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DETERMINATION OF TRACE AZAARENES IN WATER BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The method in which trace two-, three- and five-ring azaarenes in water samples can be determined was investigated by gas chromatography (GC). The trace azaarenes in water were concentrated with an Amberlite XAD-2 resin column and separated into azaarenes with two and three rings and those with five rings by a solvent extraction, followed by a clean-up procedure using an alumina column. The azaarenes thereby separated were determined by GC with a flame thermionic detector (GC–FTD) and GC–mass spectrometry with a selected ion monitor detector (GC–MS–SIM). Detection limits of the azaarenes by GC–FTD were in the range 0.5–3 ng and those of GC–MS–SIM in the range 0.02–0.5 ng. Utilization of GC–MS–SIM was very useful for the determination of trace azaarenes in environmental samples.

INTRODUCTION

Aza heterocyclic hydrocarbons (azaarenes) are known to exhibit mutagenic and carcinogenic activity[1–3], just as do neutral polyaromatic hydrocarbons. As azaarenes are formed during incomplete combustion, the contents of automobile exhausts[4], industrial effluents[5], urban suspended particulate matter[6–9] and sedimentary samples have been investigated[10–12]. In water, however, even the presence of azaarenes has not previously been found. Information concerning azaarenes in environmental water is needed in order to clarify their fate in aquatic systems. Although the separation of azaarenes has been attempted by thin-layer chromatography[6,13], high-performance liquid chromatography[9,14,15], and gas chromatography (GC)

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using capillary columns[11,16], the development of a method for concentrating azaarenes in water and the use of a detection method with high specificity and sensitivity are required because azaarenes would be present in water at very low levels.

In this investigation, the analytical procedure was based on the concentration of azaarenes in water by use of Amberlite XAD-2 resin, separation by solvent partition and determination by GC using a flame thermionic detector (GC–FTD) and GC–mass spectrometry with a selected ion monitor (GC–MS–SIM).

EXPERIMENTAL

Reagents

All solvents were distilled before use. Quinoline, isoquinoline, 2-methylquinoline, 1-methylisoquinoline, 6-methylquinoline, 4-methylquinoline, 2,6-dimethylquinoline, 2,4-dimethylquinoline, benzo[*h*]quinoline, acridine and benzo[*f*]quinoline were obtained from Tokyo Kasei Kogyo; 4-azafluorene and phenanthridine were from Aldrich and dibenz[*a,j*]acridine from K&K Labs. (Plainview, NY, U.S.A.). Dibenz[*a,h*]acridine, dibenz[*c,h*]acridine and 10-azabenzo[*a*]pyrene were kindly donated by Dr. K. Syudo (Department of Pharmaceutical Sciences, University of Tokyo). Structures of the azaarenes except for quinolines and isoquinolines are shown in Fig. 1. Amberlite XAD-2 resin (20–50 mesh) (Rohm & Haas, Philadelphia, PA, U.S.A.) was washed by sequential solvent extraction as described by Junk *et al.*[17]. Neutral alumina (activity grade, super I; ICN Pharmaceutics, G.F.R.) was used after its activity had been altered by the addition of various amounts of water. Water used for the recovery test was obtained by passing distilled water through an XAD-2 resin column (50 × 2 cm I.D.).

