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ANALYTICAL STUDY OF LOW-CONCENTRATION GASES

III*. DETERMINATION OF LOWER FATTY ACIDS AND THEIR TRIMETHYLSILYL ESTERS IN AIR BY GAS CHROMATOGRAPHY

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SUMMARY

A gas chromatographic method for the determination of lower fatty acids as odour pollutants is discussed. A sample gas was introduced on to glass beads coated with 1% sodium hydroxide solution to collect fatty acids, which were isolated in a sampling tube and then submitted to gas chromatography without concentration. These fatty acids were reacted with trimethylsilylimidazole to form trimethylsilyl esters, which were also subjected to gas chromatography. The latter method resulted in a sensitivity 5–10-fold greater than that by the former method. The recoveries were more than 90% in both methods, with coefficients of variation of less than 5% in the former and about 8% in the latter. The gas chromatographic method gave good reproducibility, with detection limits of 0.5–1 ppb** when 200 l of gas were sampled. Air quality was determined by both the methods on samples from a hog farm and a refuse incinerator.

INTRODUCTION

The concentrations of lower fatty acids such as acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids have become important in studies of ill-smelling substances in air. For the determination of minute amounts of such compounds in air, the alkali filter method of Okita *et al.*[1], the alkali solution method of Okabayashi *et al.*[2] and the alkali beads method of Nakayama *et al.*[3,4] are of interest. However, many problems remain unsolved with respect to the chemical treatment for the gas chromatographic (GC) analysis of these compounds and hence methods for determining them remain unestablished.

* For Part II, see *J. Chromatogr.*, 265 (1983) 45.
** Throughout this article, the American billion (10^9) is meant.

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In the quantitative determination of lower fatty acids as their trimethylsilyl (TMS) derivatives, Mamer *et al.*[5] allowed lower fatty acids in serum and urine to react with trimethylsilylimidazole in order to analyse them in the form of TMS esters. There are few reports on the determination of lower fatty acids in air in the form of TMS derivatives.

We absorbed free fatty acids from air using glass beads coated with 1 % sodium hydroxide solution, then separated the fatty acids with hydrogen chloride–diethyl ether solution in a sampling tube and submitted them to GC analysis. We also prepared the TMS derivatives of low-concentration lower fatty acids in air prior to GC analysis. We found that by this method lower fatty acids in air could be determined quantitatively and simply in a short time.

EXPERIMENTAL

Apparatus

A Shimazu 5A gas chromatograph equipped with a flame-ionization detector (FID) and a Nippon Denshi JMS-D 300/JMA-200 gas chromatograph–mass spectrometer were used.

Reagents

All reagents were of special grade.

Acetic acid, propionic acid, isobutyric acid, *n*-butyric acid, isovaleric acid, *n*-valeric acid and sodium hydroxide were obtained from Katayama, anhydrous sodium sulphate (pesticide residue analysis grade) also from Katayama and N-trimethylsilylimidazole (TSIM) from Pierce (5-g vial).

Hydrogen chloride saturated solution of diethyl ether. To concentrated sulphuric acid was added concentrated hydrochloric acid dropwise, and the resulting hydrogen chloride gas was dried over calcium chloride and absorbed into 100 ml of absolute diethyl ether.

Fatty acid standard solutions. A 20–30 μg/ml diethyl ether solution of fatty acids was prepared.

Analytical method

A sample of air was collected by passing it through glass beads coated with 1 % sodium hydroxide solution. The hydrogen chloride-saturated diethyl ether solution was then added to the sample to separate lower fatty acids, an aliquot of which was subjected to GC. When the sample contained low-concentration lower fatty acids, they were gas chromatographed after trimethylsilylation with TSIM.

Sampling method. Thoroughly washed glass beads (12–16 mesh) were coated with 1 % (w/v) sodium hydroxide solution. A sampling tube as shown in Fig. 1 (10 mm in diameter, 60 mm long) was filled with 1.2 g of the glass beads to a height of 1.5 cm. Before collection of the sample, 500 μl of distilled water were dropped on to the beads with a pipette. The sample was passed into the tube arranged as shown in Fig. 2 at an aspiration rate of 10 l/min to collect fatty acids.

