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QUANTITATION OF LONG-CHAIN ALKYL BENZENES IN ENVIRONMENTAL SAMPLES BY SILICA GEL COLUMN CHROMATOGRAPHY AND HIGH-RESOLUTION GAS CHROMATOGRAPHY

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SUMMARY

A high-resolution (capillary) gas chromatographic method with preceding separation by silica gel column chromatography was established for quantitation of alkylbenzenes with normal C₁₀–C₁₄ and branched C₁₁–C₁₃ alkyl chains in sediment and suspended matter. This method is applicable to environmental samples containing nanogram amounts of alkylbenzenes per gram of sample (dry weight). The recoveries of alkylbenzenes are 81–94% and the reproducibility is good (relative standard deviation less than 12%).

INTRODUCTION

The presence of anthropogenic hydrocarbons in recent marine and coastal sediments has been reported by many authors^{1–7}. We recently found a series of alkylbenzenes with C₁₀–C₁₄ alkyl chains in coastal (Tokyo Bay) and river sediments and proposed that these alkylbenzenes are supplied into the sediments by the use of synthetic detergents (ABS) which contain trace amounts of alkylbenzenes as contamination⁸. A similar idea was presented by Eganhouse *et al.*⁹ for the alkylbenzenes in the sediments of the coastal zone of southern California.⁵ Therefore, these long-chain alkylbenzenes (hereafter described as C₁₀–C₁₄ alkylbenzenes) can be used as a good tracer of pollution by synthetic detergents and/or domestic wastes in the aquatic environment.

In the case of environmental samples, it is generally difficult to separate C₁₀–C₁₄ alkylbenzenes from aliphatic and aromatic hydrocarbons because the former are present in smaller amounts than the latter. In this context, only one paper¹⁰ has reported quantitative analyses of this type of alkylbenzenes in mixtures of organic compounds in environmental samples. Two different methods were employed: silver nitrate thin-layer chromatography (TLC) to isolate the alkylbenzene fraction from the total lipid extract, and direct analysis of hydrocarbon fractions by high-resolution gas chromatography–mass spectrometry (GC–MS) without separating these alkylbenzenes from other hydrocarbons. However, TLC is generally not suitable for

routine analyses and may not be appropriate for analysis of environmental samples. We considered column chromatography to be better than TLC.

The purpose of the present study was to establish conditions for isolating C₁₀–C₁₄ alkylbenzenes by the use of silica gel column chromatography, with pre-treatment, which can be applied to environmental (sediment and suspended matter) samples.

EXPERIMENTAL

Chemicals

All organic solvents used in this study were redistilled in an all-glass still. Activated copper was prepared according to the procedure of Blumer¹¹ and stored in benzene. Florisil (100–200 mesh, Wako Fine Chemicals) was washed with benzene–methanol (6:4), heated at 155–160°C for 5 h and stored in benzene. Silica gel (100–200 mesh; Mallinckrodt) was baked at 450°C, heated at 155–160°C for 5 h and stored in *n*-hexane.

The following standards were obtained commercially and used without further purification: 1-phenyloctane (1-C₈AB), 1-phenylnonane (1-C₉AB), 1-phenyldecane (1-C₁₀AB), 1-phenylundecane (1-C₁₁AB), 1-phenyldodecane (1-C₁₂AB), 1-phenyltridecane (1-C₁₃AB), 1-phenyltetradecane (1-C₁₄AB), biphenyl, acenaphthene, fluorene, pyrene, phenanthrene, benzo[*a*]fluorene, chrysene, benzo[*a*]pyrene, perylene, coronene, *n*-docosane, 1-docosene and *n*-dotriacontane.

A standard mixture of linear alkylbenzenes with known isomeric composition was supplied by Mitsubishi Petrochemical Company and used for the identification of alkylbenzenes from environmental samples. A standard mixture of branched alkylbenzenes was prepared by extracting alkylbenzenes with *n*-hexane from a synthetic detergent (Neopelex No. 6) containing branched alkylbenzene sulphonates (hard-type ABS), which was supplied by Kao Corporation.

