of "total oil" in sediments, gas chromatography with packed columns may be used, although much information is lost. Inject 2 µL of the sample extract, which has been subjected to the cleanup procedure (8.6), into the gas chromatograph (see Table 2 for chromatographic conditions).

The following solution may be used as a reference sample: 2µL of a solution containing 150 mg of the crude oil or oil in question in 25 mL of hexane; 12 µg injected. The chromatograms can be integrated using an integrator or a planimeter. It has proven beneficial to integrate only a "window" of the chromatogram; for example, between n-C_{15} and n-C_{26}, therefore, one should integrate and determine the area per µg reference oil for the "total oil" estimation using the area between n-C_{15} and n-C_{26}.

The reference oil must be analyzed using conditions identical to those applied for the actual samples.

Table 2. Conditions for packed column gas chromatography.

A Gas chromatograph equipped with a flame ionization detector

Carrier gas	: Nitrogen ∿17 mL/min
Column	: 200 cm x 4 mm i.d. 3% SP 2100 on Chromosorb W HMDSO 80-100 mesh
Injection temp.	: 300°C
Manifold temp.	: 350°C
Temp.programming	: Initial value 80°C Initial time 3 min. Prgm. rate 8°C/min. Final time 15 min.
Injected volume	: 2 µL

8.9 Gas chromatography using fused silica capillary columns

1-2 µL of the sample extract, which has been subjected to the separation procedure above (section 8.5), is injected by the splitless injection technique. The chromatographic conditions are given in Table 3.

Table 3. Conditions for capillary column gas chromatography.

HP 5880A Gas chromatograph equipped with a flame ionization detector.

Carrier gas	: Nitrogen 8 psi, 1.5 mL/min.
Make up gas	: Nitrogen 40 mL/min.
Column	: 25 m x 0.33 i.d. SE-54 fused silica
Injection temp.	: 280°C
Manifold temp.	: 280°C
Temp.programming	: Initial value 40°C Initial time 4 min. Prgm.rate 10°C/min. Final time 15 min.
Injected volume	: 1.5 µL splitless

The peak areas are measured using an electronic integration system and identification is achieved by comparing the gas chromatographic retention times with those of known standards and with previous gas chromatographic/mass spectrometric analyses of similar samples using identical conditions and the same column.

Quantification of the identified and integrated peaks is performed by comparing with the nearest internal standard.

8.10 Computerized gas chromatography/mass spectrometry (GC/MS/Comp)

0.3 µL of the sample extract, which has been subjected to the cleanup procedure above (8.6) is injected by the splitless injection technique. The chromatographic conditions are described in Table 4.

Table 4. Conditions for the computerized gas chromograph/mass spectrometer (GC/MS/Comp).

Finnigan GC/MS/Comp Model 9000/3200F/6100

Carrier gas	: Helium 1.5 mL/min
Column	: 25 m x 0.2 mm i.d. Sp 2100 fused silica
Injection temp.	: 280°C
Temp.programming	: Initial value 20°C, then 20-100°C in 1 min.
	Initial time 1 min.
	: Prgm.rate 6°C/min. from 100-230°C
	: Final time 5 min. isothermal
Injected volume	: 0.3 µL splitless.

The column is extended all the way through to the ion source on the Finnigan 3200 mass spectrometer. The following aromatic hydrocarbons have shown to be applicable for analysis using the selected ion monitoring (SIM) mode, Table 5.

Figures 15a and 15b page 30 and 31 are examples of chromatograms of selected aromatic hydrocarbons obtained using the selected ion monitoring (SIM) mode.

Table 5. Aromatic hydrocarbons selected for analyses.

Components	Molecular ions
Naphthalene	m/z 128
methyl-naphthalenes (C_1)	m/z 142
C_2-naphthalenes	m/z 156
C_3-naphthalenes	m/z 170
phenanthrene	m/z 178
methyl-phenanthrenes (C_1)	m/z 192
C_2-phenanthrenes	m/z 206
Methyl-dibenzothiophenes (C_1)	m/z 198
C_2-dibenzothiophenes	m/z 212
C_3-dibenzothiophenes	m/z 226

The quantification of the naphthalenes is based on the internal standard, deuterated biphenyl (ion-164). Deuterated anthracene (ion-188) is used for the quantitation of phenanthrene, methyl-phenanthrene, methyl-dibenzothiophene; and deuterated pyrene (ion-212) is used for the quantitation of dimethyl-phenanthrene, dimethyldibenzothiophene and trimethyl-dibenzothiophene.

