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CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC DETERMINATION OF PYRIDINE BASES IN ENVIRONMENTAL SAMPLES

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SUMMARY

A method is described for the determination of pyridine bases in environmental samples such as river water and bottom sediments. After distillation from environmental samples, the pyridine bases are concentrated on a rotary evaporator, extracted with *n*-hexane and determined by capillary gas chromatography–selected-ion monitoring (GC–SIM). The determination of twenty pyridine bases is possible using a 15 m × 0.53 mm I.D. capillary column coated with DBWAX (equivalent to PEG 20M) followed by SIM. The detection limits were 0.01–0.1 ng and recoveries from river water and bottom sediment were over 90% with coefficients of variation below 4% ($n = 7$) except for pyridine. This method is capable of the simultaneous determination of twenty pyridine bases with sufficient sensitivity and accuracy to be applicable to environmental samples.

INTRODUCTION

Pyridine bases are important industrial starting materials for medicines, veterinary products and surfactants. They smell unpleasant, exhibit high toxicity and are so difficult to decompose by microorganisms in nature that they remain intact when released into the environment. Therefore, there is a need for a microanalytical method for determining pyridine bases in environmental samples.

Analyses for pyridine bases have been made mainly by gas chromatography (GC)[1–8], gas chromatography–mass spectrometry (GC–MS)[9–13] and high-performance liquid chromatography (HPLC)[14,15]. Many reports have been published on methods for standard substances, coal tar and fuel oil, but there have been for fewer papers on methods for environmental samples such as river water, factory wastewater and bottom sediments; in particular, there have been little work on bottom sediments. Novotony *et al.*[9] used capillary GC–MS to analyse regenerated oils for aromatic bases. Felice[11] employed GC–MS to analyse solvent-refined coal distillates and

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aqueous extracts for alkylpyridines. Kaezvinsky *et al.*[14] applied a cation-exchange resin to extract organic bases from aqueous samples and then used GC with flame thermionic detection (FTD). Stuber and Leenheer[15] investigated the application of XAD-8 resin for the selective concentration of aromatic bases in water. All the above methods give unfavourable recovery, sensitivity and selectivity, or are difficult to apply directly to the analysis of environmental samples containing target substances at levels below 1 μg/l.

This investigation was undertaken to establish a suitable method for the simultaneous determination of pyridine bases present in environmental samples in very small amounts. Capillary columns are generally suitable for the separation and determination of pyridine bases abundant in isomers, but are inferior in sensitivity to packed columns because a large volume of sample can not be injected. In this study, a Megabore capillary column was selected, which has a large inner diameter about a fifth of that of a packed column, with similar sample injection volumes. The experimental examination was focused on the pre-treatment of environmental samples and the application of high-selectivity GC–selected-ion monitoring (SIM) to the determination of pyridine bases. We succeeded in establishing method that gives excellent sensitivity, selectivity, recovery and coefficient of variation.

EXPERIMENTAL

Reagents

The following twenty pyridine bases were obtained from Wako, Kanto Chemical or Tokyo Kasei: pyridine, 2-, 3- and 4-methylpyridine, 2-, 3- and 4-ethylpyridine, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethylpyridine, 2- and 4-*n*-propylpyridine, 2,4,6-trimethylpyridine, 3-ethyl-4-methylpyridine, 2-methyl-5-ethylpyridine and 2- and 4-vinylpyridine. *n*-Hexane and anhydrous sodium sulphate were of the grade suitable for the detection of pesticide residues. Sodium hydroxide was of analytical-reagent grade.

Apparatus and measurement conditions

GC–MS was carried out with a Model JMS-D300 instrument (Japan Electric Optics Laboratory) and GC–FTD with a Shimadzu Model GC-9A instrument.

The GC conditions were as follows: column, DBWAX (J & W Scientific), 15 m × 0.53 mm I.D.; column temperature, initially 60°C for 5 min, increased to 90°C at 5°C/min and held at 90°C for 10 min; injection port temperature, 200°C; and carrier gas (helium) flow-rate, 8 ml/min.

The SIM conditions were as follows: ionization voltage, 27.5 eV; ion multiplier voltage, 2.5 kV; temperature of the ion source and enricher, 250°C; and monitor ions, *m/z* 79, 93, 105, 106, 107, 120 and 121.

