Bull. Environm. Contam. Toxicol. 25, 416-423 (1980)

Mass Fragmentographic Determination of Polymethylnaphthalene and Polymethylphenanthrene in a Crude Oil and in Marine Organisms

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For marine organisms have been contaminated with the environmental various pollutants in recent years, the investigation of the petroleum contamination in marine organisms requires the trace level quantitative determination of the indicative substances to be identified by a fingerprint of petroleum.

Presently, a variety of analytical techniques exist for the determination of petroleum components in environmental samples (RASMUSSEN 1976, WISE et al. 1977, OVERTON et al. 1977, GIGER and SCHAFFNER 1978, TIMOTHY and CARPENTER 1979). Paraffines, polyaromatic hydrocarbons such as benzpyrene, and organic sulfur compounds are attempted as the indicative substances for oil pollution of marine organisms (ANDELMAN and SUESS 1970, BLUMER et al. 1970, WARNER 1976, NAKAMURA and KASHIMOTO 1978).

Polymethylnaphthalene (PMN) and polymethylphenanthrene (PMP) were chosen because of their simple separation from a crude oil by means of an activated charcoal chromatography, their relatively high existence in aromatic compounds, and their persistence relative to other petroleum components in the tissue of oil contaminated marine organisms.

The purpose of this paper is to present an activated charcoal chromatographic separation procedure of PMN and PMP in a crude oil and a trace level quantitative analytical method of them in marine organisms by mass fragmentography (MF).

EXPERIMENTAL

Reagents. All solvents used were Pesticide Residue Grade obtained from Wako Pure Chemical Industry. Benzene was redistilled. Reference compounds were obtained from Tokyo Kasei Kogyo Co. The coconut shell charcoal, 250-300 mesh size, was activated by heating at 600°C for 3 hours and let stand overnight.

Tissue Extraction and Cleanup. 50 g of wet tissue cut into small pieces was homogenized in 100 ml ethanol using a Polytron homogenizer, and then 5 g KOH was added. After the mixture was saponified for 3 hours, it was filtered through cotton into a separatory funnel containing 200 ml water. The filtrate was extracted three times with 100 ml n-hexane. The combined n-hexane extracts were washed with appropriate amount of water and the concentrate was passed through 10 g Florisil (Floridin Co., activated at 200°C for 3 hours) column with 200 ml of 5% ether

0007-4861/80/0025-0416 $01.60

in n-hexane as eluting solvent. The eluate was concentrated.

Activated Charcoal Column Chromatographic Separation. The column was a 1.5 cm x 25 cm glass tube fitted with a stopcock and pluged with small wad of cotton previously washed with benzene and dried. 0.5 g activated charcoal was added into the column with n-hexane, on which cotton was placed. The concentrated tissue extract was transferred to the column and eluted with 200 ml methylene chloride, followed 250 ml benzene (Fraction 5). Methylene chloride fraction was concentrated, and then poured to 2 g activated charcoal column prepared as noted above. This fraction was eluted as follows to give four fractions shown in Fig. 1. Each eluate was concentrated to 1 ml.

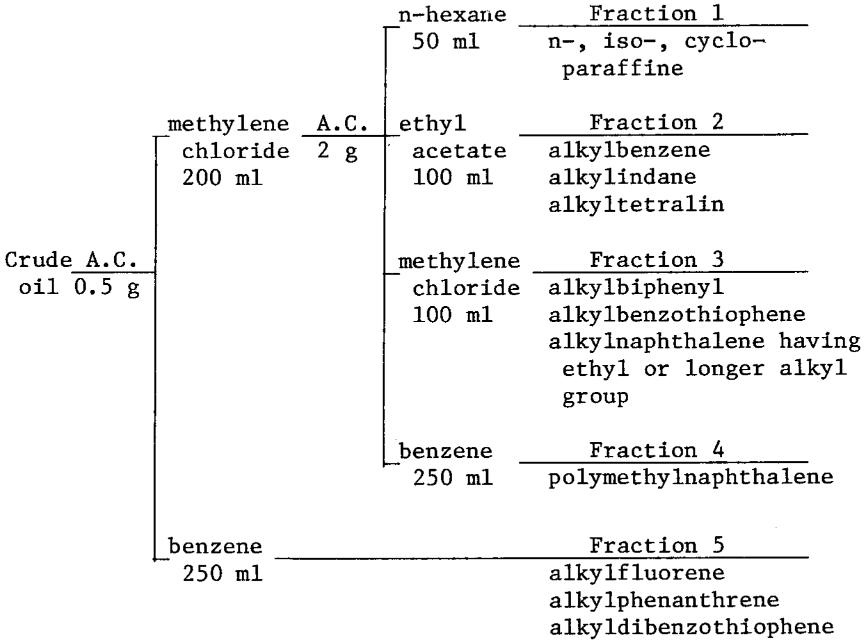

Fig. 1 Activated charcoal (A.C.) column chromatographic separation scheme for a crude oil.