The areas under the different peaks are calculated using a Finnigan 6100 computer.

The response factors for the different aromatic hydrocarbons relative to the deuterated standards, biphenyl, anthracene and pyrene, are determined by injecting known amounts of the chosen aromatic components in a mixture of Ekofisk crude (rich in naphthalenes and phenanthrenes) and Arabian light crude (rich in dibenzothiophenes).

Ekofisk crude, 20 mg, is chromatographed on a short silica column (2.5 cm silica in a Pasteur pipette). The silica gel (120-230 mesh) is activated over night at 105°C before use. The alkanes (and some of the lower naphthalenes) are eluted with 7 mL pentane. The aromatic fraction is then eluted with 5 mL pentane. The eluate is taken to dryness and then dissolved in 100 µL hexane.

The Arabian Light crude oil is treated in the same manner to obtain an aromatic mixture high in dibenzothiophenes.

These two mixtures are analysed using gas chromatography having a flame ionization detector (FID), capillary column and the same conditions as used for the GC/MS analyses. It is an assumption that components that resemble one another give the same response per unit weight. The composition of the aromatic mixtures (in percent) is calculated from the areas of the GC-diagrams. When the composition is known, exact amounts of naphthalene, 2.6-dimethylnaphthalene and 2,3,6- trimethylnaphthalene are added so that the naphthalenes can be determined. Phenanthrene is added to determine methylphenanthrene and methyl-dibenzothiophene, and fluoranthene is added for the determination of dimethylphenanthrene, di- and trimethyldibenzothiophene. The new mixtures are now analysed by GC and the peak areas integrated. The information is now sufficient to determine the naphthalenes, phenanthrenes and dibenzothiophenes in the aromatic mixture.

To be able to calculate the responsefactors for the different aromatic hydrocarbons using GC/MS, known amounts of the three deuterated standards are added to the aromatic mixtures and the samples are then analysed by GC/MS in the SIM mode. Since both the amount of aromatic hydrocarbons and the deuterated standards now are known, the responsefactors can be calculated.

Example:
The responsefactor (rf) for naphthalene in relation to biphenyl-d_{10} is the number you multiply by the area of the 128-

fragment (naphthalene is detected using ion-128) to obtain the true amount.

The responsefactor for naphthalene is calculated as follows:

$$rf = \frac{\text{known amount naphth/area naphth}}{\text{known amount I.S. (biphenyl-d}_{10}\text{)/area I.S.}}$$

$$rf = \frac{18.5/733351^*}{37.3/980978} = 0.66$$

* the amount of naphthalene, phenanthrene etc. originates from the standard mixture.

The responsefactor for phenanthrene is calculated using

I.S. = anthracene-d_{10}.

The standard aromatic mixture is made using mixtures of Ekofisk and Arabian Light crude oils where known amounts of the deuterated standards are added. The aromatic hydrocarbons in the crude oil mixtures are quantified using GC/MS/SIM and the responsefactors then calculated (see above). The resulting mixture is now used as a standard for the calculation of the response factors used for the quantitation of naphthalenes, phenanthrenes and dibenzothiophenes.

Table 6. Amount in µg/mL of the different aromatic hydrocarbons in the standard oil as determined by GC(FID).

Compound	µg/mL	Compound	µg/mL	Compound	µg/mL
Naphthalene	18.5	Phenanthrene	6.2	C_2-phenanthr.	20.5
meth.naphth.	81.6	ANTHRACENE-D10	33.0	C_2-dibenzothioph.	47.3
C_2- naphth.	115.6	meth.-phenanthrene	20.7	C_3dibenzothioph.	29.8
C_3- naphth.	75.7	meth.-dibenzothioph	29.3	PYRENE-D10	33.2
BIPHENYL-D10	37.3				

