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LIQUID CHROMATOGRAPHIC DETERMINATION OF ALIPHATIC DIAMINES IN WATER VIA DERIVATIZATION WITH ACETYLACETONE

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SUMMARY

Traces of C_2–C_6 aliphatic diamines were derivatized with acetylacetone in an aqueous medium containing 1–4% dipotassium hydrogenphosphate, and the resulting derivatives were extracted with a Sep-Pak C_{18} cartridge and determined by reversed-phase high-performance liquid chromatography. The diamines could be determined in the range 1.23–14.32 ng/ml in water with relative standard deviation of 0.4–4.9%. The detection limits were 0.14–1.78 ng/ml for 100-ml water samples. The recovery of diamine derivatives from river-water and sea-water was 88–96% with relative standard deviation of 1.3–4.0% and 34–93% with relative standard deviations of 1.3–9.8%, respectively.

INTRODUCTION

Aliphatic diamines have received much attention as chelating agents, chemical reagents and metabolic in biomedical studies. As they are highly soluble in water and have no absorption in the visible (VIS) or ultraviolet (UV) region, for increased sensitivity in chromatographic analysis it is preferable that the diamines are derivatized in an aqueous medium. High-performance liquid chromatography (HPLC)[1–7], ion-exchange and ion-pair chromatography[8–12] and thin-layer chromatography[13–15] are widely used to determine traces of diamines or polyamines by derivatization procedures employed either for fluorescence or UV–VIS detection. In previous work[16], an HPLC technique was used to determine traces of C_1–C_4 aliphatic amines in air via their derivatization with 7-chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl). Though, the application of this method to diamines in water samples was investigated[17], considerable difficulty was encountered owing to the poor solubility of NBD-Cl in water.

Acetylacetone, soluble to 15% in water at 30°C, reacts with the diamines affording condensation products. These derivatives are known as Schiff bases and have strong absorption bands in the UV region. This make it possible to determine diamines sensitively.

However, few reports have been published concerning the determination of aliphatic diamines in environmental water samples by HPLC via acetylacetone derivatives. In this paper, a convenient method is presented for the determination of C_2–C_6 aliphatic diamines in water at ng/ml levels by reversed-phase HPLC via acetylacetone derivatization.

EXPERIMENTAL

Reagents and materials

The dihydrochlorides of the C_2–C_6 diamines and 1,2-diaminopropane used as standards were of special grade from Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan). Methanol, ethanol and water were of liquid chromatographic grade. The other reagents used were of commercially available special grade. Acetylacetone was purified by distillation. A Sep-Pak C_{18} (SP) cartridge for extraction of the acetylacetone derivatives from water samples was obtained from Waters Assoc. (Milford, MA, U.S.A.). Stock solutions containing 1.23–4.78 mg/ml of the C_2–C_6 diamines were made in water. Standardsof lower concentrations were made by appropriate dilution in water.

Apparatus

A 1084B liquid chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) with an absorbance detector adjusted to 315 nm was employed. The analytical column was a 25 cm × 4.6 mm I.D. stainless-steel tube packed with Develosil ODS-5 (5 μm) (Nomura Kagaku, Aichi, Japan). The column temperature was 35°C. The mobile phase was methanol–water (55:45, v/v) and the flow-rate was 1.0 ml/min.

