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Isolation and Gas-Chromatographic Determination of Saturated and Polycyclic Aromatic Hydrocarbons in Mussels

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The method here presented is intended for application in the analysis of trace quantities of petroleum hydrocarbons in oil-polluted mussels.

It is a technique incorporating saponification, pentane extraction and silica gel cleanup, and represents a modification and *ad hoc* synthesis of other procedures (from, e.g., Black *et al.* (1979), Brown RA *et al.* (1979), Brown LR *et al.* (1979), Cretney *et al.* (1980), Dunn (1976), Farrington & Tripp (1975), Overton & Laseter (1980) Warner (1976)). Recovery efficiencies of the method are 80% or greater for C_{15} to C_{38} n-alkanes, and vary between 0% for naphthalene and 84% for pyrene.

Isolation and quantification of the polycyclic aromatic hydrocarbon (PAH) fraction are of importance in environmental monitoring; some compounds in this group are either known or suspected to be carcinogenic agents (Lee *et al.* (1981)).

Concomitant analysis for the saturated hydrocarbons fraction can provide essential clues to the petrogenic or pyrolytic origin of the PAHs.

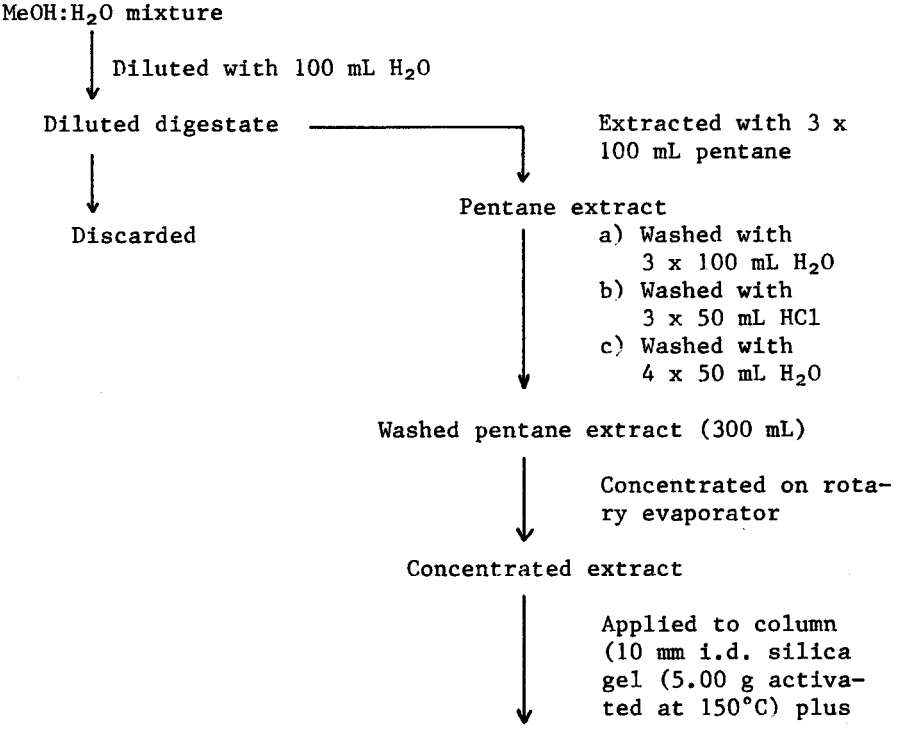
MATERIALS AND METHODS

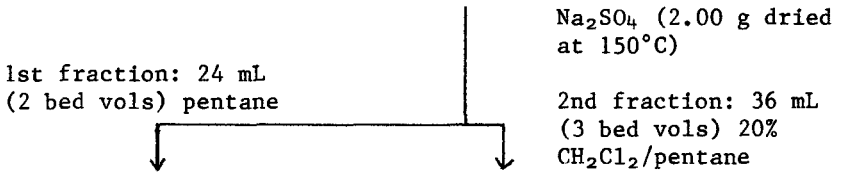
Sodium sulphate (anhydrous) - Merck G.R.
Potassium hydroxide - Merck G.R.
Silica gel - Merck silica gel 60 extra pure for column chromatography
Hydrochloric acid - Merck G.R.
Methanol - Merck G.R.
Dichloromethane - Merck G.R.
Cyclohexane - Merck G.R. or redistilled BDH Laboratory Grade
Pentane - Technical grade (∿25% iso and ∿75% n-isomers) redistilled through glass fractionating column, 1 m x 55 mm, packed with glass wool

* Correspondence and reprint requests.