Method for recovering free fatty acids. The sampling tube was dried in a drier at 80–100°C, the lower outlet was then tightly stoppered, dry sodium sulphate was placed on the glass beads and the tube was allowed to cool in a desiccator. Into the

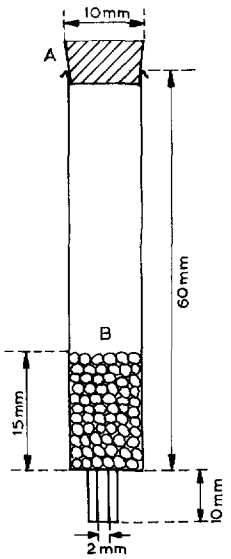

Fig. 1. Sampling tube. A, Silicone rubber; B, glass beads.

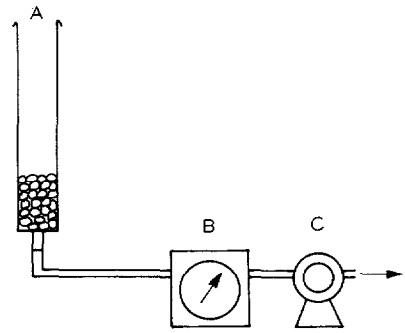

Fig. 2. Sampling apparatus. A, Sampling tube; B, gas meter; C, pump.

tube was added 1–2 ml of hydrogen chloride-saturated diethyl ether solution precisely from the upper inlet, which was then immediately stoppered tightly with a silicone-rubber stopper, this stopper being used as a packing (Gasukuro Kogyo, Cat. No. M-10100) for GC injection. A portion of 1–2 μl of the ether solution was sampled through the silicon-rubber stopper with a microsyringe for GC.

The amount of hydrogen chloride-saturated solution injected requires discussion. A small amount injected causes problems in sampling the ether solution; large amounts injected made the sensitivity adequate because of the decreased concen-

TABLE I

OPERATING CONDITIONS FOR GC ANALYSIS OF C_2–C_5 FATTY ACIDS AND THEIR TMS ESTERS

Fatty acids	*TMS-fatty acids*
Column: 5% Thermon-1000 + 0.5% H_3PO_4 on Chromosorb W (80–100 mesh), 3 m × 3 mm I.D., glass	Column: 3% OV-17 on Chromosorb G (60–80 mesh), 2 m × 3 mm I.D., glass
Temperatures: Column, 120°C; injection, 250°C	Temperature: Column, 35–110°C at 6°C/min, 2 min hold; injection, 200°C
Flow-rates: N_2, 1 kg/cm^2; H_2, 60 ml/min; Air, 0.8 l/min	Flow-rates: N_2, 1 kg/cm^2; H_2, 60 ml/min; Air, 0.8 l/min
Detector: FID	Detector: FID

trations of fatty acids in the ether solution. Our experiments revealed that the optimal amount to be injected was 1–2 ml, which neutralized the fatty acids and caused no problems in sampling with a microsyringe. The volume of hydrogen chloride gas in 1 ml of the solution[6] was greatly in excess for the neutralization of sodium hydroxide in the sampling tube.

When the fatty acids were analysed in the form of TMS derivatives, 300 μl of TSIM were injected into the sampling tube, to which the hydrogen chloride-saturated ether solution was added and of which the upper inlet was stoppered tightly with a thoroughly dried silicone-rubber stopper. The tube was then heated in a drier at 60°C for 20 min to effect trimethylsilylation. Care was taken to prevent unstoppering. After trimethylsilylation, 1–2 μl of the solution was sampled by piercing the stopper with a microsyringe, followed by GC analysis.

Gas chromatographic conditions

The GC conditions used for the free acids and their TMS derivatives are shown in Table I.

RESULTS AND DISCUSSION

Recovery and precision

Definite concentrations of fatty acid standard solutions (50 μl–1 ml) were injected into the apparatus shown in Fig. 3. Each of the fatty acids which was heated and vaporized was collected in a sampling tube using a stream of 200 l of nitrogen at a flow-rate of 10 l/min. Recoveries and coefficients of variation were obtained based on the peak heights on the gas chromatograms obtained. Table II shows the results when 100 μl of a 30 μg/ml standard solution was injected directly into a gas chromatograph. The reproducibility was acceptable, with recoveries of more than 90% and coefficients of variation of less than 5% for all the fatty acids.