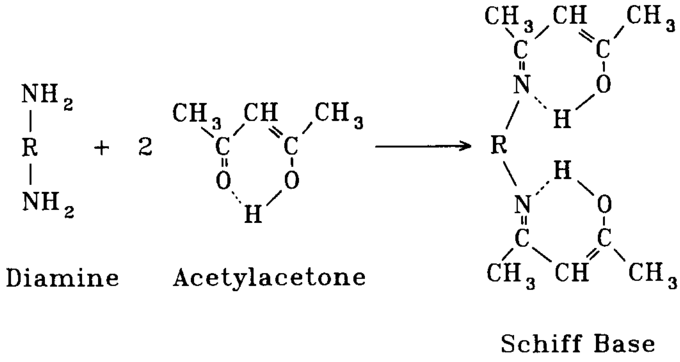
Analytical procedure

A 100-ml volume of the water sample was mixed with 1–4 g of dipotassium hydrogenphosphate and 1 ml of acetylacetone and then allowed to stand for 16 h at room temperature. For sea-water, a white precipitate was observed when dipotassium hydrogenphosphate was added. This was removed by filtration with a No. 5C cellulose filter (Toyo Roshi, Tokyo, Japan), and then acetylacetone was added to the filtrate. A 100-ml volume of the reaction mixture was passed at 30–50 ml/min through the SP cartridge which was washed with 5 ml of ethanol and then 10–20 ml of distilled water before use. The cartridge was washed with 10 ml of distilled water in order to eliminate excess of acetylacetone. The adsorbed acetylacetone derivatives of diamines were eluted with ethanol and 2 ml of the eluate was collected. Then, a 10-μl aliquot of the eluate was analyzed by HPLC. The diamines were identified by their retention times and quantified by their peak heights.

RESULTS AND DISCUSSION

Derivatization reaction

The reaction of aliphatic diamines with acetylacetone occurs in an aqueous solution at around neutrality (pH *ca.* 8) to produce Schiff bases. The reaction mechanism is as follows:



The derivatization of diamines for HPLC analysis was examined at appropriate dipotassium hydrogenphosphate and acetylacetone concentrations in 100 ml of the reaction medium containing 0.6–2.4 μg diamines. Dipotassium hydrogenphosphate was used to adjust the pH. Aspects of the derivatization of four typical diamines at 0–4% dipotassium hydrogenphosphate are shown in Fig. 1. The absorbance of the diamines was independent of the dipotassium hydrogenphosphate concentration in the range 1–4%. Fig. 2 shows the effects of the acetylacetone concentration on the absorbance of the diamines in the reaction media. The absorbance of 1,2-diaminoethane and 1,2-diaminopropane were constant at 1.0–2.0% acetylacetone, while the other diamines were independent of the acetylacetone concentration in the range 1.0–2.5%. Accordingly, dipotassium hydrogenphosphate and acetylacetone were added to the water sample at concentrations of 1–4 and 1–2%, respectively. The reaction of the diamines with acetylacetone proceeded slowly at room temperature (20°C). The apparent absorbances of the diamines reached a maximum overnight (16 h) and were constant for 1 week. An unknown peak next to 1,5-diaminopentane

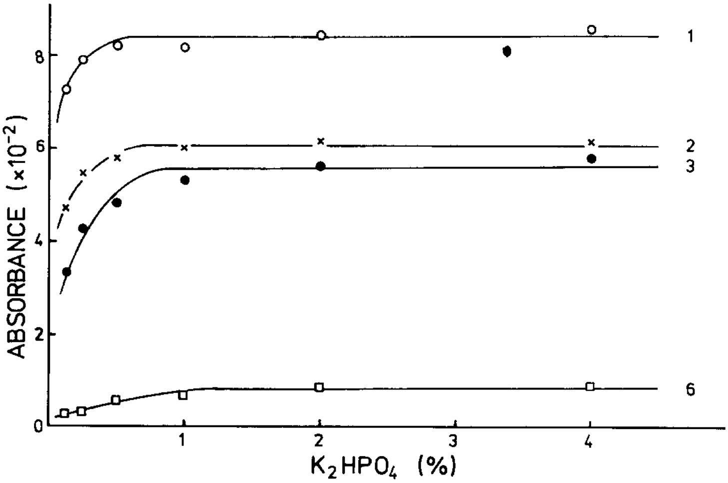


Fig. 1. Effects of dipotassium hydrogenphosphate on the absorbances of the diamine acetylacetone derivatives in the reaction medium: (1) 1,2-diaminoethane; (2) 1,2-diaminopropane; (3) 1,3-diaminopropane; (6) 1,6-diaminohexane.