Standard procedure

Aqueous samples. A 500-ml volume of sample water is placed in a distillation flask and 5 ml of 10% w/v sodium hydroxide solution is added. Distillation is carried out into 5 ml of 0.5 *M* sulphuric acid as the absorption liquid until *ca.* 120 ml has been distilled. The distillate is transferred into a 300-ml flask and concentrated to *ca.* 5 ml on a rotary evaporator below 60°C. The concentrate is washed out into a 50-ml separating funnel and diluted to 15 ml with distilled water, then 5 ml of *n*-hexane and 12 ml of 75% w/v sodium hydroxide solution are added with ice–water cooling. After cooling, followed by shaking for 5 min, the *n*-hexane layer is removed and dried with anhydrous sodium sulphate. A 2-μl volume of the dried *n*-hexane extract is injected into the gas chromatograph and determined by SIM.

Bottom sediments. A 50-g moist sediment sample is weighed into a distillation flask to which 300 ml of distilled water, 5 ml of 10% w/v sodium hydroxide solution and 2–3 drops of silicone oil are added. The mixture is distilled until 100 ml of distillate have been obtained. The distillate is treated similarly to the aqueous sample as described above.

RESULTS AND DISCUSSION

Examination of distillation conditions

The following examinations were conducted in order to obtain the optimal distillation conditions. For all examinations conducted, a standard mixture was employed that contained the following seven pyridines at the same concentration: pyridine, 4-methylpyridine, 4-ethylpyridine, 3,4-dimethylpyridine, 4-*n*-propylpyridine, 4-vinylpyridine, and 3-ethyl-4-methylpyridine.

First, an examination was conducted to establish a suitable amount of alkali to be added for distillation. To a 1-l flask containing 500 ml of distilled water were added 100 μg of the standard mixture and various amounts of 10% w/v sodium hydroxide solution, the solution was distilled until 100 ml of distillate were obtained and the distillate was analysed directly by GC–FTD. The relationship between the amount of alkali added and the recovery was examined.

For bottom sediment samples, to 50 g of moist sediment were added 300 ml of distilled water and 100 μg of the standard mixture, and the mixture was treated similarly as described for the aqueous sample. It was found that the pyridine bases are distilled out unless the system is acidic and that no appreciable increase in recovery is brought about by increasing the amount of alkali added. As real samples are sometimes acidic, it was concluded that 5 ml of 10% w/v sodium hydroxide solution should be added before distillation.

Next, an examination was conducted to establish a suitable amount of distillate. With the amount of alkali added set at 5 ml of 10% w/v sodium hydroxide solution, the same operation as described above was employed, collecting successive 20-ml fractions of distillate, the relationship between the amount of distillate and the recovery being examined. It was found that 100% recovery was obtained if distillation was continued until 100 ml of distillate for bottom sediment samples (Fig. 1) and 120 ml for aqueous samples (Fig. 2) were collected.

Examination of conditions for concentration with a rotary evaporator

Pyridine bases will be vaporized unless the concentration step is conducted under acidic conditions. Therefore, an examination of the relationship between amount of acid added and recovery was carried out.

To 150 ml of distilled water were added 20 μg of a standard mixture and volume of 0.5 *M* sulphuric acid in the range 0–10 ml, concentration was carried out on a rotary evaporator below 60°C and subsequent treatments were conducted ac-

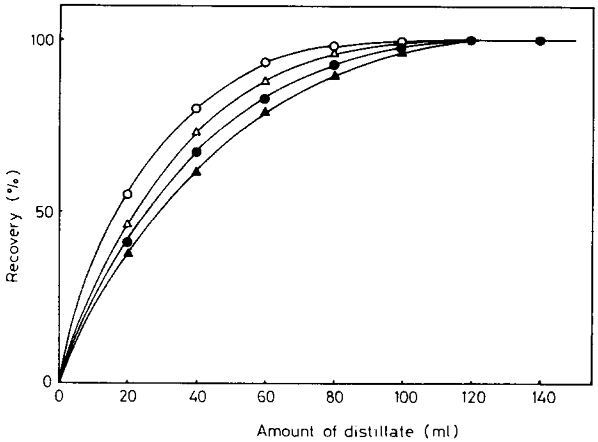


Fig. 1. Relationship between recovery and amount of distillate from aqueous sample. ▲, Pyridine; ●, 4-ethylpyridine; △, 4-vinylpyridine; and ○, 3-ethyl-4-methylpyridine.

cording to the standard procedure. It was found that the addition of more than 2.0 ml of 0.5 *M* sulphuric acid gave 100% recovery. Environmental samples such as bottom sediments may contain ammoniacal nitrogen, so it was concluded that 5 ml of 0.5 *M* sulphuric acid should be employed for security.