Environmental samples

Three kinds of environmental samples (two river sediments, river suspended matter and bay sediment) were used. The river sediments were taken from River Tamagawa and River Arakawa with an Ekman dredge in 1983. The suspended matter was taken by filtering 200 l of river-water (River Tamagawa) through a pre-baked glass fibre filter (GF/C, pore size 0.6 μm). Rivers Tamagawa and Arakawa were representative of polluted rivers in Japan, flowing through or adjacent to the Tokyo city area into Tokyo Bay. The population of the drainage basin is approximately 3 and 0.5 million, respectively¹².

The Tokyo Bay sediment sample was collected with a dredge near the central part of the bay (location 2) in 1981 and stored at –20°C until use. The area of Tokyo Bay is approximately 1200 km² and the population of its drainage basin is 23 million¹².

Extraction and isolation procedures

Fig. 1 gives an outline of the procedures for extraction and isolation of alkylbenzene from environmental samples. A freeze-dried sediment sample (*ca.* 5 g) was Soxhlet-extracted in a glass-fibre thimble with 150 ml of benzene–methanol (6:4) for

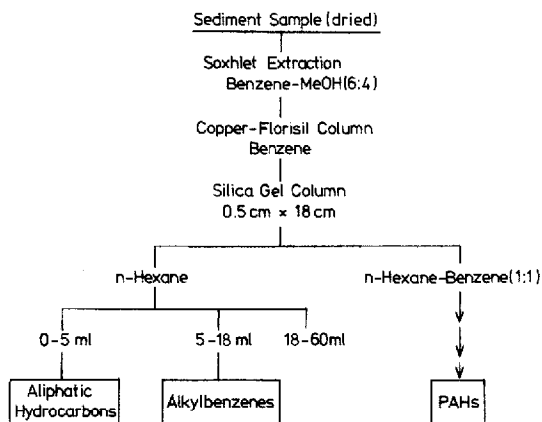


Fig. 1. Analytical procedure for alkylbenzenes.

18 h. In the case of samples of suspended matter, a freeze-dried glass-fibre filter containing the suspended matter was Soxhlet-extracted with 1.5 l of benzene-methanol (6:4) for 18 h. The organic solvent extract was concentrated to dryness in a rotary evaporator at 30°C and then taken up in 5 ml of benzene. Elemental sulphur in the extract was removed as copper sulphide by addition of 0.5 g of activated copper. The extract in benzene was then applied on a Florisil column (8 × 1.0 cm I.D.) for removal of copper sulphide and polar material (pigments). The first 30 ml of benzene eluate was taken and evaporated to dryness in a rotary evaporator at 30°C.

The eluate was taken up in 0.3 ml of *n*-hexane and then subjected to silica gel column chromatography using a 18 × 0.5 cm I.D. column. A slight pressure of nitrogen gas was employed, giving a flow-rate of *ca.* 0.2 ml/min. *n*-Hexane was used as an eluent to give three fractions: 0–5 ml, 5–18 ml and 18–60 ml. Then, 6 ml of *n*-hexane-benzene (1:1) was used to elute polycyclic aromatic hydrocarbons (PAHs). The second *n*-hexane fraction was shown to contain C₁₀–C₁₄ alkylbenzenes.

Qualification and quantitation

The second *n*-hexane fraction (alkylbenzene fraction) was evaporated just to dryness under reduced pressure at 30°C and taken up in an appropriate volume (50–100 μl) of isooctane solution containing biphenyl (13 mg/ml) as an internal standard. A small portion (2 μl) was then injected for GC on a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector. A 25 m × 0.3 mm I.D. fused-silica capillary column coated with SE-54 was used for the analysis under the following conditions: detector temperature, 310°C; injector temperature, 300°C; initial column temperature, 50°C, isothermal for 2 min, then programmed to 120°C at 30°C/min, to 200°C at 3°C/min and then to 300°C at 30°C/min and maintained for 20 min; helium carrier gas flow-rate, 1.5 ml/min; splitless injection.