Gas Chromatography-Mass Spectrometry. A LKB Model 9000, GC-MS instrument was employed. The chromatographic column was 3 mm x 2 m glass column packed with 2% OV-17 liquid phase on 80-100 mesh, acid-washed and silanized Chromosorb W. Temperatures of sample injection port, molecular separator, and ion source were 280°C, 290°C, and 270°C, respectively. The flow rate of carrier gas (helium) was 30 ml/min. The accelerating voltage was 3.5 kV and the trap current 60 μA. The electron-impact ionization mass spectra were recorded at an electron energy of 70 eV.

The GC oven was programmed from 130°C to 210°C at 3°C/min of MF of PMN and from 190°C to 240°C at 2°C/min for PMP.

The quantitative information of each peak by MF was performed by the reading data processor Chromatopac C-R1A (Shimadzu Co.) which gave the mass fragmentograms, peak areas, retention times, and amounts relative to the standard concentration.

RESULTS AND DISCUSSION

Activated Charcoal Column Chromatographic Separation. An important problem of the adsorbent material used in a routine analysis is to retain the stable adsorption activity of separation. Charcoal activated was constant and reproducible during 24 hours in our experiment. The high and specific adsorptivity of activated charcoal allowed the applicability of a variety of polar solvents to separate the relatively low molecular weight compounds. A number of papers on the fractionation method of petroleum components using mainly silica gel, alumina and other materials were published (SYNDER 1965, MAIR and MAYER 1966, HIRSCH et al. 1972, JONES et al. 1977). The application of them required the definite activating conditions and the use of mixing solvents is not suitable for the separation of PMN and PMP from a crude oil and the quantitative and reproducible analysis of them as a routine method.

Validation study of the separation procedure and the recovery by an activated charcoal column chromatography was carried out using a standard mixture containing 20 µg of each of 29 compounds in n-hexane and determined by gas chromatography. n-Eicosane and n-octylcyclohexane appeared in Fraction 1. Xylene, three trimethylbenzene isomers, indane, tetralin, and n-hexylbenzene appeared in Fraction 2. Benzothiophene, biphenyl, two ethylnaphthalene isomers, and isopropylnaphthalene appeared in Fraction 3. Naphthalene, 1- and 2-methyl, 2,6-, 1,3-, 2,3-, and 1,5-dimethyl, and 2,3,5-trimethylnaphthalene appeared in Fraction 4. The first column chromatography with 0.5 g activated charcoal was performed in order to separate fluorene, dibenzothiophene, phenanthrene, 1- and 2-methyl, and 3,6-dimethylphenanthrene. Anthracene and polyaromatic compounds than triaromatic could be too strongly adsorbed to be eluted sufficiently by 250 ml benzene. Recoveries of standard compounds from the activated charcoal column were greater than 80%.

The procedure described above was applied to a grade A crude oil. Examination of GC-MS confirmed that alkylnaphthalene in Fraction 3 was distinguishable from PMN in Fraction 4, comparing with the MS intensities of molecular ion and other fragment ions. The molecular ion peak of PMN and PMP is of generally higher intensity than the fragment ion corresponding to [M-15] or [M-29]. As observed in the validation study, the presence of methyl group increases the retention volume in the activated charcoal column. Alkylphenanthrene in Fraction 5 was identified as PMP by GC-MS.

Quantitative Determination by Mass Fragmentography. Because of the petroleum contamination at total oil levels as low as a few μg/g in marine organisms, the gas chromatographic procedure is not applicable by the reason of the presence of interfering biogenic compounds and ambiguous contaminants and the complexity of petroleum even after cleanup and fractionation. The separativity, high sensitivity, and linearity of MF enable us to eliminate above problems.

For MF of PMN and PMP, the single ion detection attachment was used. The ions used for PMN are the molecular ion peaks at m/e 128, 142, 156, 170, and 184. Similarly, the ions used for PMP are the molecular ion peaks at m/e 178, 192, and 206.

In order to obtain accurate measurement of amounts of their homologues and isomers, their sensitivities must be known. Schiller reported that low voltage mass spectrometric sensitivities increased either slightly or remained constant with the addition of methyl group to polyaromatic nucleus (1977). The sensitivities of molecular ions at 70 eV for isomers of methylnaphthalene, dimethylnaphthalene, and methylphenanthrene were approximately identical as shown in Fig. 2. And only minor differences among homologues was observed. Although there would be some differences between trimethyl and tetramethylnaphthalene in molecular ion peak intensity, the differences would be not considered great, with due regard to little differences among mono-, di-, and trimethylnaphthalene. Pure compounds of each peak of PMN and PMP in a crude oil were lacking for the calibration work in the quantitative analysis, but the total amounts of isomers and homologues of them would be approximately measurable by introducing above reasonable assumption.