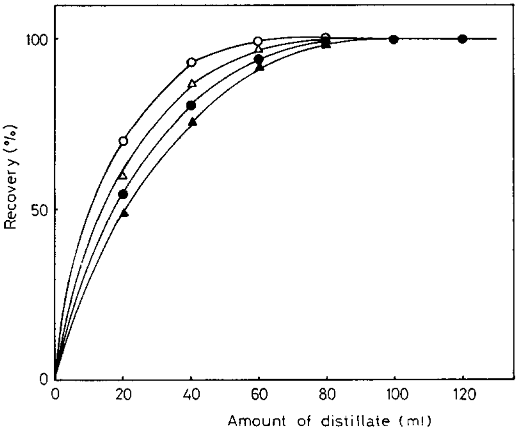


Fig. 2. Relationship between recovery and amount of distillate from bottom sediment. Compounds as in Fig. 1.

Examination of extraction conditions

Pyridine bases, most of which are water soluble, are difficult to extract with *n*-hexane unless the system is highly basic. Fig. 3 shows the relationship between amount of alkali added and the percentage extraction; this result was obtained by experiments in which a volume of 75% w/v sodium hydroxide solution in the range

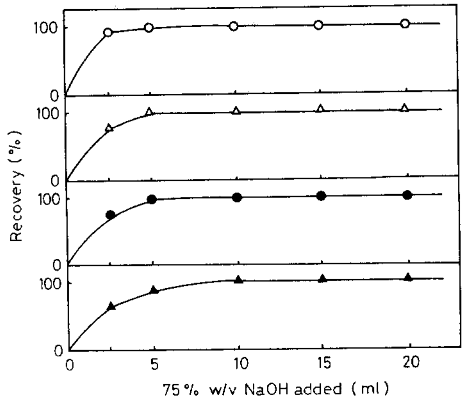


Fig. 3. Relationship between recovery and amount of 75% w/v sodium hydroxide solution added for extraction. Compounds as in Fig. 1.

0–20 ml was added to 15 ml of 0.16 *M* sulphuric acid containing 5 μg of a standard mixture and the mixed solution was extracted with 5 ml of *n*-hexane. It was found that the addition of more than 10 ml of 75% w/v sodium hydroxide solution gives a constant recovery.

Examination of GC–SIM conditions

Attempts to separate the twenty pyridine bases using high-polarity column packings such as PEG 6000, PEG 20M, FFAP and glycerol failed. Another attempt at separation was made using a Megabore capillary column, which is comparable to a packed column with respect to sample injection volume. It was found that separation using a DBWAX column followed by SIM gives almost complete separations.

To select monitor ions for determination, each pyridine base was subjected to an electron-impact mass spectral measurement. Ions of *m/z* 79, 93, 105, 106, 107, 120 and 121 were selected as the monitor ions on the basis of the spectral intensity and selectivity of the fragments. To determine the ionization voltage that allows the monitor ions to give their highest sensitivities, an examination was conducted by using ionization voltages of 15–30 and 70 eV. It was been found that 27.5 eV gave the highest sensitivities. Table I summarizes the monitors ions and detection limits for each pyridine base.

Recovery experiments and application to real samples

Recovery experiments were conducted by adding a constant amount of each pyridine base to 500 ml of river water or 50 g of bottom sediment and measuring the recovery according to the standard procedure (Table II); pyridine was added in a large amount as its blank value was high. As can be seen from Table II, recoveries of over 90% with coefficients of variation of below 4% were obtained for all 20 pyridine bases, expect pyridine.

The proposed method was applied to river waters, factory wastewaters, laboratory wastewaters and bottom sediments (n = 10). SIM chromatograms of pyridine bases, wastewaters from our laboratory and bottom sediments from Tokyo Bay are

TABLE I

MONITOR IONS AND DETECTION LIMITS FOR PYRIDINE BASES

The parenthesized *m/z* value is for a monitor ion used to confirm the identity of the unparenthesized *m/z* value.