Water - collected directly in glass from stainless steel/enamelled cast iron still
n-Alkane Standards:
C_{15} to C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, C_{36} and C_{38} - Alltech Associates, Illinois
PAH Standards:
Naphthalene - Merck scintillation grade (≧99%)
Acepnaphthene - Fluka AG (∿98%)
Fluorene - Fluka AG (≧97%)
Anthracene - Merck scintillation grade (≧99,5%)
Fluoranthene - Fluka AG (≧98%)
Pyrene - Fluka AG (∿97%)
Benzo(a)pyrene - Fluka AG (∿97%)
Benzo(ghi)perylene - Fluka AG (> 98%)
Isoprenoid Hydrocarbon Standards:
Pristane (2, 6, 10, 14 - Tetramethylpentadecane) - Aldrich
Phytane (2, 6, 10, 14 - Tetramethylhexadecane) - Sigma
Gas chromatograph: Pye-Unicam Model 204
Detector: Flame ionization
Column: J & W DB-5 fused silica capillary: 30 m x 0.245 mm i.d. x 0.25 µm film thickness
Carrier gas: High-purity nitrogen
Integrator: Hewlett-Packard 3388A

The scheme for the isolation of the hydrocarbons follows:





The amounts of reagents used throughout the separation scheme appear, from empirical observation, to be optimum for 10 g of sample or less.

The presence of 25% water in the methanolic potassium hydroxide saponification mixture prevents cross-esterification with the methanol. (Farrington & Tripp 1975).

The 1:1 dilution of the digestate with water is to reduce the solubility of both the pentane extractant and the nonpolar target compounds in the saponification mixture.

Washing of the pentane layer with hydrochloric acid is essential to remove (by hydrolysis alone?) a considerable number of compounds which elute from the cleanup column with the solvent front of the first fraction, masking any hydrocarbons present. Sulphuric acid cannot be substituted for HCl, as, although the interferent compounds are removed from the pentane layer, the PAHs are also found to be eliminated, presumably (at the low concentrations encountered) by sulphonation.

The washed pentane extract is concentrated very carefully just to dryness on a rotary evaporator, the water bath being set at 30°C and the condenser water at 1-4°C. Evaporation to dryness is necessary in order to ascertain the mass of the total extract, to ensure that the mass of silica gel used in cleanup is at least 100 times greater than this value.

The cleanup columns are dry-packed, as even at sub-tropical winter day-time temperatures (~20°C), spontaneous bubbling of the pentane in slurry-packed columns results in channelling and local drying of the bed.

Use of dichloromethane in elution of the PAHs from the cleanup column is advantageous in two respects: its boiling point (39.8°C) is close to that of pentane (36.1°C), thus enabling concentration of eluates with minimum losses, and it can generally be used as supplied by Merck (i.e., is more easily purified than, for example, cyclohexane).

Virtually all of the reagents used have to be freed from impurities, using the pentane and/or the distilled water, the only two components of the separation scheme which are sufficiently pure for trace analysis. The sodium sulphate and silica gel require elution in a glass chromatographic column with at least 4 or 5 bed

volumes of pentane. The saponification mixture is extracted with its own volume of pentane (divided into three separate washes) to remove waxes from the potassium hydroxide and unidentified interferents from the methanol, while the hydrochloric acid is similarly washed, and stored in glass, rather than its original plastic, bottles. Saturated aqueous sodium chloride solution, used in amounts of 1- 5 mL for breaking of emulsions at all stages of the separation scheme, is extracted with pentane and stored in glass.