The above results demonstrate the satisfactory separation of the fatty acids from the sampling tube with the hydrogen chloride–diethyl ether solution. Unlike alkali solution and alkali filter-paper methods, the method requires no concentration. Accordingly, there was little loss of lower fatty acids owing to vaporization on concentration, and the recoveries and the coefficients of variation were nearly equal to those in Table II, irrespective of the amounts of the fatty acids injected (50 μl–1 ml). The above results suggest a sufficient precision for air pollution analysis. Fig. 4 shows an example of a gas chromatogram obtained after the separation and recovery of fatty acids.

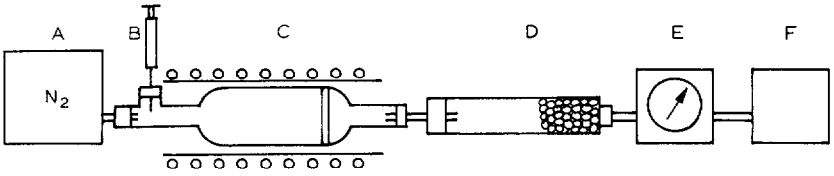

Fig. 3. Apparatus for preparation of standard gas and sampling set (D, E, F). A, Mylar bag; B, microsyringe; C, heater and glass tube; D, sampling tube; E, gas meter; F, pump.

TABLE II

RECOVERIES AND COEFFICIENTS OF VARIATION

Concentration of lower fatty acids, 30 μg/ml; 100 μl injected.

Parameter	*Sample*					
	C_2	C_3	*Iso*-C_4	*n*-C_4	*Iso*-C_5	*n*-C_5
Minimum and maximum recoveries (%)	92.4–104	93.6–103	86.2–96.4	84.9–96.5	87.5–92.6	89.4–102
Average recovery (%) (n = 5)	97.7	96.8	90.5	90.1	92.8	95.5
Coefficient of variation (%)	4.85	4.38	4.53	4.70	4.91	4.88

Trimethylsilylation

In order to trimethylsilylate lower fatty acids, Mamer *et al.*[5] extracted the acids from serum and urine with diethyl ether, distilled off the ether in a stream of nitrogen and heated the residue with TSIM at 60°C for 15 min to obtain TMS derivatives. Because this method may cause vaporization of the fatty acids during the distillation of the ether, however, we considered the possibility of the reaction of fatty acids with TSIM in a sampling tube to give trimethylsilylated fatty acids and attempted to carry out the following procedures.

Into the sampling tube were injected 50 μl–1.5 ml of fatty acid standard solutions, the ether in the tube was distilled off and the residue was dried at 80–100°C. The tube, the lower outlet of which was tightly stoppered, was allowed to cool in a desiccator. A small amount of dry sodium sulphate was placed on the top of the glass

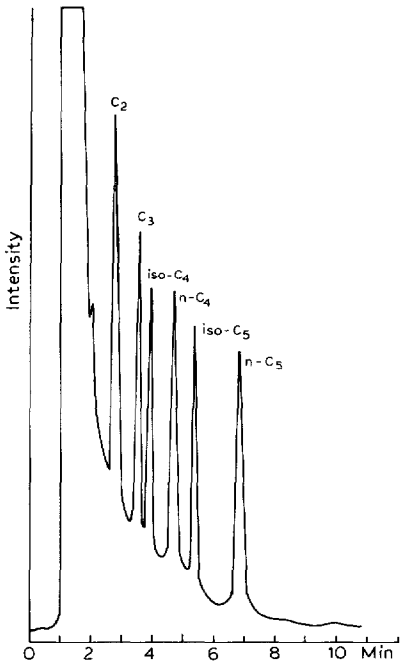

Fig. 4. Typical gas chromatogram of free fatty acids.

beads, to which 1 ml of hydrogen chloride-saturated diethyl ether was accurately added and the upper inlet was immediately stoppered tightly with a silicone-rubber stopper. TSIM was injected into the sampling tube through the stopper using a thoroughly dried microsyringe to effect trimethylsilylation. This method confirmed the formation of TMS fatty acids in the tube and investigations were made on the reaction conditions such as reaction time and the amount of TSIM added.