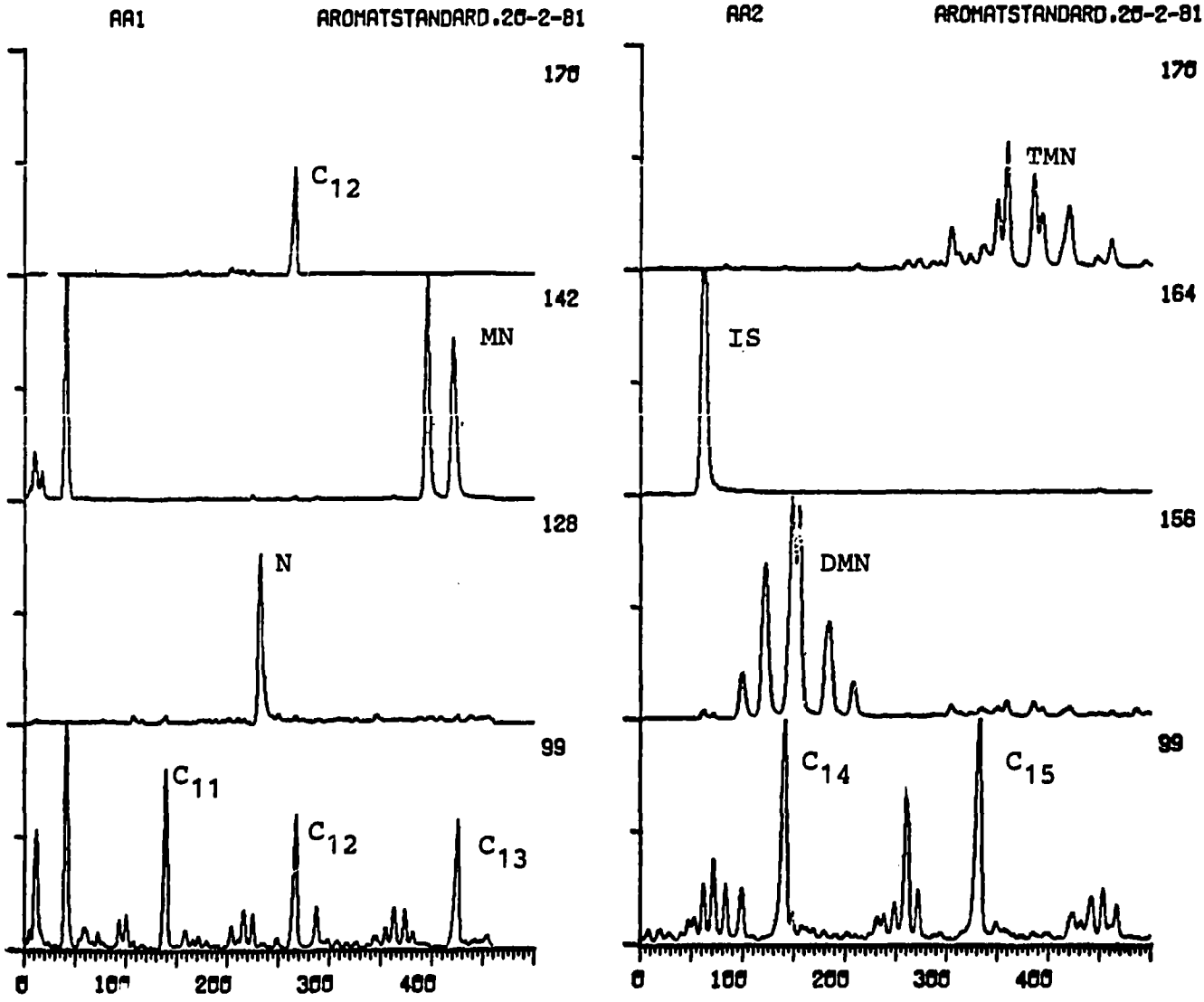


Fig. 15a. Gas chromatogram of the standard aromatic solution from a capillary column with mass spectrometric SIM detection representing the following peaks:

File AA1*: 99-alkanes
128-naphthalene
142-methylnaphthalenes
170-trimethylnaphthalenes

File AA2: 99-alkanes
156-dimethylnaphthalenes
164-diphenyl-d_{10} (internal standard)
179-trimethylnaphthalenes

*File AA1 is the storage space in the data system.