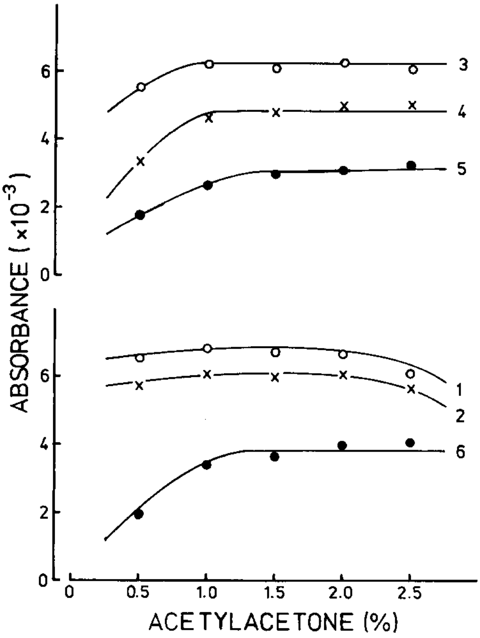


Fig. 2. Effects of acetylacetone on the absorbances of the diamine acetylacetone derivatives in the reaction medium. Sample volume injected onto the column, 10 μl: (1) 1,2-diaminoethane, 3.1 ng; (2) 1,2-diaminopropane, 3.5 ng; (3) 1,3-diaminopropane, 3.9 ng; (4) 1,4-diaminobutane, 4.8 ng; (5) 1,5-diaminopentane, 5.3 ng; (6) 1,6-diaminohexane, 11.9 ng.

however, increased daily and interfered with the measurement of this diamine. To assure analytical precision, the absorbances of the derivatives were determined 16 h after the commencement of reaction.

TABLE I

ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS FOR THE ACETYLACETONE DERIVATIVES OF ALIPHATIC DIAMINES

Extinction coefficients of each derivative were calculated from the separate peak in the chromatogram as follows: $\varepsilon = (A/L)(WR/M)$, where A is the absorbance at the peak height, L the path length of the cell (1.0 cm), M the amount (mol) of diamine injected, W the peak width at half-height (min) and R the flow-rate of the mobile phase (0.001 l/min).

Diamine	*Wavelength of absorption maximum (nm)*	ε_{315} *(l mol^{-1} cm^{-1})*
1,2-Diaminoethane	320	22 700
1,2-Diaminopropane	316	23 600
1,3-Diaminopropane	314	25 300
1,4-Diaminobutane	312	22 300
1,5-Diaminopentane	314	18 900
1,6-Diaminohexane	310	18 700

TABLE II

STABILITY OF THE ACETYLACETONE DERIVATIVES OF THE DIAMINES

Diamine	*Concn. as diamine** *(ng/ml)*	*Absorbance** · 10^{-3}*			
		0	*1*	*3*	*7 days*
1,2-Diaminoethane	3.1	3.60	3.60	3.84	3.76
1,2-Diaminopropane	3.5	3.92	3.88	4.08	4.00
1,3-Diaminopropane	3.9	4.00	3.96	4.16	3.96
1,4-Diaminobutane	4.8	2.60	2.56	2.64	2.40
1,5-Diaminopentane	5.3	1.36	1.36	1.44	1.36
1,6-Diaminohexane	11.9	1.60	1.56	1.64	1.60

* Concentration of diamine in an aqueous sample prior to derivatization.

** Calculated from peak height for 10 μl of final sample injected, at various storage times at room temperature (20–25°C) without protection against light.

Spectrophotometric properties

The UV spectra of the acetylacetone derivatives were obtained after HPLC separation by determining their absorbances at every 2 nm in the range 220–400 nm. Table I list the absorption maxima and extinction coefficients at 315 nm of these derivatives. Since the absorption maxima occurred at 310–320 nm, the derivatives were detected at 315 nm in the HPLC analysis. The extinction coefficients of the diamine derivatives were 25300–18700 l mol^{-1} cm^{-1}. The strong absorption bands of the diamine acetylacetone derivatives in the UV region are attributed to hydrogen-bonded conjugated chelate rings. A reasonable sensitivity is obtained by use of acetylacetone for ultratrace determination of the aliphatic diamines. The acetylacetone