Preparation of alumina columns

To 24 g of neutral alumina in a flask was added 1 ml of the water, and the flask

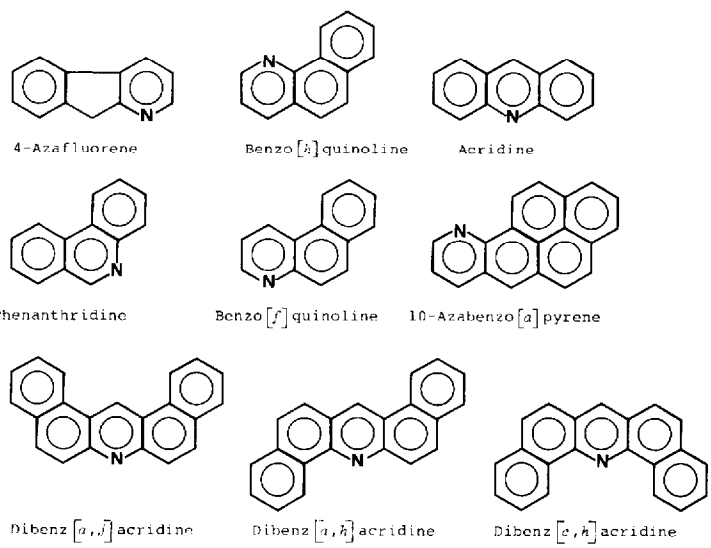

Fig. 1. Chemical structures of azaarenes.

was stoppered, shaken for 30 min and left overnight. An alumina column (10 × 1 cm I.D.) was prepared by pouring a slurry of deactivated alumina with *n*-hexane into a glass column (30 × 1 cm I.D.).

Concentration and pre-separation of azaarenes

By using a glass apparatus as described by Junk *et al.*[17], a 5-l volume of water sample was passed through an XAD column (15 × 1.5 cm I.D.) at a flow-rate of 40–60 ml/min. After loading of all of the sample on to the XAD column, the glass reservoir walls were rinsed with 200 ml of dichloromethane and the solvent was poured into the XAD column. The dichloromethane was allowed to equilibrate with the resin for more than 10 min, then the solvent was passed through the column and both water and dichloromethane eluted from the column were collected in a 250-ml separating funnel. After the removal of the water layer, the dichloromethane layer was extracted twice with 50-ml portions of 1 *N* hydrochloric acid.

The dichloromethane layer containing dibenzacridines was concentrated to less than 2 ml with a Kuderna–Danish (KD) evaporator, the concentrate being transferred into a 10-ml round-bottomed flask containing approximately 0.6 g of deactivated alumina. The contents of the flask were dried with a gentle stream of nitrogen gas, then placed on the top of the alumina column (10 × 1 cm I.D.). After the column had been washed with 450 ml of *n*-hexane, the dibenzacridines were eluted with 50 ml of *n*-hexane solution containing 5% of acetone. The eluate was concentrated to about 2 ml with the KD evaporator, the solution obtained thereby being submitted to GC.

The acidic solution containing quinolines and benzoquinolines was washed with dichloromethane and rendered alkaline with 5 *N* sodium hydroxide solution. The alkaline solution was extracted twice with 50-ml portions of dichloromethane, and the dichloromethane solution thus obtained was dried by means of a sodium sulphate column (5 × 1.5 cm I.D.) and then concentrated to about 2 ml with the KD evaporator.

GC–FTD procedure

A Hewlett-Packard Model 1100 gas chromatograph with an FTD was used for the analysis of all azaarenes. The GC conditions for the quinolines and benzoquinolines were as follows: a glass column (1.8 m × 2 mm I.D.) packed with 5% PEG-HT on Uniport HPS (Gasukuro Kogyo Co., Tokyo, Japan) was used, the oven temperature was programmed from 120 to 240°C at 4°C min, and the flow-rate of nitrogen as the carrier gas was kept at 25 ml/min. The GC conditions for the dibenzacridines were as follows: a glass column (0.9 m × 2 mm I.D.) packed with 2% PZ-179 + 1% OV-17 on Uniport HPS was used, the oven temperature was maintained at 280°C and the flow-rate of nitrogen was maintained at 40 ml/min.

GC–MS–SIM procedure

GC–MS–SIM was measured with a JMS-01SG-2 type GC–MS system (Nihon Denshi Co., Tokyo, Japan). A molecular ion of each azaarene was used for the SIM measurement. The column packing used for GC–FTD was also employed for GC–MS–SIM. The column temperature was kept constant throughout the analysis: for the quinolines and the methylquinolines at 150°C, for the dimethylquinolines at

170°C, for 4-azafluorene at 200°C and for 10-azabenzo[*a*]pyrene and the dibenzacridines at 280°C. The temperatures of the ion source and the enricher were kept at 210 and 290°C, respectively. The ionizing voltage was set at 75 eV, the ionizing current was 200 μA and the pressure in the ion source was $4 \cdot 10^{-6}$ Torr.

