Note

Determination of Trace Lower Fatty Acids by Capillary Column Gas Chromatography-Mass Spectrometry

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The determination of lower fatty acids is important in environmental and biological studies. In particular, volatile fatty acids with four up to six carbon atoms are strongly malodorous and their odor threshold values are fairly low. Therefore, determination of trace amounts of these acids is necessary. Many investigations on the gas chromatographic (GC) determination of lower fatty acids have already been reported.^{1~6)} But the methods used are not sufficient for sensitive and selective analysis.

The average detection limits for lower fatty acids on GC using a capillary column have been of nanogram level. Therefore, derivatization of these acids is very necessary for trace analysis because of the sharpness of peaks. However, methylation or trimethylsilylation of lower fatty acids is not so effective because of the high volatility of the products and the lack of diagnostic ions for selected ion monitoring (SIM). In order to overcome these problems, Burke and Halpern⁷⁾ recently performed the butylation of lower fatty acids for mass spectrometry (MS). But the detection limits for five C₄-C₅ acids with their method were 30.4 to 52.1 nM.

In this study, lower fatty acids were converted to benzyl esters with benzyl alcohol and the esters formed were analyzed by SIM using a capillary column. The detection limits were around sub-picogram level and the selectivity was very high.

MATERIALS AND METHODS

Apparatus. A JEOL JMS-DX300 mass spectrometer was used with a Hewlett Packard 5710A gas chromatograph and a JEOL JMA3500 mass data analysis system. The operating conditions for GC/MS were as follows: column, Hewlett Packard ultra #1 fused silica capillary (0.20 mm i.d. $\times$ 25 m $\times$ 0.33 μ m); column temperature, 70°C to 230°C, with a linear increase of 4°C/min; injection port temperature, 250°C; head pressure of carrier gas (He), 1.0 kg/cm²; injection mode, splitless with a delay time of 60

sec; temperature of ion-source, 180°C; pressure of ion-source, 1.0~2.0 $\times$ 10⁻⁶ torr; ionizing current, 300 μ A; ionizing energy, 70 eV; accelerating voltage, 3 kV; and resolution of the mass spectrometer, 500. The column was directly connected to the ion-source. SIM was carried out at *m/z* 108.

Reagents. Acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, pivalic acid, benzyl alcohol, sodium hydroxide, *p*-toluenesulfonic acid and hydrochloric acid were of special analytical grade and purchased from Wako Pure Chemical Industries, Ltd. All solvents were of analytical grade for residual pesticides and purchased from Wako Pure Chemical Industries, Ltd. Silica Gel 60 for column chromatography was purchased from Merck Co. All benzyl esters of lower fatty acids were synthesized by heating benzyl alcohol and the corresponding fatty acid in the presence of *p*-toluenesulfonic acid.

General procedure for sample preparation. An air sample (10~20 l) is passed through Chromosorb 103 (1.5 g) packed in a glass tube (10 mm i.d., 10 cm length). The Chromosorb 103, which has been washed sufficiently with methanol and then acetone before packing, should be washed again with ethyl ether (100 ml) immediately before use. After sampling, compounds adsorbed on the Chromosorb 103 are eluted with ethyl ether (100 ml), and then the resultant solution is concentrated to around 1 ml under atmospheric pressure.

A water sample (100 ml), which is not highly polluted, is made alkaline by the addition of sodium hydroxide (4 g) and then washed twice with dichloromethane (40 ml) in order to remove organic substances. The aqueous layer is concentrated to around 10 ml with a rotary evaporator and then conc. hydrochloric acid (20 ml) is added. Organic substances are extracted twice with dichloromethane (40 ml). The extracted solution is concentrated to around 1 ml under atmospheric pressure after drying over anhydrous sodium sulfate.

For a liquid sample such as polluted water and urine, sodium chloride (20 g) is dissolved in the sample (150 ml) and then conc. hydrochloric acid (10 ml) is added. The solution is distilled under stirring until the volume of the distillate reaches 100 ml. Sodium hydroxide (4 g) is dissolved in the distillate and the organic substances are eliminated by shaking the solution twice with dichloromethane (40 ml). Then, the aqueous solution is concentrated to around 10 ml with a rotary evaporator. After adding conc. hydrochloric acid (20 ml), organic substances are extracted twice with dichloromethane (40 ml). The extracted solution is dried over anhydrous sodium sulfate and then concentrated to around 1 ml under atmospheric pressure.

Solid samples (1~20 g) such as soil and feces are suspended in water (150 ml) containing sodium chloride (15 g) and conc. hydrochloric acid (10~20 ml), and then

distillation under atmospheric pressure is carried out until the volume of the distillate reaches 100 ml. The following procedures are the same as described for liquid samples.