Compound	*m/z*	*Detection limit (ng)*
Pyridine	79 (52)	0.01
2-Methylpyridine	93 (66)	0.01
3-Methylpyridine	93 (66)	0.01
4-Methylpyridine	93 (66)	0.01
2-Ethylpyridine	106 (107)	0.02
3-Ethylpyridine	107 (92)	0.05
4-Ethylpyridine	107 (106)	0.04
2,3-Dimethylpyridine	107 (106)	0.03
2,4-Dimethylpyridine	107 (106)	0.02
2,5-Dimethylpyridine	107 (106)	0.02
2,6-Dimethylpyridine	107 (106)	0.01
3,4-Dimethylpyridine	107 (106)	0.02
3,5-Dimethylpyridine	107 (106)	0.03
2-*n*-Propylpyridine	93 (106)	0.01
4-*n*-Propylpyridine	93 (121)	0.02
2-Vinylpyridine	105 (79)	0.02
4-Vinylpyridine	105 (78)	0.02
2,4,6-Trimethylpyridine	121 (79)	0.03
2-Methyl-5-ethylpyridine	106 (121)	0.02
3-Ethyl-4-methylpyridine	106 (121)	0.02

TABLE II

RECOVERY OF PYRIDINE BASES FROM RIVER WATER AND BOTTOM SEDIMENT

Compound	*River water*			*Bottom sediment*		
	Added (μg)	*Recovery (%)*	*C.V. (%)**	*Added (μg)*	*Recovery (%)*	*C.V. (%)**
Pyridine	10	87.0	4.4	10	84.0	4.7
2-Methylpyridine	1	94.0	3.2	1	92.2	3.6
3-Methylpyridine	1	94.2	3.9	1	93.1	3.1
4-Methylpyridine	1	92.2	3.2	1	90.2	3.1
2-Ethylpyridine	1	96.8	3.9	1	97.5	3.4
3-Ethylpyridine	1	96.5	2.9	1	97.1	3.4
4-Ethylpyridine	1	96.0	3.0	1	97.8	2.2
2,3-Dimethylpyridine	1	97.2	2.8	1	97.2	2.7
2,4-Dimethylpyridine	1	96.4	3.6	1	94.5	3.4
2,5-Dimethylpyridine	1	97.3	2.2	1	97.0	2.5
2,6-Dimethylpyridine	1	96.7	2.0	1	93.2	2.1
3,4-Dimethylpyridine	1	96.5	3.6	1	94.8	3.7
3,5-Dimethylpyridine	1	97.1	2.7	1	95.1	3.0
2-*n*-Propylpyridine	1	93.9	3.3	1	94.7	2.1
4-*n*-Propylpyridine	1	94.6	3.3	1	93.8	2.5
2-Vinylpyridine	1	95.3	2.9	1	97.2	2.4
4-Vinylpyridine	1	93.4	2.6	1	97.1	2.4
2,4,6-Trimethylpyridine	1	94.5	3.2	1	94.7	3.9
2-Methyl-5-ethylpyridine	1	97.0	2.4	1	95.9	2.2
3-Ethyl-4-methylpyridine	1	91.8	3.0	1	92.5	3.1

* Coefficient of variation (n = 7).