All laboratory ware used in sample preparation is either pentane-washed metal or glass cleaned with chromic acid and rinsed with distilled water - plastic, rubber, cork, silicone grease, etcetera are avoided, and Teflon liners are used in plastic caps for bottles and sample vials.

For the analytical component of the work, a crude Grob-type splitless capillary injector was devised, and a 'J & W' DB-5 fused silica capillary column (25 m x 0.245 mm i.d. x 0.25 μm film thickness) installed on the gas chromatograph. Figures 1 and 2 show the separations obtained on the saturated hydrocarbons and the PAHs (at concentrations of approximately: a) 10 ppm per component and b) 1 ppm per component (the latter being about the lowest standard concentration practicable)).

RESULTS AND DISCUSSION

Six replicates of 10 g of mussel homogenate (from a composite sample of ∿120 g of mussel tissue from a non-polluted beach) were fortified with 1 mL of cyclohexane solution containing ∿0.5 ppm of each of the standard compounds. These samples were then taken through the extraction/isolation procedure as outlined above.

After careful evaporation to dryness (under the conditions specified above), each of the fractions was taken up in 0.5 to 1.0 mL cyclohexane, and these solutions introduced into the gas chromatograph. The run conditions are given in the captions to Figures 1 and 2. External-standard quantification was used.

The results for the saturated and PAH fractions may be found in Tables 1.1 and 1.2, respectively.

It will be seen that the percentage recovery of the saturated compounds varies from 78 to 111%. The coefficient of variation for the C_{15} to C_{26} range, at 14 to 22%, is felt to be relatively good, including as it does all the steps in the extraction procedure and the imprecision implicit in a G.C. sampling technique which precludes sample placement directly in the mouth of the analytical column. The 24 to 40% coefficient of variation of the C_{28} to C_{38} n-alkane values is attributed to the usual retention time imprecision of late-eluting peaks (which can alter the measured area of small peaks), together with the inescapable molecular mass discrimination occurring in the splitless injector, allied with the impossibility of manually positioning the end of

Table 1. Results of Extraction Efficiency Tests - Mussel Tissue Homogenate Fortified With Both Saturated and Polycyclic Aromatic Hydrocarbon Standards

1.1 SATURATED HYDROCARBONS	Standard concentration (mg/L)	Measured concentration (mg/L)	Measured / Standard %	Sample standard deviation (mg/L)	Coefficient of variation (%)
C_{15}	8.3	6.8	83	0.98	14
C_{16}	15.8	14.3	91	1.9	14
C_{17}	9.2	10.2	111	1.7	16
Pristane	14.6	12.4	85	1.9	15
C_{18}	9.9	9.7	99	1.4	15
Phytane	5.7	4.4	78	0.79	18
C_{19}	9.5	9.4	99	1.5	17
C_{20}	9.6	8.8	92	1.4	16
C_{21}	9.9	9.0	91	2.0	22
C_{22}	10.1	10.1	100	2.0	20
C_{23}	11.4	11.0	97	2.2	20
C_{24}	9.6	9.3	97	1.9	20
C_{25}	9.7	9.4	97	1.9	21
C_{26}	9.8	9.1	93	1.9	21
C_{28}	9.8	8.9	90	2.1	24
C_{30}	9.8	8.6	88	2.4	28
C_{32}	9.4	8.4	89	3.2	38
C_{34}	9.5	8.8	93	3.5	39
C_{36}	9.1	7.3	80	2.9	40
C_{38}	9.2	7.4	80	2.4	33
1.2 POLYCYCLIC AROMATIC HYDROCARBONS					
Naphthalene	9.6	0.0	-	-	-
Acenaphthene	20.7	5.8	28	2.9	49
Fluorene	9.3	4.7	51	1.5	33
Anthracene	10.9	6.0	55	2.5	42
Fluoranthene	10.9	8.7	80	2.4	28
Pyrene	10.9	9.2	84	2.7	29
Benzo(a)pyrene	10.2	7.1	70	2.6	37
Benzo(ghi)perylene	10.0	7.8	78	2.8	36