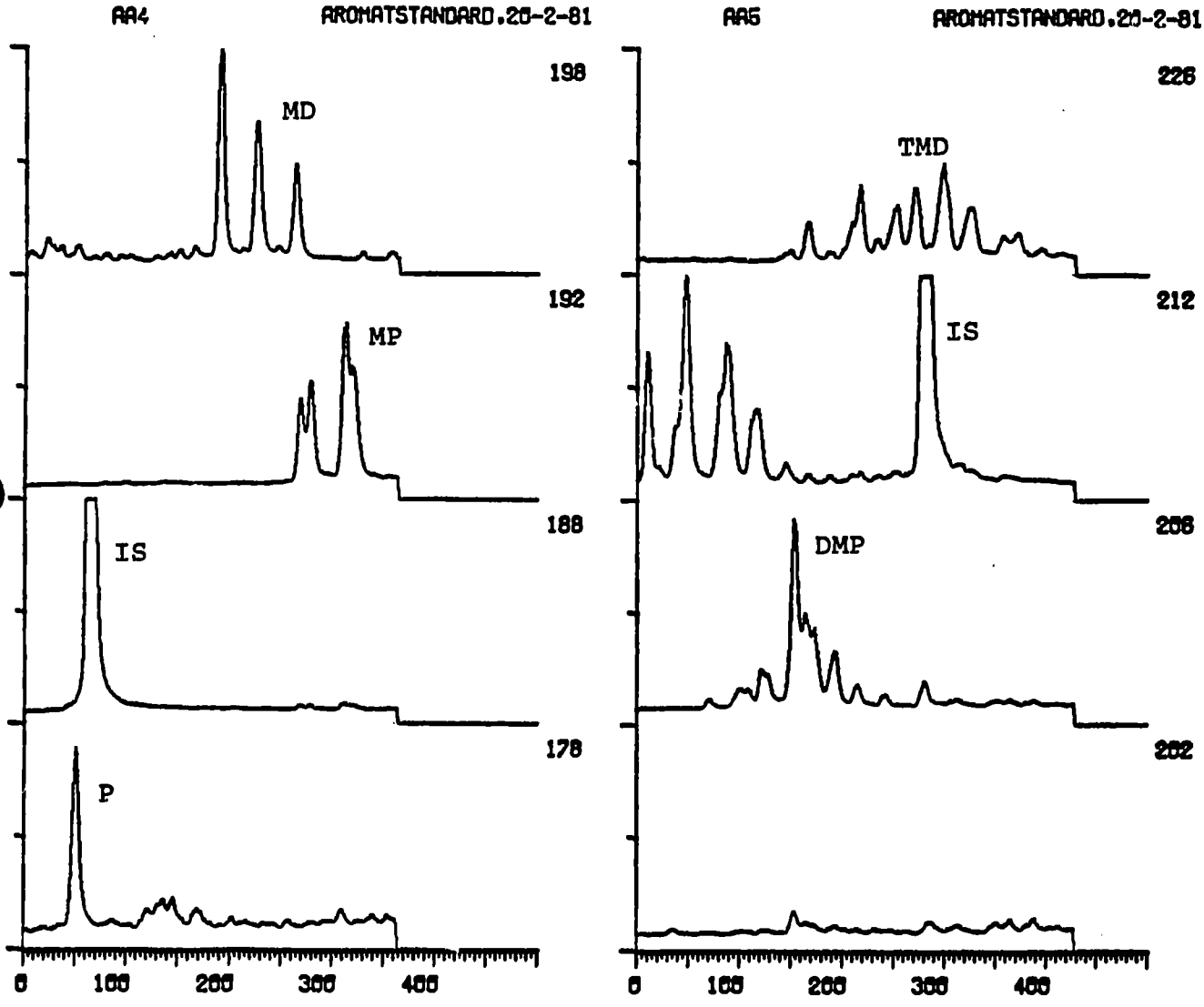


Fig. 15b. Gas chromatogram of the standard aromatic solution from a capillary column with mass spectrometric SIM detection representing the following peaks:

File AA4: 178-phenanthrene
188-anthracene-d_{10} (internal standard)
192-methylphenanthrenes
198-methyldibenzothiophene

File AA5: 202-fluoranthene, pyrene
206-dimethylphenanthrenes
212-dimethyldibenzothiophene, pyrene-d_{10} (internal standard)
226-trimethyldibenzothiophene

9. BASIS FOR OBTAINING QUALITY DATA

To ensure the best possible quality of the environmental analytical measurements carried out, the American Chemical Society (ACS) Committee on Environmental Improvement directed its Subcommittee on Environmental Analytical Chemistry to develop a set of guidelines: "Guidelines for Data Acuisition and Data Quality Evaluation in Environmental Chemistry". These guidelines are helpful in developing reliable chemical analysis of environmental samples and should therefore allow more confident interlaboratory exchange and use of the obtained data. This chapter is based mainly on the ideas given in these guidelines.