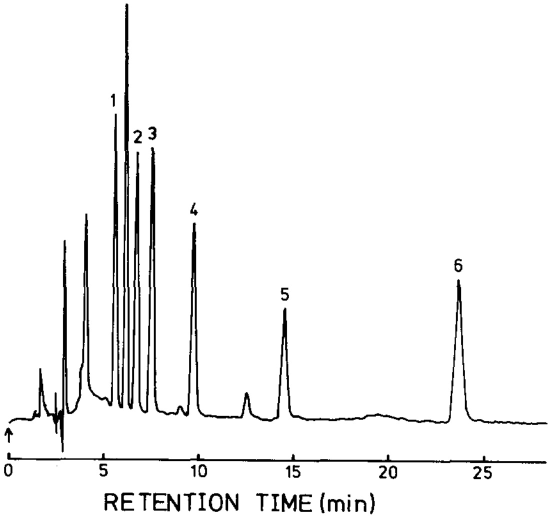


Fig. 3. Typical liquid chromatogram of the acetylacetone derivatives of the diamines. Amounts of diamines: 3.1–11.9 ng. Mobile phase: methanol–water (55:45, v/v); flow-rate, 1.0 ml/min. For the derivatives, see Fig. 2.

derivatives were stable in ethanol, Table II showing that their UV absorbances remained constant at least for 1–7 days.

Chromatographic separations

Separation of the acetylacetone derivatives of the C_2–C_6 diamine standards by HPLC was investigated by changing the ratio of the solvent components in the mobile phase on a Develosil ODS-5 (5 μm) column (25 cm × 4.6 mm I.D.). Excellent separations were obtained in the mobile phase of methanol–water (55:45, v/v). Fig. 3 shows a typical liquid chromatogram of acetylacetone derivatives under these conditions. Table III reports the retention times and analytical precision for these derivatives. The same-day relative standard deviation of the retention times was less than 0.55%. The diamines were determined at 4.93–19.10 ng levels with relative standard deviations of 1.39–2.36% by the HPLC technique.

Extraction with an SP cartridge

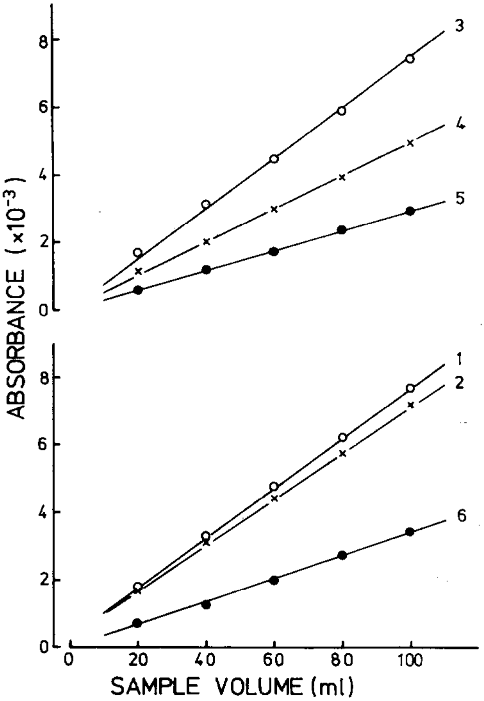
The acetylacetone derivatives in the reaction mixture were extracted with an SP cartridge and quantified by HPLC. When the aqueous reaction mixture was passed through the SP cartridge, the acetylacetone derivatives were selectively retained and most of the acetylacetone that did not reacted passed through. The acetylacetone derivatives were completely eluted by less than 2 ml of ethanol and none was found in the later eluate. Therefore, the initial 2 ml of the eluate was collected for the analysis. The breakthrough volume of the C_2–C_6 diamines at 3.1–31.8 ng/ml was investigated by passage through the SP cartridge. Figs. 4 and 5 show analytical data for diamines in 20–100 and 100–600 ml water samples, respectively. Excellent linearity was obtained for sample volumes less than 100 ml. In the case of sample volumes of 100–600 ml, breakthrough or saturation phenomena were observed for C_2 and C_3 diamines. The breakthrough volume was 150–200 ml for 1,2-diaminoethane and 300 ml for 1,2- and 1,3-diaminopropane; the other diamines were completely retained in less than 600 ml. Thus, a sample volume of 100 ml was used for extracting acetylacetone derivatives to assure simultaneous determination of the C_2–C_6 diamines.