Linear alkylbenzenes were identified by MS as well as by coinjection with the standard mixture of linear alkylbenzenes for GC. The mass spectra were recorded with a Shimadzu-LKB 9000 GC-MS system. The operating conditions were as fol-

lows: GC column, 25 m \times 0.3 mm I.D., fused silica coated with SE-54; injector temperature, 280°C; oven temperature programmed from 100 to 300°C at 6°C/min, helium carrier gas pressure at column head, 0.6 kg/cm²; MS in the electron impact (EI) mode at 70 eV with scanning from 50 to 400 a.m.u.; temperature of ionization chamber, 330°C.

Branched alkylbenzenes were identified by coinjection with the standard mixture of branched alkylbenzenes which had been identified by GC-MS.

All quantitative data were obtained by measuring GC peak areas using a Hewlett-Packard 5880A Series GC Terminal integrator. Biphenyl was used as an internal standard for the quantification of C₁₀-C₁₄ alkylbenzenes. Their concentrations were calculated by using the response factors of 1-C₁₀AB, 1-C₁₁AB, 1-C₁₂AB, 1-C₁₃AB and 1-C₁₄AB relative to biphenyl and assuming that the relative response factors of C₁₀ABs is the same that of 1-C₁₀AB, C₁₁ABs that of 1-C₁₁AB, C₁₂ABs that of 1-C₁₂AB, C₁₃ABs that of 1-C₁₃AB and C₁₄ABs that of 1-C₁₄AB, respectively. The response factors of 1-C₁₀AB, 1-C₁₁AB, 1-C₁₂AB, 1-C₁₃AB and 1-C₁₄AB relative to biphenyl were determined separately using authentic alkylbenzene standards.

RESULTS AND DISCUSSION

Isolation of alkylbenzenes by silica gel column chromatography

In general, a benzene-methanol (6:4) extract of a sediment sample contains a large amount of polar materials (mainly chlorophyll pigments). Since these polar materials interfere with the isolation of alkylbenzenes in silica gel column chromatography, a Florisil column was used to remove them. The appropriate conditions are given in the Experimental section.

The optimum conditions for silica gel chromatography of the alkylbenzenes was examined by using a mixture of standard *n*-alkanes, *n*-alkenes, alkylbenzenes and PAHs (each 10 μ g) dissolved in 0.3 μ l of *n*-hexane. This was applied on a silica gel column (18 \times 0.5 cm I.D.) and eluted with *n*-hexane at a flow-rate of 0.2 ml/min. A 1- or 2-ml volume of eluate was taken and evaporated just to dryness under a stream of nitrogen, and then immediately taken up in an appropriate volume of *n*-hexane. A small portion (2 μ l) was then subjected to GC for determination of the

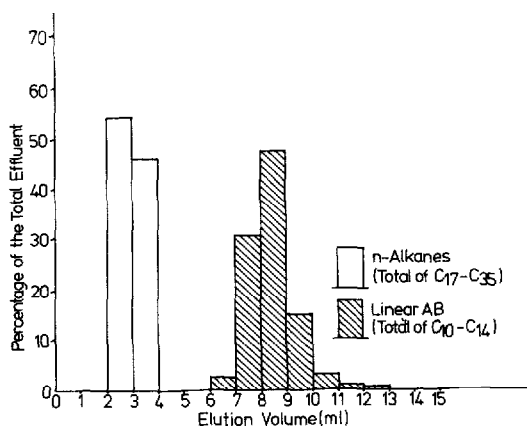


Fig. 2. Elution diagram of *n*-alkanes and alkylbenzenes extracted from River Tamagawa sediment.

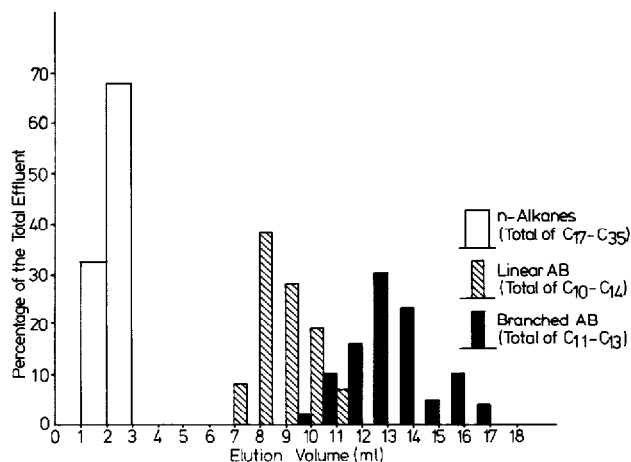


Fig. 3. Elution diagram of *n*-alkanes and alkylbenzenes extracted from Tokyo Bay sediment.