Sampling of sea water

Water samples were collected from Dohkai Bay in Kitakyushu City, which is one of the most industrialized areas in Japan. Water samples were taken in 20-l glass bottles and stored at 5°C until taken for analysis.

RESULTS AND DISCUSSION

Selection of the solvent for the elution of azaarenes from XAD-2 resin

The efficiency of the elution of azaarenes from the XAD-2 resin column with diethyl ether and dichloromethane was tested. A test solution, which had been prepared by adding 1-ml portions of acetone solutions containing 500 μg each of the azaarenes to be tested to 100 ml of water, was loaded on to the resin column and then the azaarenes were eluted with diethyl ether or dichloromethane. Volumes of 5 ml of the effluent were collected in small test-tubes and the azaarenes in each solution were analysed by GC with a flame-ionization detector (FID). Elution patterns of the azaarenes are shown in Figs. 2 and 3. Although more than 140 ml of diethyl ether were required in order to elute dibenz[*a*,*j*]acridine completely, most of the azaarenes were eluted within 30 ml of dichloromethane, which was more efficient than diethyl ether generally used with XAD-2 resin[17,18].

Recovery of azaarenes from XAD-2 resin

The recovery of azaarenes added to water was tested by the following pro-

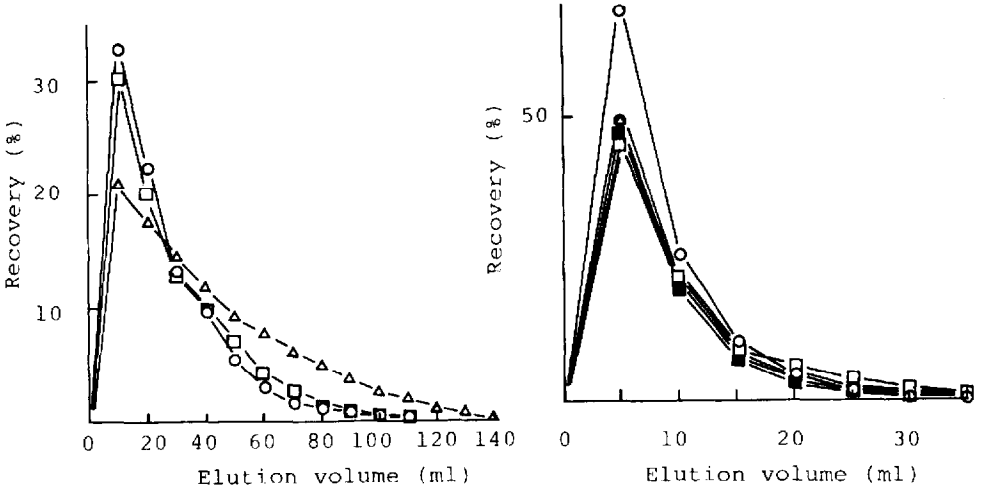

Fig. 2. Elution patterns of azaarenes eluted from an XAD-2 resin column with diethyl ether. ○, Acridine; □, benzo[*f*]quinoline; △, dibenz[*a*,*j*]acridine.

Fig. 3. Elution patterns of azaarenes eluted from an XAD-2 resin column with dichloromethane. ○, 1-Methylisoquinoline; □, 4-azafluorene; △, acridine; ●, benzo[*f*]quinoline; ■, dibenz[*a*,*j*]acridine.

cedure. A test solution, which had been prepared by adding 1-ml portions of acetone solutions containing 50 μg/ml each of the azaarenes to 5 l of water, was passed through the resin columns. After treatment by the method described under Experimental, the recovery of the azaarenes was determined by GC–FID or GC–FTD. The recovery data are shown in Table I.