No. of replicates: 6

Total no. of G.C. runs for all replicates: 27 for saturated hydrocarbons, 31 for PAHs

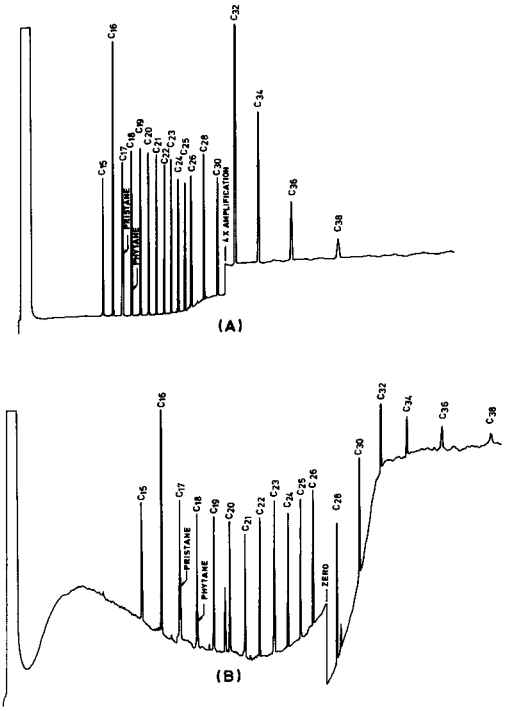


Figure 1. Chromatograms of the saturated hydrocarbon standards. C_{15} - C_{38}: n-alkanes. Column: J & W DB-5 fused silica, 30 m x 0.25 mm x 0.25 µm film thickness. After preheating the empty needle for 3 s, a splitless injection was performed over 3 s., 2 µL of a cyclohexane solution of the compounds being introduced. Split vent closed for 30 s after injection. Injector: 200°C. Detector: 300°C. Column flow of N_2 carrier at 60°C and 2.00 kg/cm^2 inlet pressure: 37 cm/s. Split flow: 40 cm^3/min. N_2 make-up flow: 35 cm^3/min.

A) Standard solution containing ∿10 mg/L of each component. Temperature programme: 60°C initial, rate 12°C/min, final 300°C held for 40 min. F.I.D. amplifier range 1, attenuation 128.

B) Standard solution containing ∿1 mg/L of each component. Temperature programme: rate 6°C/min, otherwise identical to that of (A) above. F.I.D. amplifier range 1, attenuation 8.

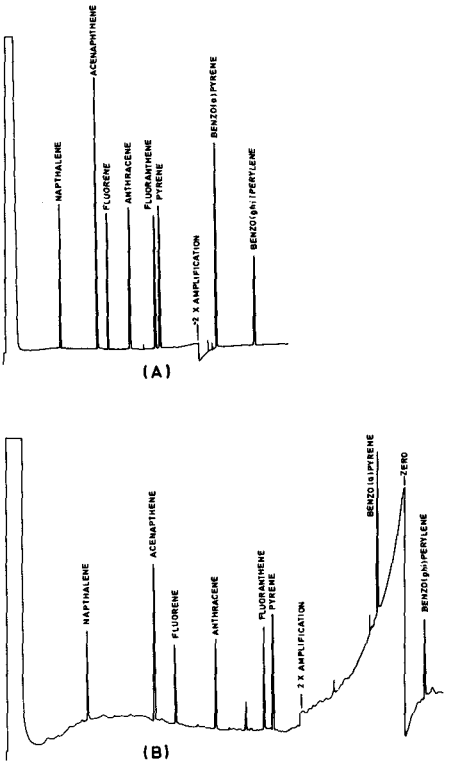


Figure 2. Chromatograms of the polycyclic aromatic hydrocarbon standards.

A) Standard solution containing ∿10 mg/L of each component. Conditions identical to those of Fig. 1(A), except attenuation 256.