ABs. The results indicated that *n*-alkanes and *n*-alkene were eluted in the first 4 ml, alkylbenzenes between 7 and 13 ml and PAHs were eluted after 70 ml.

In order to test whether the above elution conditions were also valid for sediment extracts, a sediment extract from River Tamagawa (location: Rokugo) was applied on a silica gel column. Fig. 2 illustrates the elution curve obtained. This indicates that the elution volumes of linear alkylbenzenes in the sediment extract are almost the same as those for the standards. Similar results were obtained for an extract from Tokyo Bay sediment (Fig. 3). In this case, the elution volumes of linear ABs as well as branched ABs were determined because branched ABs were present in sufficient amounts. Consequently, aliphatic hydrocarbons were eluted in the first 4 ml of *n*-hexane, linear ABs in the second 7–11 ml and branched ABs in 10–17 ml. The elution volume of PAHs was larger than 70 ml. The alkylbenzene fraction ob-

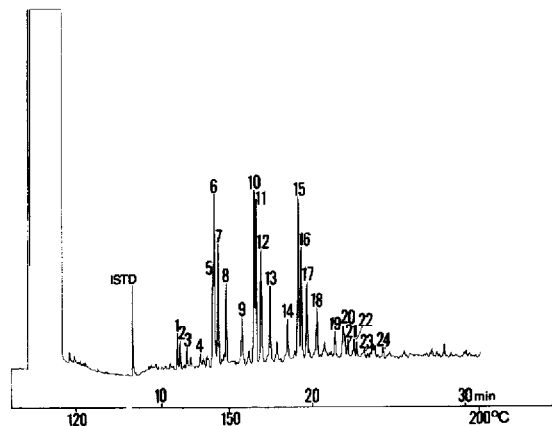


Fig. 4. Capillary gas chromatogram of alkylbenzenes extracted from River Tamagawa sediment. Numbers refer to the compounds listed in Table I. ISTD = Biphenyl as an internal standard. GC conditions: 25 m × 0.3 mm I.D. Fused-silica capillary column coated with SE-54; 0.7 atm helium as carrier gas; flame ionization detector; splitless mode; column temperature 50°C for the initial 2 min, then programmed to 120°C at a rate of 30°C/min, to 200°C at a rate of 3°C/min and then to 300°C at a rate of 30°C/min.

tained by this procedure is pure enough for quantitation by GC, as shown in Fig. 4. Thus, it was concluded that the second 5–18 ml of *n*-hexane eluate corresponds to the alkylbenzene (linear as well as branched) fraction.

Retention indices of alkylbenzenes

Retention indices of alkylbenzenes were calculated for GC identification. 1-C₈AB, 1-C₉AB, 1-C₁₀AB, 1-C₁₁AB, 1-C₁₂AB, 1-C₁₃AB and 1-C₁₄AB were used as the internal standards for determining retention indices of alkylbenzenes, and their retention indices are defined as 80.00, 90.00, 100.00, 110.00, 120.00, 130.00 and 140.00, respectively. The retention index, *I*, is defined as

$$I = 10 \times \frac{RT(\text{substance}) - RT(1-C_n\text{AB})}{RT(1-C_{n+1}\text{AB}) - RT(1-C_n\text{AB})} + 10n$$

where RT(substance) is the retention time of the substance for which the retention index is to be determined, RT(1-C_{*n*}AB) and RT(1-C_{*n*+1}AB) are the retention times