TABLE I

RECOVERY OF AZAARENES FROM WATER USING AN XAD-2 RESIN COLUMN

Compound	*Recovery (%)*	*Coefficient of variation (%)*
Quinoline	92.4	1.1
2-Methylquinoline	91.7	1.2
4-Methylquinoline	95.0	0.7
6-Methylquinoline	96.0	2.2
1-Methylisoquinoline	79.9	1.0
2,4-Dimethylquinoline	83.5	0.8
2,6-Dimethylquinoline	95.1	1.8
4-Azafluorene	92.9	3.3
Benzo[*h*]quinoline	93.8	3.7
Acridine	90.1	2.5
Phenanthridine	93.9	2.1
Benzo[*f*]quinoline	92.6	2.0
10-Azabenzo[*a*]pyrene	89.6	3.1
Dibenz[*a,j*]acridine	73.4	4.1
Dibenz[*a,h*]acridine	89.3	1.2
Dibenz[*c,h*]acridine	88.9	2.9

During the elution of azaarenes from the XAD-2 resin with dichloromethane, impurities might be released from the resin, as James *et al.* reported[19]. In the blank test using pure water, however, no peak was found at least at the retention time of the azaarenes tested with an FID or FTD.

Separation and removal of interfering substances

In order to remove interfering substances such as polyaromatic hydrocarbons, which are closely related to the formation of azaarenes in the environment, liquid–liquid partition under acidic conditions and column chromatography were attempted as follows. The quinolines, isoquinolines and their methyl derivatives and the benzoquinolines were transferred from the dichloromethane layer into a 1 *N* hydrochloric acid layer, but the five-ring azaarenes were not transferred at all into the acidic layer because azaarenes of larger molecular size hardly exhibit alkaline properties. The separation of the five-ring azaarenes from polyaromatic hydrocarbons was attempted by column chromatography using alumina as the adsorbent. Polyaromatic hydrocarbons, which are frequently formed in the environment, were mixed with two azaarenes reference, and an elution solvent suitable for the separation of the azaarenes from the polyaromatic hydrocarbons was carefully selected.

A standard acetone solution containing 100 μg each of polyaromatic hydrocarbons (phenanthrene, fluorene, pyrene, chrysene, benzo[*a*]pyrene) and azaarenes (dibenz[*c,h*]acridine and dibenz[*a,j*]acridine) was added to 0.6 g of alumina which had

been placed in a round-bottomed flask. The solvent in the flask was removed with a gentle stream of nitrogen gas, and the alumina thus obtained was transferred on to the top of a deactivated alumina column (10 × 1 cm I.D.). *n*-Hexane and mixtures of *n*-hexane and acetone were used as the elution solvents, and azaarenes and polyaromatic hydrocarbons in each fractionated eluate were measured by GC–FID or GC–FTD. The elution patterns under identical chromatographic conditions are shown in Fig. 4.