TABLE III

RETENTION TIMES AND ANALYTICAL PRECISION FOR THE ACETYLACETONE DERIVATIVES

Diamine	*Retention time (min)**		*Analytical precision**		
	Av. ± S.D.	*R.S.D. (%)*	*Amount of diamine (ng)*	*Absorbance*** · 10^{-3} *(Av. ± S.D.)*	*R.S.D. (%)*
1,2-Diaminoethane	5.70 ± 0.031	0.54	4.93	8.78 ± 0.122	1.39
1,2-Diaminopropane	6.75 ± 0.037	0.55	5.60	7.97 ± 0.188	2.36
1,3-Diaminopropane	7.56 ± 0.040	0.53	6.19	7.81 ± 0.156	2.00
1,4-Diaminobutane	9.59 ± 0.051	0.54	7.69	5.85 ± 0.129	2.21
1,5-Diaminopentane	14.06 ± 0.059	0.42	8.50	3.81 ± 0.075	1.97
1,6-Diaminohexane	22.36 ± 0.102	0.46	19.10	4.71 ± 0.087	1.85

* Average of seven experiments; S.D. = standard deviation, R.S.D. = relative standard deviation.
** Calculated from peak height for 10 μl of final sample injected.



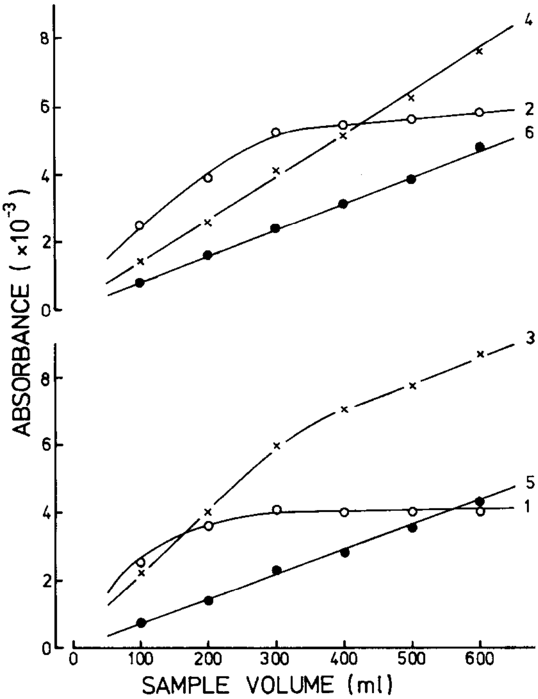


Fig. 4. Plots of the sample volume passed through the SP cartridge *vs.* the absorbance of the diamine acetylacetone derivatives in the eluates. Concentrations of diamines in water sample: 8.2–31.8 ng/ml. For the derivatives, see Fig. 2.

Fig. 5. Plots as in Fig. 4 for diamine concentrations in water sample of 3.1–11.9 ng/ml.

Quantitative response

Table IV reports the detection limits, analytical precision and calibration data for the C_2–C_6 aliphatic diamines. A blank test was performed using 100 ml of distilled water and the other chemicals used in the analysis. The reaction mixture was passed through the SP cartridge and the eluate was analyzed in a similar way. No peaks corresponding to diamines were observed in the blank chromatogram. The aliphatic diamines were determined in the range 1.23–14.32 ng/ml with relative standard deviations 0.4–4.9% in water samples, and excellent linearity was obtained. The estimated detection limits of these diamines in water are 0.14–1.78 ng/ml for 100 ml of a water sample.

Determination of diamines in real samples

Analyte recovery was investigated by using 100 ml of river-water and sea-water spiked with 369–1432 ng of the C_2–C_6 diamines. Table V shows that 88–96% of the diamines was recovered from the river-water with relative standard deviations of 1.3–4.0%. Table VI reveals that 34–93% of the diamines was recovered from sea-water with relative standard deviations of 1.3–9.8%. In the case of sea-water, the recovery was decreased in going from C_2 to C_6 diamines, possibly due to matrix

TABLE IV

DETECTION LIMITS, ANALYTICAL PRECISION AND CALIBRATION DATA FOR THE DIAMINES

Detection limits (DL) of each derivative were calculated from the sensitivity of response estimating standard deviation as follows

$$D = t(n - 1, 0.05)\ \sigma/\sqrt{n} \cdot dC/dR$$

$$DL = 3\bar{D}$$

where D is the detection power and $\bar{D}$ is the avarage value of D calculated from different concentrations (detection limits were defined as three times the detection power), $t(n - 1, 0.05)$ the t-distribution at 95% reliability, σ the standard deviation of the response, n the number of replicates, C the concentration of the diamines and R the absorbance at the peak height.