TABLE I
LINEAR ALKYL BENZENES IDENTIFIED IN A TAMAGAWA SEDIMENT SAMPLE

Peak No.	Compound	Retention* index	Concentration	
			Average (ng/g dry sediment)	R.S.D.* (%)
1	5-C ₁₀ AB	86.70	114	9
2	4-C ₁₀ AB	87.54	123	9
3	3-C ₁₀ AB	89.37	70	8
4	2-C ₁₀ AB	92.84	67	1
5	6-C ₁₁ AB	95.92	233	} 11
6	5-C ₁₁ AB	96.20	531	
7	4-C ₁₁ AB	97.15	354	11
8	3-C ₁₁ AB	99.15	244	13
9	2-C ₁₁ AB	102.71	237	15
10	6-C ₁₂ AB	105.31	642	9
11	5-C ₁₂ AB	105.76	547	10
12	4-C ₁₂ AB	106.90	440	12
13	3-C ₁₂ AB	108.95	272	10
14	2-C ₁₂ AB	112.63	205	10
15	7-/6-C ₁₃ AB	114.75	748	10
16	5-C ₁₃ AB	115.42	446	10
17	4-C ₁₃ AB	116.59	284	12
18	3-C ₁₃ AB	118.81	193	10
19	2-C ₁₃ AB	122.52	147	5
20	7-/6-C ₁₄ AB	124.20	315	8
21	5-C ₁₄ AB	125.01	86	10
22	4-C ₁₄ AB	126.37	96	14
23	3-C ₁₄ AB	128.67	53	3
24	2-C ₁₄ AB	132.46	69	5
	Total		6516	

* Average calculated from five determinations.

** Triplicate analyses; R.S.D. = relative standard deviation.

for the internal standards which bracket the substance of interest and n is alkyl carbon number of the alkylbenzene internal standard eluted prior to the substance of interest.

Table I gives retention indices for linear alkylbenzenes from River Tamagawa (location: Rokugo) sediment. These alkylbenzenes had been identified by MS and coinjection with the standard alkylbenzenes in GC, as mentioned before (see Experimental section). The reproducibility of the retention indices was within 0.07 for five replicate analyses of the sediment extracts.

TABLE II
ALKYL BENZENE CONCENTRATIONS (ng/g DRY MATERIAL) IN ENVIRONMENTAL SAMPLES

<i>Linear alkylbenzenes</i>		<i>Recent sediment</i>		<i>Suspended solid</i>
<i>Peak No.</i>	<i>Compound</i>	<i>R. Arakawa (Shinkasai-bashi)</i>	<i>Tokyo Bay</i>	<i>R. Tamagawa (Chofu-seki)</i>
1	5-C ₁₀ AB	46	25	103
2	4-C ₁₀ AB	37	6	8
3	3-C ₁₀ AB	38	4	34
4	2-C ₁₀ AB	63	19	—*
5	6-C ₁₁ AB	156	142	591
6	5-C ₁₁ AB	279	96	1126
7	4-C ₁₁ AB	187	50	841
8	3-C ₁₁ AB	168	55	423
9	2-C ₁₁ AB	276	n.d.**	310
10	6-C ₁₂ AB	353	206	2375
11	5-C ₁₂ AB	286	130	2075
12	4-C ₁₂ AB	240	86	1450
13	3-C ₁₂ AB	178	38	900
14	2-C ₁₂ AB	123	5	680
15	7-/6-C ₁₃ AB	385	306	4288
16	5-C ₁₃ AB	245	218	2488
17	4-C ₁₃ AB	157	99	1638
18	3-C ₁₃ AB	144	38	1613
19	2-C ₁₃ AB	113	22	1099
20	7-/6-C ₁₄ AB	302	224	1689
21	5-C ₁₄ AB	110	68	678
22	4-C ₁₄ AB	31	58	490
23	3-C ₁₄ AB	47	13	469
24	2-C ₁₄ AB	54	5	278
	Total	4009	1913	25 650
<i>Branched alkylbenzenes</i>				
	C ₁₁ AB	40	55	—
	C ₁₂ AB	907	1471	—
	C ₁₃ AB	175	485	—
	Total	1122	1983	—

* Not detected.