The percentage relative intensity of molecular ion peak was calculated from the ten most intense peaks in reference data (CORNU and MASSOT 1975, STENHAGEN et al. 1974, HELLER and MILNE 1978). The average and its deviation of molecular ion peak intensity of isomers are 38.5 ± 1.2, 39.3 ±1.0, 43.0 ± 1.5, and 39.8 ± 3.9 for eight dimethylnaphthalenes except 1,2-dimethylnaphthalene, four trimethylnaphthalenes, four methylphenanthrenes except 4-methylphenanthrene, and five dimethylphenanthrenes except 4,5-dimethylphenanthrene, respectively.

In order to measure PMN and PMP at one sample injection, at the specified retention time, when the first mass fragmentographic experiment is ended, the magnetic field is switched manually to the next designated value by means of mass maker and the second mass fragmentographic analysis is performed. This sequence of events proceeds until all of the analysis have been performed.

The initial work of MF is to define the optimum magnetic field at the individual molecular ion and calibrate the reference compounds. The replicate analysis (at least three times) of the standard sample must be examined to achieve sufficient reproducibility (within 10%) of the mass fragmentographic method prior to measurements.

Quantitative aspect of the determination must be accomplished by peak area measurements compared to standard compounds under the identical conditions, because the total amounts of unresolved isomers in a sample must be measured.

TABLE 1.

Average recovery (%) based on mass fragmentographic analysis in triplicate of standard PMN and PMP from the overall procedure.

Compound	Amount added, µg			
	0.08	0.4	4	20
Naphthalene	72	85	88	74
1-Methylnaphthalene	80	81	86	82
2-Methylnaphthalene	76	83	84	81
2,6-Dimethylnaphthalene	78	82	93	91
1,3-Dimethylnaphthalene	82	91	90	84
2,3-Dimethylnaphthalene	76	79	81	86
1,5-Dimethylnaphthalene	75	81	92	84
2,3,5-Trimethylnaphthalene	73	80	75	74
Phenanthrene	79	84	81	84
1-Methylphenanthrene	83	92	87	85
2-Methylphenanthrene	77	89	85	83
3,6-Dimethylphenanthrene	71	78	75	76

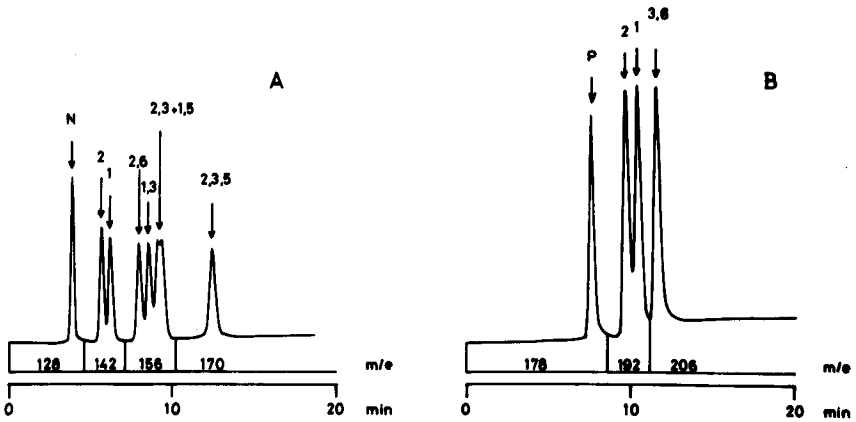

Fig. 2 Mass fragmentograms of standard PMN and PMP. (A) 80 pg each of PMN. (B) 400 pg each of PMP.

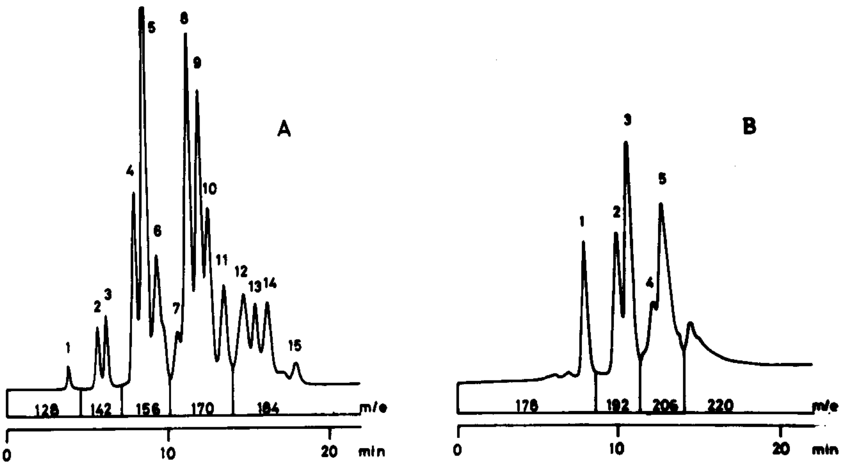

Fig. 3 Mass fragmentograms of PMN and PMP in a grade A crude oil. (A) 1 µl injection of Fraction 4 (concentrated to 1 ml) and (B) 4 µl injection of Fraction 5 (concentrated to 1 ml) from 0.5 mg crude oil.