Environmental analytical measurements are developed for a variety of purposes (see introduction). This broad range of need for analytical data and the many ways of using the information may require a variety of analytical certainty. It is therefore important to establish the need for accuracy in each situation. There are many sources of difficulties in environmental analyses: a large number of organic compounds; a wide variety of parameters, lack of or gaps in technical knowledge, sampling bias, systematic and human errors are among those listed in the "Guidelines".

9.1 Planning. On the background of all these difficulties, accuracy is very difficult to achieve. It is therefore of great importance that every step of the task is well planned. Such a plan or model is implicitly or explicitly involved in every measurement process. Data are generated for use in answering questions from which conclusions may be drawn. If the model, that is the interrelations of the data to the problem, is faulty, the conclusions drawn will also be faulty, even if the measurements are of good quality.

The plan model should be based on the cooperative effort by the analyst, who knows the measurement techniques, the scientist who will use the data, and the statistician who eventually will evaluate the data. Further, no measurement program should be

undertaken until such a plan model is established. The plan should contain the general aspects of the problem and the analytical system to be used to solve the problems, preferably with reference to written details of the plans and procedures (protocols).

9.2 Quality assurance. To identify and correct problems a quality assurance program should be designed and it should include:

1. Maintenance of skilled personell, written and validated methods, and properly constructed, equipped and maintained laboratory facilities.
2. Provision of representative samples and controls.
3. Use of high-quality glassware, solvents, and other testing materials.
4. Calibration. adjustment, and maintenance of equipment.
5. Use of control samples and standard samples, with proper records.
6. Directly observing the performance of certain critical tests.
7. Review and critique of results.
8. Tests of internal and external proficiency testing.
9. Use of replicate samples.
10. Comparison of replicate results with other laboratories (intercalibration).
11. Response to user complaints.
12. The monitoring of results.
13. Corrections of departures from standards of quality.

These basic elements of quality assurance define the framework that written protocols, including all analytical procedures, must follow to obtain reliable results.

Accurate data are far more likely to be obtained when supported by the competent use of internal and external standards, and when the laboratory has demonstrated close agreement with acceptable levels of accuracy proved by participation in an intercalibration exercise.

9.3 Definitions of some statistical terms

9.3.1 The specificity of a analytical chemical method is the decree to which the mean value of the measurements is due to the substance to be determined and not to other substances that may be present in the sample being analysed.

9.3.2 The sensitivity of a analytical chemical method is the smallest change in the quantity to be measured which produce a detectable change in the output. In this case it is synonymous with the term minimum detectability.

9.3.3 The precision of a analytical chemical method is the degree to which one representative determination of a substance in a sample will yield a measurement that approaches the average measurement of an infinite number of determinations of the same sample. (The precision in other words means the reproducibility of the analytical results).

9.3.4 The accuracy of a analytical chemical method is the degree to which the mean value of the measurements obtained by the method approaches the true value for the measured substance (the effects of other substances interfering being eliminated physically or mathematically).

10. CONCLUDING REMARKS

The selection of methods for proper analyses of petroleum hydrocarbons is not great and the choice being dictated by the situation at hand and the object of the analysis. The analytical methodology available, however, is sensitive and selective so that individual aromatic hydrocarbons may be determined in sediments at levels down to 10^{-9} g/g dry weight of sediment. Fig. 16 gives an overview of the most common way of analysing sediment samples today.

Today it is feasible to separate hundreds of individual aromatic components, but there are very few standards commercially available at the present. However, if the components that can