** Not determined.

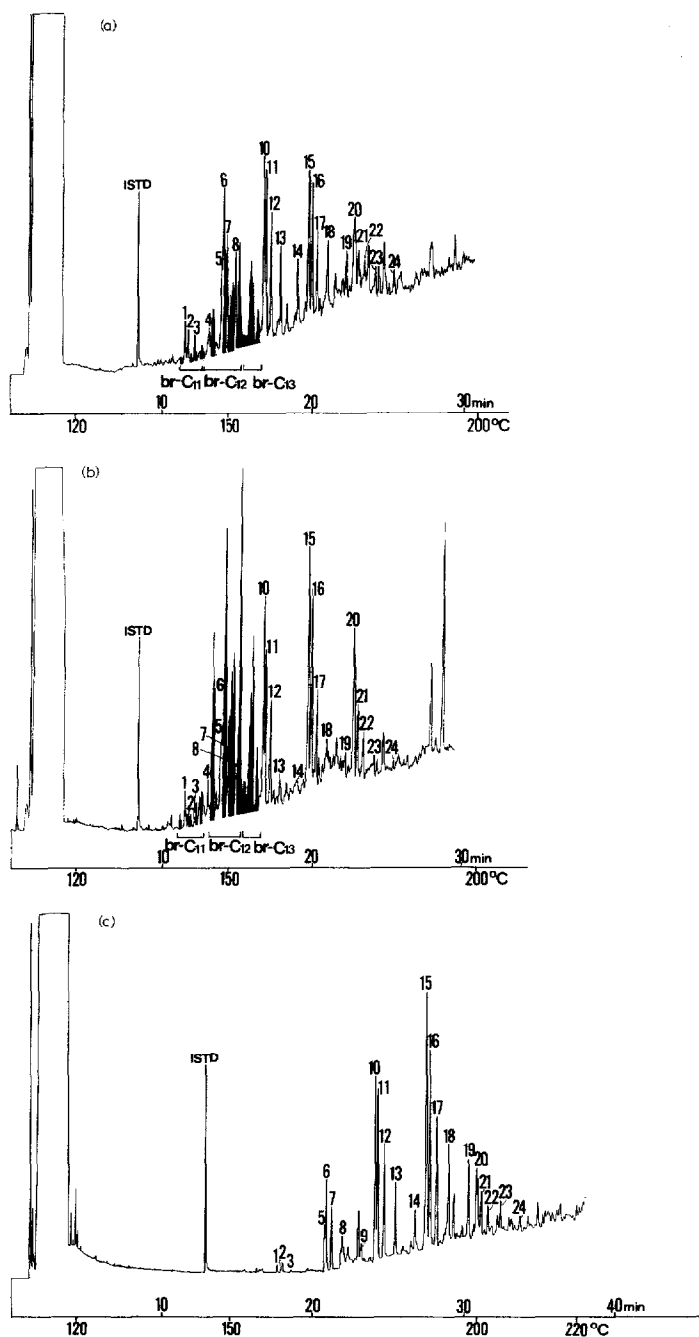


Fig. 5. Capillary gas chromatograms of alkylbenzenes extracted from River Arakawa sediment (a), Tokyo Bay sediment (b) and River Tamagawa suspended matter (c). Numbers refer to the compounds listed in Table I. Shaded peaks correspond to branched alkylbenzenes. Br-C₁₁ = Alkylbenzenes with branched C₁₁ chain; br-C₁₂ with branched C₁₂ chain and br-C₁₃ with branched C₁₃ chain. GC conditions as in Fig. 4.

Calibration curve

The linearity of the calibration curve for GC analysis was examined by using a mixture of equal concentrations (2–50 mg/l) of standard 1-C₁₀, 1-C₁₁, 1-C₁₂ and 1-C₁₃ alkylbenzenes in isooctane. A 2- μ l volume of each solution was injected. A linear relationship was obtained between the GC response and the amount of alkylbenzenes injected. The detection limit is 0.1 ng of alkylbenzenes. As described below, a common sediment sample contains 1–1000 ng of linear alkylbenzene per g dry material. Thus the calibration curve for alkylbenzenes can be used for their determination in sediment samples.