Diamine	*DL (ng/ml)*	*Analytical precision*			*Regression line*** and corr. coeff.*
		Concn. as diamine (ng/ml)*	*Absorbance** · 10^{-3}*	*R.S.D. (%)*	
1,2-Diaminoethane	0.26	0	0		
		1.23	1.03	2.1	$Y = 0.741X + 0.051$
		2.46	1.90	2.3	$r = 0.998$
		3.69	2.75	2.2	
1,2-Diaminopropane	0.35	0	0		
		1.40	1.09	1.5	$Y = 0.629X + 0.089$
		2.80	1.88	3.8	$r = 0.996$
		4.20	2.68	2.1	
1,3-Diaminopropane	0.14	0	0		
		1.55	1.01	2.1	$Y = 0.524X + 0.085$
		3.10	1.75	1.3	$r = 0.996$
		4.64	2.46	0.4	
1,4-Diaminobutane	0.44	0	0		
		1.92	0.65	1.5	$Y = 0.288X + 0.037$
		3.84	1.14	3.4	$r = 0.997$
		5.77	1.69	2.1	
1,5-Diaminopentane	0.44	0	0		
		2.12	0.38	2.3	$Y = 0.164X + 0.013$
		4.25	0.72	1.2	$r = 0.999$
		6.37	1.05	2.8	
1,6-Diaminohexane	1.78	0	0		
		4.77	0.45	4.9	$Y = 0.089X + 0.010$
		9.55	0.89	4.7	$r = 0.998$
		14.32	1.28	3.1	

* Concentration of diamine in an aqueous sample prior to derivatization.

** Calculated from peak height for 10 μl of final sample injected. Average of four experiments.

*** X = Concentration (ng/ml) of diamine in water; Y = absorbance at peak height. The number of samples was 16.

efects; however, the diamines could be determined by the standard addition method to compensate for the matrix effects, if necessary.

The SP cartridge was easy to handle in extracting and analyzing a large number

TABLE V

RECOVERY OF THE ACETYLACETONE DERIVATIVES OF DIAMINES FROM RIVER-WATER

A 100-ml volume of river-water was used.

Diamine	*Derivative (ng)*		*Recovery** *(%)*	*R.S.D.* *(%)*
	Added	*Found**		
1,2-Diaminoethane	369	348	94	1.4
1,2-Diaminopropane	420	397	95	1.5
1,3-Diaminopropane	464	425	92	1.3
1,4-Diaminobutane	577	507	88	1.7
1,5-Diaminopentane	637	601	94	2.7
1,6-Diaminohexane	1432	1374	96	4.0

* Average of four experiments. The amount found is the difference between the amount detected in spiked water sample (S) and that in the non-spiked water sample (N). Recovery (%) = $(S-N)/A \cdot 100$, where A is the amount added.