At 60°C, similar to the temperature in the method by Mamer *et al.*[5], an investigation was made of the relationship between the amount of TSIM added (50–500 μl) and the peak heights on the gas chromatogram of the resulting TMS fatty acids. Nearly constant peak heights were obtained with more than 300 μl of TSIM for 1–30 μg of each of the fatty acids. A nearly quantitative peak height was observed for a reaction time of 20 min when more than 300 μl of TSIM were added. When the peaks obtained under these reaction conditions were compared with those obtained on reaction at 60°C for 3 h, the peak heights were nearly equal. The results are given in Tables III and IV, showing the percentage production of TMS fatty acids in terms of peak heights on the gas chromatograms; for the results in Table III, 1 ml of TSIM was reacted with the fatty acids for 60 min, and for those in Table IV, 300 μl of TSIM were reacted with the fatty acids for 3 h.

The above results confirmed the formation of TMS fatty acids in sampling tubes in which 300 μl of TSIM was reacted with the fatty acids at 60°C for 20 min. Less than 0.5 μg of fatty acids collected in sampling tube on trimethylsilylation permitted the quantitative determination of minute concentrations of fatty acids in air, because of improved peak sharpness and GC sensitivity, in spite of poor reproducibility. More than 0.5 μg of fatty acids collected gave satisfactory reproducibility with coefficients of variation below 8%. These low fatty acid concentrations are considered to have sufficient precision for atmospheric analysis by taking the volume of air sampled into consideration.

An investigation was made on the effects of sodium sulphate on absorption into and decomposition of the TMS fatty acids produced during trimethylsilylation. To 2 ml of a diethyl ether solution of TMS fatty acids was added 1 g of dry sodium sulphate and the peak heights after the solution had been allowed to stand for 5 h at room temperature were compared with those before it had been allowed to stand. As shown in Table V, the average recoveries of the fatty acids were 99–100% with

TABLE III

REACTION EFFICIENCY (%) WITH DIFFERENT AMOUNTS OF TSIM

Reaction temperature, 60°C; reaction time, 60 min; amount of lower fatty acids, 2 μg.

Amount of TSIM (μl)	*Sample*					
	C_2	C_3	*Iso*-C_4	*n*-C_4	*Iso*-C_5	*n*-C_5
50	23	28	34	38	27	29
100	54	48	42	50	33	39
200	95	96	97	99	64	59
300	100	100	100	100	100	100
500	100	100	100	100	100	100

TABLE IV

REACTION EFFICIENCY (%) WITH DIFFERENT REACTION TIMES

Reaction temperature, 60°C; amount of TSIM, 300 μl; amount of each fatty acid, 2 μg.

Reaction time (min)	*Sample*					
	C_2	C_3	*Iso*-C_4	*n*-C_4	*Iso*-C_5	*n*-C_5
5	78	64	64	32	19	8
10	100	91	89	73	62	42
15	100	95	99	88	83	71
20	100	100	100	100	100	100
30	100	100	100	100	100	100

coefficients of variation of 1.3–2.9%. It appeared that dry sodium sulphate had a negligible effect on the recoveries for an analytical period of about 1 day. The TMS fatty acids, because of their instability, should not be allowed to stand for more than 5 h.

Evaluation of GC–MS results

GC–MS was carried out under the following conditions: glass column (2 m × 2 mm I.D.), 3% OV-17 on Chromosorb G (60–80 mesh); column temperature, programmed from 35 to 110°C at 6°C/min, 2 min hold; injection temperature, 200°C; flow-rate (helium), 1.5 kg/cm^2; ionizing current, 100 μA; ionizing voltage, 20 eV; ion source temperature, 220°C.

The mass spectra were evaluated to confirm the identities of TMS derivatives after each of the fatty acids had reacted with TSIM. The mass spectra are shown in Fig. 5A–F, and indicate that all the fatty acids developed molecular ion peaks as TMS derivatives. Accordingly, the chromatograms confirmed the presence of TMS derivatives.

TABLE V

RECOVERY OF LOWER FATTY ACIDS FROM DRY SODIUM SULPHATE IN DIETHYL ETHER SOLUTION

n	*Sample*					
	C_2	C_3	*Iso*-C_4	*n*-C_4	*Iso*-C_5	*n*-C_5
	Concentration (μg/ml)					
	30	*30*	*20*	*20*	*20*	*20*
1	98	99	101	99	100	99
2	101	103	101	102	102	104
3	99	98	95	98	97	100
4	100	101	99	99	100	97
Average (%)	100	100	99	100	100	100
Coefficient of variation (%)	1.3	2.2	2.9	1.7	2.1	2.9