Derivatization procedure. The concentrated solution of an extract (around 1 ml) is mixed with benzyl alcohol (0.5 ml) and *p*-toluenesulfonic acid (more than 10 mg), followed by refluxing for 3 hr. The reaction products are cleaned up directly by column chromatography on Silica Gel 60 (Merck, 15 g). Benzyl esters of lower fatty acids are eluted with carbon tetrachloride (300 ml). The eluted solution is concentrated and the internal standard (benzyl pivalate, 10 μ g) is added.

Samples. An air sample (181) was collected from a garbage container. Swine feces (20 g) were gathered in a pig house. Poultry manure (20 g) were purchased at a fertilizer shop. Human urine (150 ml) was obtained from a middle-aged male. Pond water (100 ml) was taken from a pond near the author's institute.

RESULTS AND DISCUSSION

Elimination of interfering compounds by steam distillation or solvent extraction are necessary. Otherwise, the derivatization yield decreases and the separation efficiency on column chromatography becomes poor. The derivati-

TABLE I. CONCENTRATION OF LOWER FATTY ACIDS IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

Compound	Sample				
	A (mg/g)	B (ng/g)	C (ng/ml)	D (ng/ml)	E (μ g/l)
Acetic acid	1.75	26.0	1.24	48.1	253
Propionic acid	2.02	28.0	5.17	92.2	0.102
Isobutyric acid	0.699	57.0	179	122	0.019
Butyric acid	4.65	84.0	52.6	42.5	0.066
Isovaleric acid	0.825	111	113	11.0	0.021
Valeric acid	2.02	143	97.9	9.40	ND
Isocaproic acid	0.015	184	266	ND	ND
Caproic acid	0.407	1020	460	12.6	0.015

Sample notation: A, swine feces; B, poultry manure; C, human urine; D, pond water; E, headspace gas in a garbage container. ND means "not detected." The concentrations were corrected as to the recovery coefficient for liquid and solid samples and as to the derivatization yield for the gas sample.

FIG. 1. Selected Ion Monitoring of Benzyl Esters in an Extract of Swine Feces.

The setting mass number was 108. Peak notation: 1, benzyl acetate; 2, benzyl propionate; 3, benzyl isobutyrate; 4, benzyl pivalate (internal standard); 5, benzyl butyrate; 6, benzyl isovalerate; 7, benzyl valerate; 8, benzyl caproate.

zation yield were around 66~82%. The overall recovery (%) was 64 for acetic acid, 65 for propionic acid, 54 for isobutyric acid, 56 for butyric acid, 57 for isovaleric acid, 57 for valeric acid, 60 for isocaproic acid and 62 for caproic acid. The mass spectra of benzyl esters of lower fatty acids show strong peaks at m/z 91 and 108. Ion monitoring at m/z 91 was unsatisfactory because of the high back ground level and the poor selectivity. On the other hand, ion monitoring at m/z 108 was excellent.

Quantification was carried out by the internal standard method.

Amount of benzyl ester $= (A_i/A_s) \times W_s \times f_i$
where W_s , f_i , A_i and A_s are the amount of internal standard added, relative response factor for benzyl ester i , peak area of benzyl ester i and peak area of the internal standard, respectively. The f_i values were as follows: benzyl acetate, 0.0662; benzyl propionate, 0.0758; benzyl isobutyrate, 0.158; benzyl butyrate, 0.0781; benzyl isovalerate, 0.0935; benzyl valerate, 0.0962; benzyl isocaproate, 0.168; and benzyl caproate, 0.128. Benzyl pivalate was selected as an internal standard. The most appropriate liquid phase for good separation and a low background was cross-linked methyl silicone. The detection limits were around 0.10~0.56 pg. Lower fatty acids at concentrations below the odor threshold values can be determined by this method. The relative standard deviation on five duplicate measurements using a standard solution was only 1.0~5.4% for 1000~100 pg and 5.1~19.0% for 10 pg.

This method was applied for swine feces, poultry ma-

nure, human urine, pond water and the headspace gas in a garbage container. The concentrations are shown in Table I. Selected ion monitoring of an extract of swine feces is shown in Fig. 1 as an example. There were more lower fatty acids in swine feces than in poultry manure. This result is consistent with the data in the literature.⁸⁾ It is very interesting that fatty acids with larger numbers of carbon atoms were more abundant both in poultry manure and human urine. Acetic acid was the most abundant in pond water and the headspace gas in the garbage container. The concentrations of acetic acid and butyric acid in the headspace gas of the container were much higher than their odor threshold values.

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