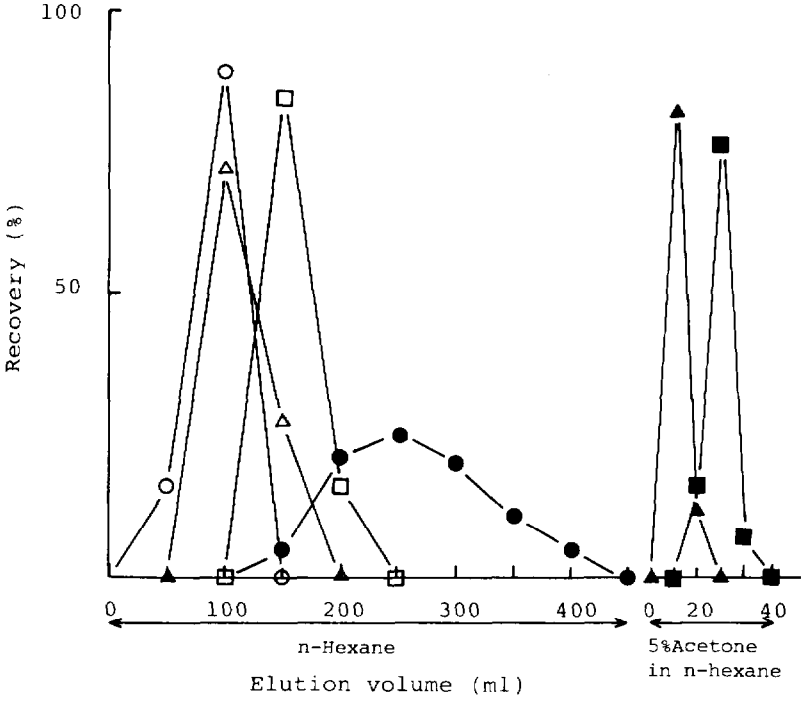

Fig. 4. Alumina column chromatograms of azaarenes and polyaromatic hydrocarbons. ○, Phenanthrene; △, pyrene; □, chrysene; ●, benzo[*a*]pyrene; ▲, dibenz[*c*,*h*]acridine; ■, dibenz[*a*,*j*]acridine.

Polyaromatic hydrocarbons of lower molecular weight than benzo[*a*]pyrene were almost completely eluted with 450 ml of *n*-hexane, the dibenzacridines subsequently being quantitatively recovered with 50 ml of *n*-hexane solution containing 5% of acetone. As the activity of alumina varies markedly with the amount of added water, the elution volume of benzo[*a*]pyrene should be determined in order to establish the conditions necessary for the separation before the analysis of samples whenever the deactivated alumina is to be prepared by the addition of water.

GC–FTD conditions

In the GC of nitrogen-containing compounds, it is generally difficult to obtain symmetrical peaks quantitatively because of their strong adsorption onto the active surface of a solid support and/or a glass column. Also in the GC of azaarenes, an adequately deactivated solid support was required in order to avoid tailing problems. As shown in Fig. 4, by use of 5% PEG-HT (thermally stabilized PEG 20M) on

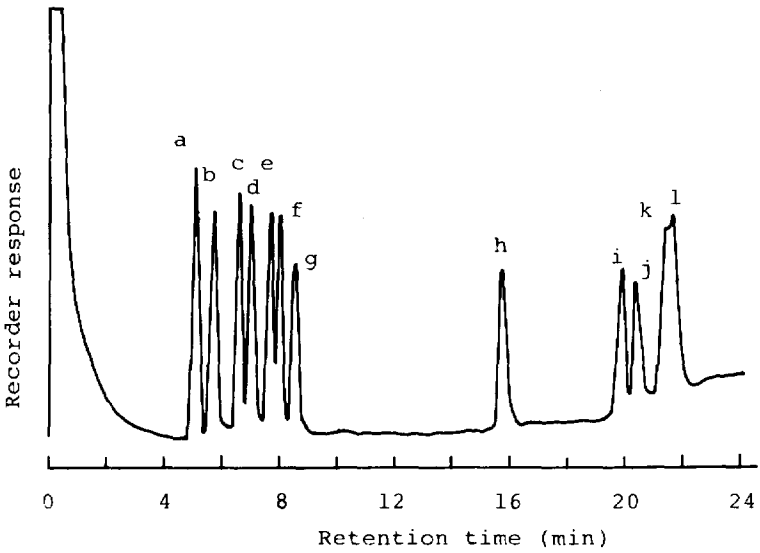

Fig. 5. FTD gas chromatograms of azaarenes. a = Quinoline; b = 2-methylquinoline; c = 1-methylisoquinoline; d = 6-methylquinoline; e = 2,6-dimethylquinoline; f = 4-methylquinoline; g = 2,4-dimethylquinoline; h = 4-azafluorene; i = benzo[*h*]quinoline; j = acridine; k = phenanthridine; l = benzo[*f*]quinoline. Amount of azaarenes injected, 5 ng. GC conditions as described under Experimental.