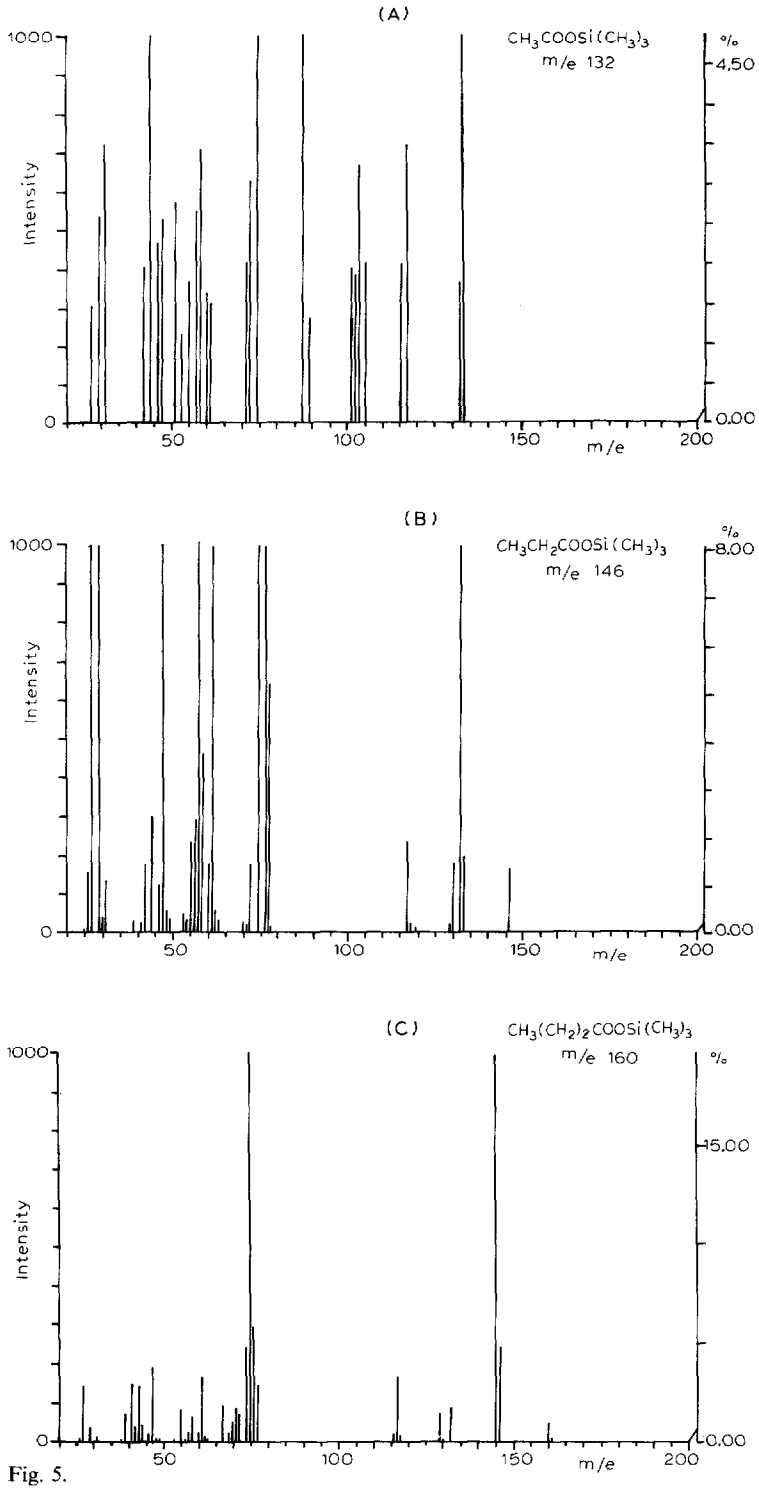
(A)
CH3COOSi(CH3)3
m/e 132
%
4.50
0.00
1000
0
Intensity
50
100
150
200
m/e
(B)
CH3CH2COOSi(CH3)3
m/e 146
%
8.00
0.00
1000
0
Intensity
50
100
150
200
m/e
(C)
CH3(CH2)2COOSi(CH3)3
m/e 160
%
15.00
0.00
1000
0
Intensity
50
100
150
200
m/e

Fig. 5.