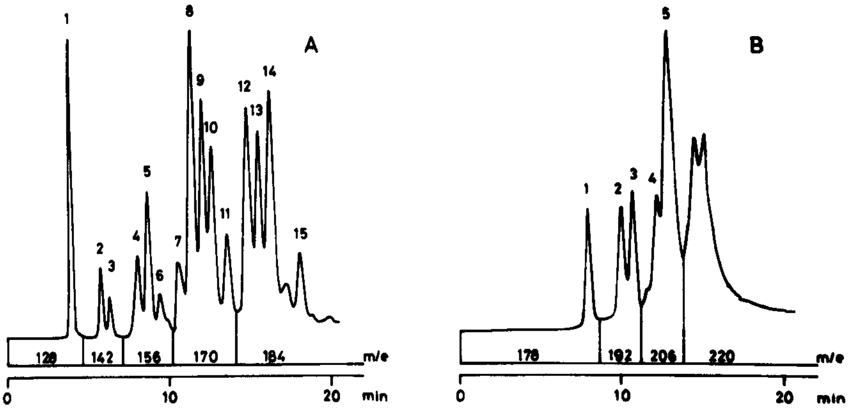

Fig. 4 Mass fragmentograms of PMN and PMP in oyster tissues. (A) 1 µl injection of Fraction 4 (concentrated to 1 ml) and (B) 1 µl injection of Fraction 5 (concentrated to 2 ml) from 50 g of oyster tissues.

The plot of the sensitivity of the molecular ion against introduced amounts is linear from 0.08 to 20 ng. The average recovery of standard PMN and PMP from the overall procedure was shown in Table 1.

In the next place, amount of each peak of PMN and PMP in a crude oil within individual scanning retention volume was calculated. Amounts of trimethyl and tetra methylnaphthalene isomer were calculated relative to 2,3,5-trimethylnaphthalene.

TABLE 2

Amounts (µg) of PMN and PMP calculated in 0.5 mg of a crude oil and 50 g of oyster tissues.

Peak No	Crude oil	Oyster tissues
Naphthalene (peak 1)	0.008	0.116
2-Methylnaphthalene (peak 2)	0.036	0.036
1-Methylnaphthalene (peak 3)	0.047	0.025
Dimethylnaphthalene		
peak 4	0.162	0.054
peak 5	0.423	0.105
peak 6	0.198	0.058
Trimethylnaphthalene		
peak 7	0.049	0.073
peak 8	0.385	0.334
peak 9	0.310	0.252
peak 10	0.203	0.209
peak 11	0.134	0.140
Tetramethylnaphthalene		
peak 12	0.167	0.339
peak 13	0.086	0.209
peak 14	0.107	0.298
peak 15	0.023	0.068
Phenanthrene (peak 1)	0.060	0.476
Methylphenanthrene		
peak 2	0.079	0.434
peak 3	0.116	0.431
Dimethylphenanthrene		
peak 4	0.044	0.643
peak 5	0.177	2.077

To confirm that no peaks are detected out of the selected retention volume range, the magnetic field of the mass spectrometer was fixed for one of molecular ions and the mass fragmentogram was recorded through all the elution time. The resulting mass fragmentogram of blank analysis did not disply any peaks interfering with the compounds of interest.

The mass fragmentograms and amounts of PMN and PMP obtained from a grade A crude oil are shown in Fig. 3 and Table 2.

Application. This method could be significant as "fingerprinting" identification to indicate petroleum contamination. We examined some actual samples to assess the applicability of our results. On the base of the standardized PMN and PMP in a crude oil, the mass fragmentograms and amounts of PMN and PMP of oyster tissues collected from the Seto Naikai coast were shown in Fig. 4 and Table 2. The mass fragmentogram pattern of oyster tissues is remarkably similar to that of a grade A crude oil, differing only on relative peak intensities. The nature and quantity of hydrocarbons found in marine organisms would be differed by the petroleum contamination details. The trace analytical technique presented here is available especially on the assessment of long-term effects on marine organisms as well as the environmental samples contaminated with repeated or chronic low-level discharges of petroleum.

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