Uniport HPS, the two- and three-ring azaarenes except phenanthridine and benzo[*f*]quinoline were adequately separated without tailing. Although various kinds of polar liquid stationary phases (for example, SP-1000, OV-225, Silar-10C, PZ-179, Poly-I-110 and BMBT) were used for the separation of phenanthridine and benzo[*f*]quinoline, satisfactory results were not obtained. On the other hand, the five-ring azaarenes which have low volatility were inadequately separated with only OV-17 as the stationary phase. However, a mixture of OV-17 and PZ-179 (thermally stabilized polyphenyl sulphones; maximum temperature 400°C)[20] was capable of separating the azaarenes, as shown in Fig. 6. These GC conditions could be applied without further modification to GC–MS–SIM for the determination of azaarenes isolated from environmental samples. By use of GC–FTD, the quinolines and benzoquinolines tested could be detected at the low levels of 0.5 and 1 ng, respectively, and minimum detectable levels of the five-ring azaarenes were in the range 1.5–3 ng.

Determination of azaarenes in sea water samples by GC–MS–SIM

This procedure was applied to a sample of sea water collected from Dohkai Bay. At first, the two- and three-ring azaarenes (quinolines and benzoquinolines) and the five-ring azaarenes (dibenzacridines and 10-azabenzo[*a*]pyrene) were analysed by GC–FTD as described under Experimental, and the gas chromatograms thus obtained are shown in Figs. 7 and 8. Although many peaks with retention times corresponding to the standard two- and three-ring azaarenes were observed, positive identification was difficult. GC using an FTD, which is a selective but not a specific detector, should be applied to the routine analysis and sample screening, because there are many geometric isomers of azaarenes due to the type of ring fusion and the

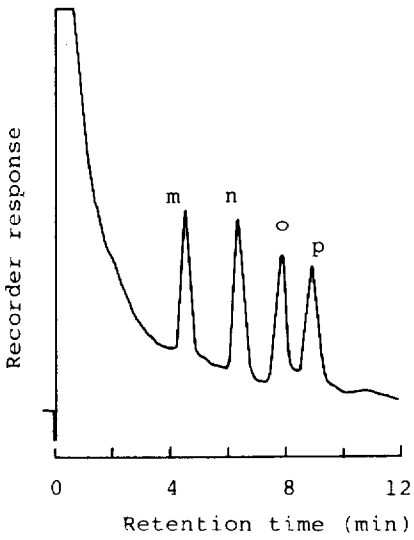

Fig. 6. FTD gas chromatograms of azaarenes. m = 10-Azabenzo[*a*]pyrene; n = dibenz[*c*,*h*]acridine; o = dibenz[*a*,*h*]acridine; p = dibenz[*a*,*j*]acridine. Amount of azaarenes injected, 5 ng. GC conditions as described under Experimental.

position of the aza-nitrogen in the molecules and their alkylated isomers. Consequently, in this study GC–MS–SIM was used for the determination of trace azaarenes in sea water.

The molecular ion peak of each azaarene which is not alkylated always becomes a base peak, as with polyaromatic hydrocarbons. As shown in Figs. 9 and 10, GC–MS–SIM using the *m/z* value of each molecular ion did not give interfering peaks on the SIM chromatograms obtained from a sample. Overall blanks were prepared for this method and examined for interfering components by GC–MS–SIM.

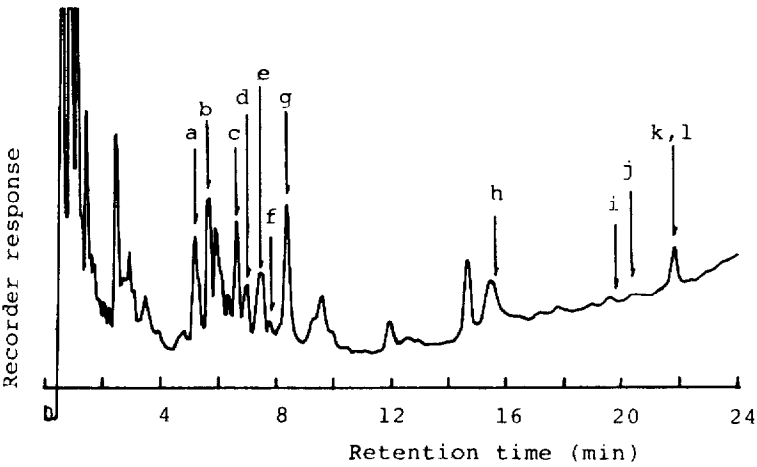

Fig. 7. FTD gas chromatograms of two- and three-ring azaarene fraction separated from sea water. a–l correspond to compounds shown in Fig. 5. GC conditions as described under Experimental.