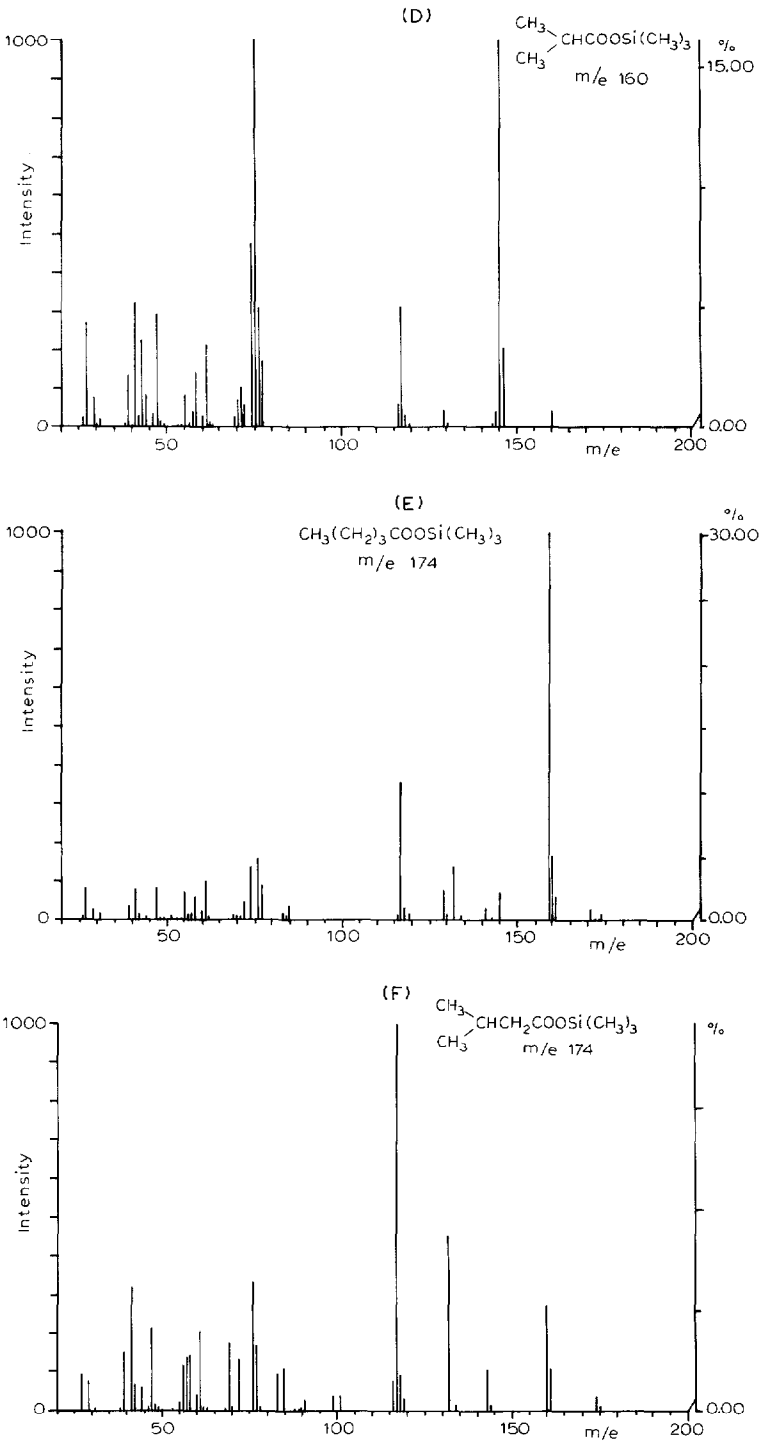

Fig. 5. Mass spectra of TMS-fatty acids.

Detection limit

Volumes of 50 μl–1.5 ml of fatty acid standard solutions (20 μg/ml) were absorbed on glass beads in sampling tubes, to which was added 1 ml of hydrogen chloride-saturated diethyl ether solution precisely to a constant volume. The a 1-μl portion was transferred by microsyringe into the gas chromatograph. When TMS derivatives were analysed, the procedure was the same as above.