Recovery and reproducibility

The recovery of alkylbenzenes was determined by duplicate analyses of a sediment sample (River Tamagawa: Rokugo). A mixture of equal amounts (2.5 μ g in 25

TABLE III
ALKYLBENZENES IDENTIFIED IN ENVIRONMENTAL SAMPLES

Peak No.	Identification	Retention index*
b-1	Branched: C ₉ AB	79.93
b-2	Branched: C ₁₂ AB	85.38
1	Linear: 5-C ₁₀ AB	86.70
b-3	Branched: C ₁₁ AB	87.06
2	Linear: 4-C ₁₀ AB	87.54
b-4	Branched: C ₁₁ AB	87.96
b-5	Branched: C ₁₁ AB	88.36
3/b-6	Linear: 3-C ₁₀ AB/branched: C ₁₁ AB	89.37
b-7	Branched: C ₁₁ AB	89.80
b-8	Branched: C ₁₂ AB	91.30
b-9	Branched: C ₁₂ AB	91.73
4/b-10	Linear: 2-C ₁₀ AB/branched: C ₁₂ AB	92.84
b-11	Branched: C ₁₂ AB	93.79
b-12	Branched: C ₁₂ AB	94.03
b-13	Branched: C ₁₂ AB	94.74
5	Linear: 6-C ₁₁ AB	95.92
6	Linear: 5-C ₁₁ AB	96.20
b-14	Branched: C ₁₂ AB	96.54
7	Linear: 4-C ₁₁ AB	97.16
b-15	Branched: C ₁₂ AB	97.55
b-16	Branched: C ₁₂ AB	98.06
b-17	Branched: C ₁₂ AB	98.67
8	Linear: 3-C ₁₁ AB	99.16
b-18	Branched: C ₁₂ AB	100.27
b-19	Branched: C ₁₃ AB	101.20
b-20	Branched: C ₁₃ AB	101.53
b-21	Branched: C ₁₃ AB	102.46
9	Linear: 2-C ₁₁ AB	102.71
b-22	Branched: C ₁₃ AB	102.95
b-23	Branched: C ₁₃ AB	104.01
b-24	Branched: C ₁₃ AB	104.58
10	Linear: 6-C ₁₂ AB	105.31

* Average of alkylbenzenes extracted from River Arakawa and Tokyo Bay sediments. The variation is within 0.18.

μl *n*-hexane) of 1-C₁₀, 1-C₁₁, 1-C₁₂ and 1-C₁₃ alkylbenzenes was added to the organic solvent extracts from 5 g of the sediment sample and analyzed by GC after taking all steps of the analytical procedure. The recoveries were 94 ± 4 , 84 ± 5 , 89 ± 3 and $81 \pm 2\%$ for 1-C₁₀AB, 1-C₁₁AB, 1-C₁₂AB and 1-C₁₃AB, which is satisfactory for their quantitation.

The reproducibility was determined by triplicate analyses of a well homogenized sediment sample (River Tamagawa: Rokugo) containing 0.05–0.7 μg of the individual alkylbenzenes per g dry sediment. The results are shown in Table I (concentrations and relative standard deviations). The reproducibility is satisfactory.

Application to environmental samples

The analytical method described is currently being used in our laboratory for studying the behaviour of alkylbenzenes in the aquatic environment. Table II shows the analytical results for alkylbenzenes in representative river sediment, bay sediment and river suspended matter to illustrate the feasibility of the present method. As shown in Fig. 5, linear C₁₀–C₁₄ alkylbenzenes are detected in all these samples. Isomers which differ in the position of substitution of the benzene ring are well separated. The relative composition of the linear alkylbenzenes in these samples is similar, C₁₂ and C₁₃ homologues being the most abundant and C₁₀ and C₁₄ homologues being minor constituents. The total amount of linear alkylbenzenes in the sediment samples is 1.9–7.1 μg per g dry sample and quite high (26 μg per g dry sample) for the suspended matter. The concentration of linear alkylbenzenes in the sediment sample is the same as of PAHs.

In contrast to the River Tamagawa sediment sample, branched alkylbenzenes were detected in the sediment samples from River Arakawa and Tokyo Bay, in concentrations of 0.4–2.0 μg per g dry sample; C₁₁–C₁₃ alkylbenzenes are present, C₁₂ being predominant. As shown in Table III, most of the branched alkylbenzenes fall in the same range on the gas chromatograms as the linear C₁₀ and C₁₁ alkylbenzenes, but are separated from them.

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