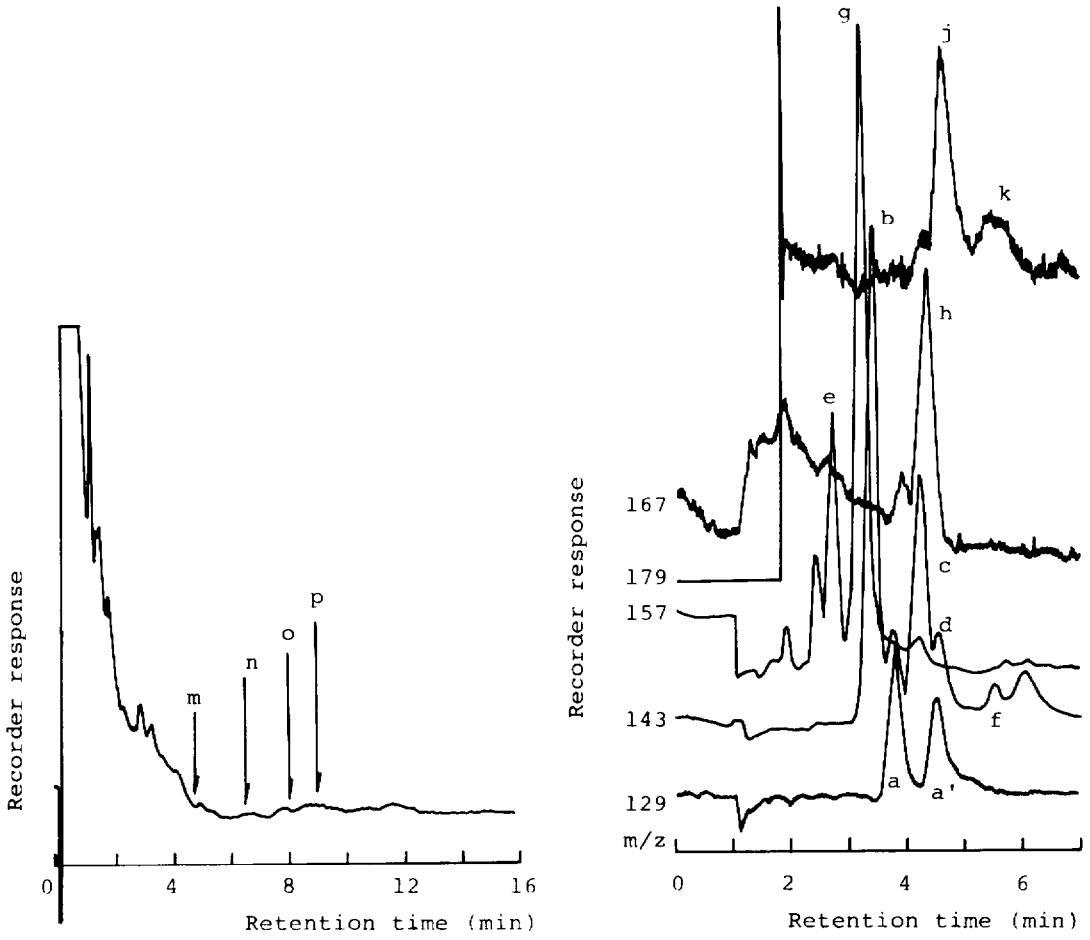

Fig. 8. FTD gas chromatograms of five-ring azaarene fraction separated from sea water. m–p correspond to compounds shown in Fig. 6. GC conditions as described under Experimental.