B) Standard solution containing ∿0.5 mg/L of each component. Conditions identical to those of Fig. 1(B), except 4 µL solution injected over 4 s, and attenuation 16.

the injection needle reproducibly over the beginning of the column with each injection.

Recovery percentages of from 28 to 84% for seven of the PAHs (naphthalene is lost completely as a result of its volatility) indicate the greater difficulty of quantitative isolation of these compounds by comparison with the saturated hydrocarbons.

The compounds are given in their elution order in Table 1.2, where an increasing percentage recovery is evident from acenaphthene to fluoranthene and pyrene (presumably due to the increasing molecular mass, and thus decreasing volatility, of the compounds), while no ready explanation exists for the anomalously low recovery of benzo(a)pyrene, except that it, together with benzo(ghi)perylene, and the n-alkanes from about C_{28} to C_{38}, may, by reason of their high molecular mass, be insufficiently soluble in the extractant pentane to be quantitatively recoverable.

All of the PAH results show high coefficients of variation, and it may be that their intermediate polarity results in losses of these compounds through surface adsorption in work-up and analysis.

In earlier developmental work, first cyclohexane and then hexane were tried as extractants, rather than the pentane used eventually. Unacceptable losses of the compounds of lower molecular mass precluded the use of cyclohexane (boiling point 80.7°C), which is a good solvent for both n-alkanes and PAHs. The boiling point of hexane (69.0°C), the next solvent tried, gave recoveries ranging from 38% for n-C_{15} to 98% for n-C_{20}, with 100% recoveries for the remaining alkanes. For the PAHs, recoveries varied from 0% for naphthalene to 100% for benzo(ghi)perylene.

An important difference between these tests and those reported above using pentane as extractant, is that the standard compounds in cyclohexane and hexane were only separated on a cleanup column and evaporated to dryness - they were not taken through the whole spiking and isolation procedure. However, hexane and cyclohexane do appear, from these results, to be rather better solvents for the heavier hydrocarbons than does pentane.

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REFERENCES

Black JJ, Dymerski PP and Zapisek WF (1979) Routine liquid chromatographic method for assessing polynuclear aromatic hydrocarbon pollution in fresh water environments. Bull Environ Contam Toxicol 22:278-84

Brown RA, Calder A, Gould JR, Gruenfeld M, Keizer P, Lee RF, Nolan M, Warner JS, Weiss FT and Zsolnay A (1979) Chemistry of spilled oil. J Environ Path Toxicol. 3:35-61

Brown LR, Heitz JR, Miles DH and Minchew CD (1979) Development of a chemical method for detecting petrogenic hydrocarbons in the presence of biogenic hydrocarbons down to the 5 pbb level. NTIS Document AD-A072 475

Cretney WJ, Christensen PA, McIntyre BW, Fowler BR (1980) Quantification of polycyclic aromatic hydrocarbons in marine environmental samples. Proceedings of International Symposium on Analysis of Hydrocarbons and Halogenated Hydrocarbons in the

Marine Environment, Ontario, Canada, May 23-25, 1978. Plenum 315-336
Dunn BP (1976) Techniques for determination of benzo(a) pyrene in marine organisms and sediments. Environ Sci Technol 10 1018-1021
Farrington JW, Tripp BW (1975) A comparison of analysis methods for hydrocarbons in surface sediments, in 'Marine Chemistry in the Coastal Environment' ed Church TM American Chemical Society, Special Symposium: Philadelphia, Penn April 8-10 1975 ACS Symposium Series 18 ACS Washington DC
Lee ML, Novotny MV, Bartle KD (1981) Analytical chemistry of polycyclic aromatic compounds. Academic Press, New York
Overton EB, Laseter JL (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', eds Petrakis L, and Weiss FT Symposium of the American Chemical Society, Miami Beach, Florida, Sept 13-14 1978. Advances in Chemistry Series 185 ACS, Washington DC
Warner JS (1976) Determination of aliphatic and aromatic hydrocarbons in marine organisms. Anal. Chem. 48(3) 578-583

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