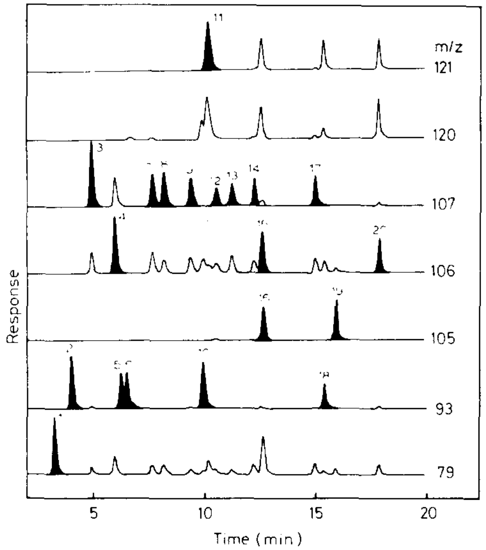
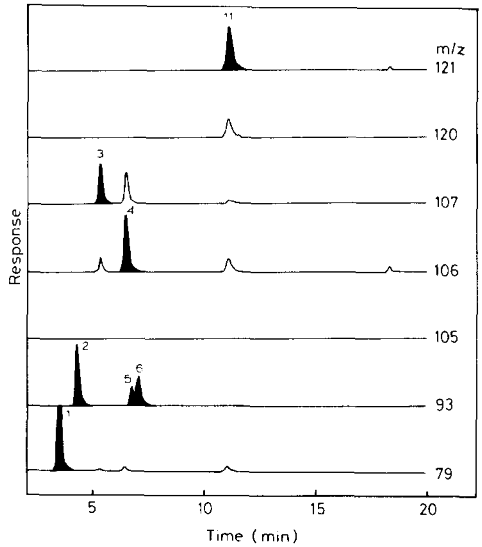


Fig. 4. SIM chromatogram of standard mixture of pyridine bases. Peaks: (1) pyridine; (2) 2-methylpyridine; (3) 2,6-dimethylpyridine; (4) 2-ethylpyridine; (5) 3-methylpyridine; (6) 4-methylpyridine; (7) 2,5-dimethylpyridine; (8) 2,4-dimethylpyridine; (9) 2,3-dimethylpyridine; (10) 2-*n*-propylpyridine; (11) 2,4,6-trimethylpyridine; (12) 3-ethylpyridine; (13) 4-ethylpyridine; (14) 3,4-dimethylpyridine; (15) 2-methyl-5-ethylpyridine; (16) 2-vinylpyridine; (17) 3,4-dimethylpyridine; (18) 4-*n*-propylpyridine; (19) 4-vinylpyridine; (20) 3-ethyl-4-methylpyridine.

shown in Figs. 4, 5 and 6, respectively. The wastewater from our laboratory was found to contain pyridine, 2-, 3- and 4-methylpyridine, 2-ethylpyridine, 2,6-dimethylpyridine and 2,4,6-trimethylpyridine at levels of 0.1–5 μg/l and the bottom sedi-



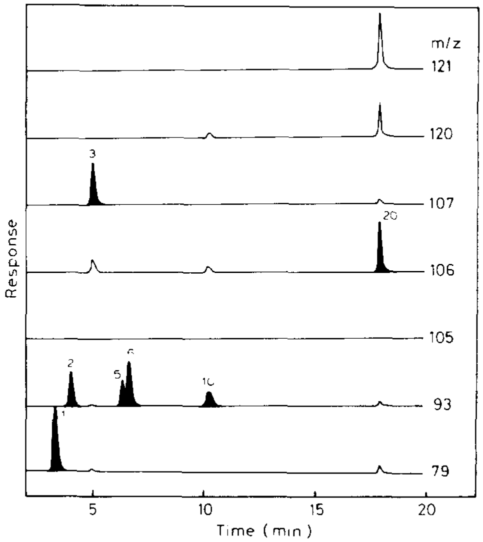


Fig. 5. SIM chromatogram of wastewater from our laboratory. Peaks as in Fig. 4.

Fig. 6. SIM chromatogram of bottom sediment from Tokyo Bay. peaks as in Fig. 4.

ments from Tokyo Bay were found to contain pyridine, 2-, 3- and 4-methylpyridine, 2-*n*-propylpyridine, 3-ethyl-4-methylpyridine and 2,6-dimethylpyridine at levels of 1–70 μg/g.

CONCLUSIONS

Pyridine bases may be distilled out under alkaline conditions, concentrated under acidic conditions and extracted with *n*-hexane under strongly alkaline conditions. The simultaneous determination of 20 pyridine bases, which was difficult with packed columns, is possible using capillary GC–SIM. Recovery experiments with environmental samples gave recoveries of over 90% with the coefficients of variation of below 4%, except for pyridine. The application of the proposed method to real environmental samples gave SIM chromatograms free from interference, demonstrating that the proposed method is suitable for practical applications.

No examination could be made of trimethylpyridines, ethylmethylpyridine, or tetramethylpyridines (except 2,4,5,6-tetramethylpyridine) because of the difficulty of obtaining standard substances; however, the proposed method is expected to be applicable to these compounds.

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