TABLE VI

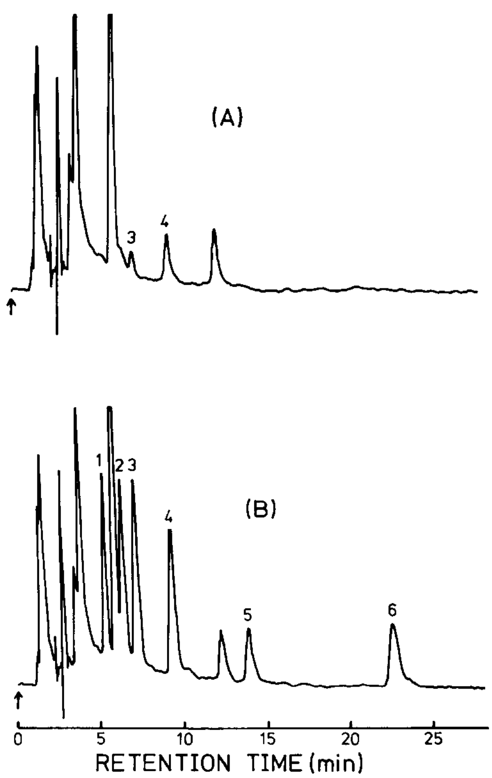
RECOVERY OF THE ACETYLACETONE DERIVATIVES OF DIAMINES FROM SEA-WATER

A 100-ml volume of sea-water was used.

Diamine	*Derivative (ng)*		*Recovery** *(%)*	*R.S.D.* *(%)*
	Added	*Found**		
1,2-Diaminoethane	369	345	93	1.3
1,2-Diaminopropane	420	364	87	1.4
1,3-Diaminopropane	464	248	53	3.5
1,4-Diaminobutane	577	199	34	4.0
1,5-Diaminopentane	637	264	41	9.8
1,6-Diaminohexane	1432	647	45	9.1

* Average of four experiments. See Table V for definition of recovery.

of water samples. Aliphatic diamines in river-water and sea-water were analyzed by use of the proposed method, and traces of diamines in these samples could be successfully determined. Fig. 6 shows typical chromatograms for 100 ml of normal and spiked river-water. The non-spiked sample peaks due to 1,3-diaminopropane and 1,4-diaminobutane corresponded to 0.45 and 2.20 ng/ml, respectively. Fig. 7 shows corresponding chromatograms for sea-water. 1,4-Diaminobutane was detected at low ng/ml levels in the unspiked sea-water.



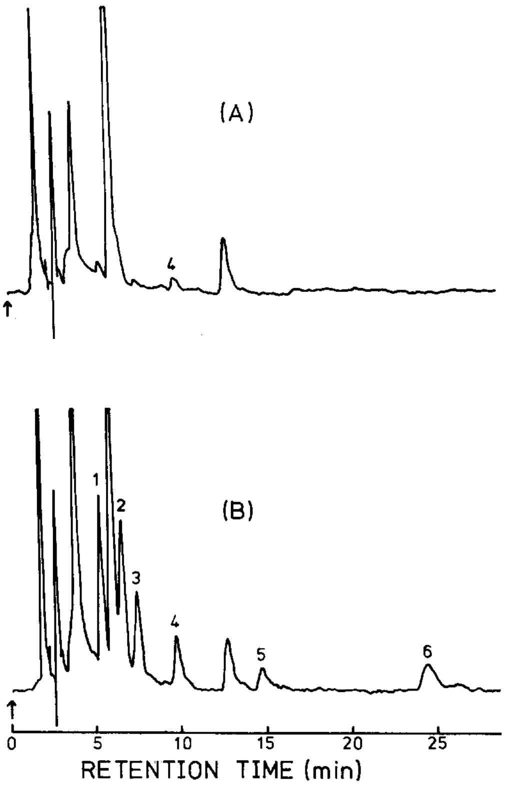


Fig. 6. Analysis of diamines in normal and spiked river-water (sample volume, 100 ml). (A) Normal river-water (the Yodo river); 3 = 1,3-diaminopropane (0.23 ng, 0.45 ng/ml), 4 = 1,4-diaminobutane (1.10 ng, 2.20 ng/ml). (B) River-water with standards added; concentrations of diamines (see Fig. 2) in the sample, 3.6–14.1 ng/ml.

Fig. 7. Analysis of diamines in normal and spiked sea-water (sample volume, 100 ml). (A) Normal sea-water (Osaka bay); 4 = 1,4-diaminobutane (0.25 ng, 0.50 ng/ml). (B) Sea-water with standards added; concentration of diamines (see Fig. 2) in the sample, 3.6–14.1 ng/ml.

CONCLUSIONS

The proposed HPLC method may be useful in routine analysis of water samples for pollution studies. Determination of the aliphatic diamines by use of this HPLC technique is probably aplicable to many other types of samples after appropriate modifications.

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