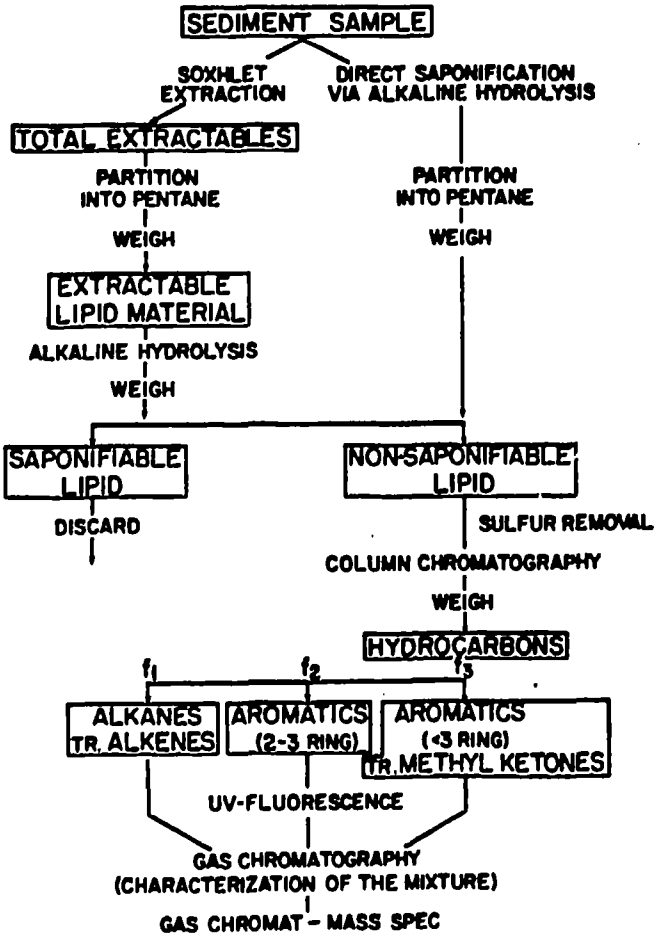


Fig. 16. Flow scheme for one type of hydrocarbon analysis for sediment samples (from Farrington, 1980).

be identified are analysed, either using standards and retention times or by GC/MS, then these components can be monitored so that an increase or decrease in concentrations in sediments over time might be discerned, and, therefore, trends established.

In the time to come the increasing offshore activity will demand increasing efforts of the analytical chemists, both in analysing field samples and doing research in the laboratory to solve questions such as solubilities of aromatic hydrocarbons in general, but also how the solubility of the different aromatic components in sediments change over time.

APPENDIX I

GEMSI Ad hoc Working Group on the Analysis of Dissolved/Dispersed Petroleum Hydrocarbons in Seawater

Woods Hole, Massachusetts, USA,
16-18 March 1981, p.4-5

Use of UVF in a petroleum pollution monitoring programme

All measurement techniques are only one part of the overall monitoring scheme, which includes planning, sampling, extraction, measurement, data reporting and interpretation. Many of these steps are the same or similar regardless of the measurement technique used in the monitoring programme. A complete assessment of simplicity, sensitivity, and cost must take all these steps into account.

The ad hoc Group felt that the following caveats must be kept in mind when a decision is taken to use UVF for dissolved/dispersed petroleum hydrocarbon monitoring:

(i) The UVF technique provides a relatively gross measurement of dissolved/dispersed petroleum hydrocarbons, and only if enough data are available (e.g., hundreds of samples for the Gulf of Mexico) is it possible to identify with adequate certainty "pollution hot spots" and trends in concentration of dissolved/dispersed petroleum hydrocarbons.

(ii) It is a technique that is easily introduced into countries with a developing expertise, and this is a major consideration in its choice as the preferred method for MARPOLMON for dissolved/dispersed petroleum hydrocarbons in seawater. However, developed countries with expertise in methods other than UVF, such as GCGC-MS, which allow for more detailed analyses, should be encouraged to apply these methods to pollu-

tion problems with the hope of increasing not only our understanding of what the UVF technique measures, but of the overall distribution of various types of individual compounds in dissolved/dispersed petroleum hydrocarbons. Additionally, the ad hoc Group felt that developed countries should aid developing countries in the acquisition of these more sophisticated instruments and in training of analysis within the IOC/TEMA framework.

(iii) The collection of data is only the first step of such a monitoring programme and provisions must exist of periodic assessment of the data and for generating recommendations based upon them. This is true for any method employed.

(iv) Important limitations are inherent in the UVF technique. For this reason, it should be considered as a starting point for this type of monitoring programme which will increase in sophistication and scope as levels of expertise and equipment availability rise to a point where more sophisticated techniques can be implemented.

(v) The programme should include a continuing effort of experimentation as to the effects of such factors as weathering of various crude oils on their fluorescence properties.

(vi) The programme should include submission of representative samples from various regions to the more detailed analysis available in laboratories with GCGC and GCGC-MS analysis, in order to assess better the types and, if possible, the sources, of compounds that cause the fluorescence measured.

It was pointed out that other methods such as glass-capillary GC and GC-MS do not have the standardization problems inherent in the UVF method. An overriding consideration in the applica-

tion of these methods to a global MARPOLMON activity is the limitations in instrument availability and technical expertise. However, since such instrumentation and technical expertise are available in some developed countries and may possibly be available in the near future in many developing countries, MARPOLMON should encourage, wherever possible, the application of these techniques in conjunction with UVF, thereby enhancing understanding of the distribution and fate of petroleum hydrocarbons in the marine environment.