Fig. 9. SIM chromatograms of two- and three-ring azaarenes separated from sea water. a = Quinoline; a′ = isoquinoline; b = 2-methylquinoline; c = 1-methylisoquinoline; d = 6-methylquinoline; e = 2,6-dimethylquinoline; f = 4-methylquinoline; g = 2,4-dimethylquinoline; h = 4-azafluorene; j = acridine; k = phenanthridine and benzo[*f*]quinoline. GC conditions as described in Experimental.

Consequently, the azaarenes tested were not found in the blanks when the analytical conditions of GC–MS–SIM specified under Experimental were used.

The detection limits of azaarenes in sea water and their estimated concentrations in the water sample collected from Dohkai Bay are listed in Table II. The detection limits were calculated by using a signal-to-noise ratio of 3:1. The relatively poor detection limit for 10-azabenzo[*a*]pyrene may be due to the high background from bleeding of the stationary phase used, the mass fragment ions of which probably include the *m/z* 253 ion. The concentrations of azaarenes in sea water were about 10–100 times greater than the detection limit. Therefore, this method can be utilized for quantitative monitoring of azaarenes in water at the nanogram level.

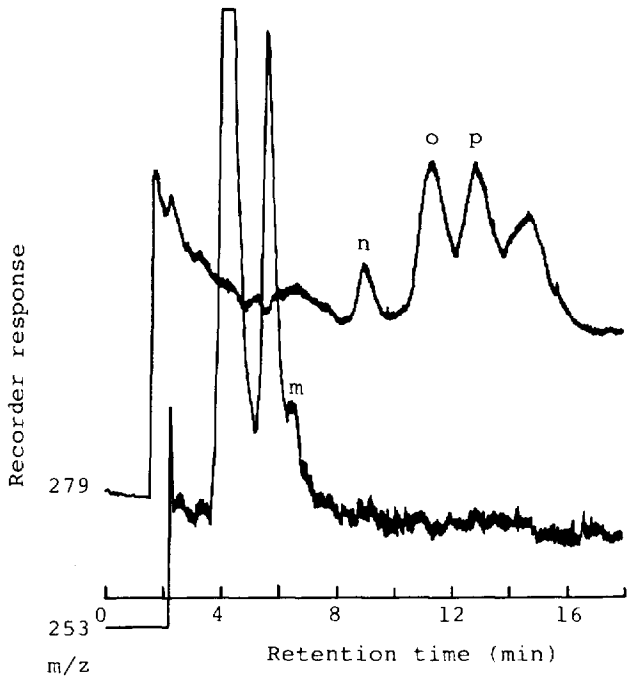

Fig. 10. SIM chromatograms of five-ring azaarenes separated from sea water. m = 10-Azabenzo[*a*]-pyrene; n = dibenz[*c*,*h*]acridine; o = dibenz[*a*,*h*]acridine; p = dibenz[*a*,*j*]acridine. GC conditions as described under Experimental.

TABLE II

DETECTION LIMITS OF AZAARENES AND THEIR CONCENTRATION IN SEA WATER FROM DOHKAI BAY, JAPAN

Compound	*Detection limit* (ng/l)*	*Concentration (ng/l)*
Quinoline	0.5	22
Isoquinoline	0.5	13
2-Methylquinoline	0.2	46
1-Methylisoquinoline	0.4	43
6-Methylquinoline	0.5	4.1
4-Methylquinoline	0.4	3.2
2,6-Dimethylquinoline	0.2	16
2,4-Dimethylquinoline	0.2	55
4-Azafluorene	0.3	5.8
Benzo[*h*]quinoline	0.3	ND**
Acridine	0.5	9.5
Phenanthridine or/and benzo[*f*]quinoline	0.3	2.4
10-Azabenzo[*a*]pyrene	2.0	ND**
Dibenz[*c*,*h*]acridine	0.4	0.66
Dibenz[*a*,*h*]acridine	0.4	3.1
Dibenz[*a*,*j*]acridine	0.5	4.1

* The volume of the sea-water sample was 20 l and the volume of the sample solution submitted to GC and the volume injected into the GC system were 0.5–1 ml and 5 μl, respectively.

** ND = Not detected.

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