Calibration graphs were prepared on the basis of the peak heights of the gas chromatograms thus obtained. Linearity was found for injections in the range 1–30 ng for both the free fatty acids and the TMS derivatives. In the former instance the sensitivity was about 5-fold higher and sharper peaks were obtained than in the latter. The detection limit, when 2 μl were microsyringed, was above 0.5 μg of fatty acids. When 200 l of sample gas were collected, the detection limits were 0.9 ppb of acetic acid, 0.8 ppb of propionic acid and 0.5 ppb of valeric acid. Figs. 6 and 7 show the gas chromatograms of an atmospheric sample in which the TMS derivatives gave more distinct and sharper peaks than the free fatty acids of nearly the same concentrations. The derivatives therefore probably gave smaller errors at low concentrations.

Practical examples

About 200 l of air were sampled in and near a hog farm and a refuse incinerator (a hopper stage) and near a treating furnace in an animal charcoal treatment plant in order to establish the air qualities by the method described the above. The results are given in Table VI.

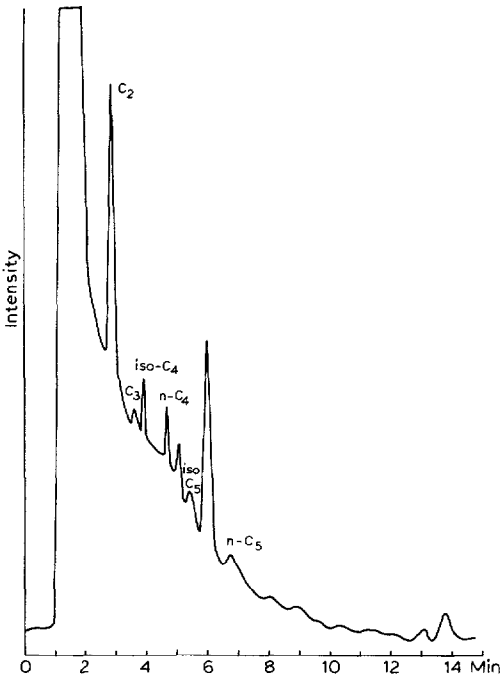

Fig. 6. Typical gas chromatogram of free fatty acids in the atomosphere.

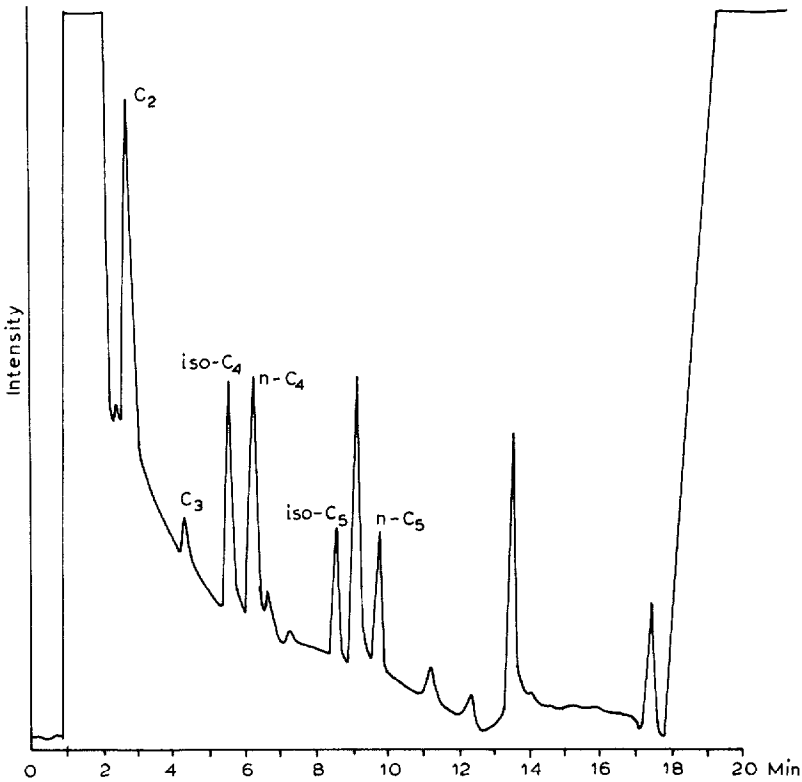

Fig. 7. Typical gas chromatogram of TMS-fatty acids in the atomosphere.

TABLE VI

CONCENTRATIONS OF LOWER FATTY ACIDS IN AIR (ppb)

Conditions: 0°C, 760 mmHg.

Location	C_2	C_3	*Iso*-C_4	*n*-C_4	*Iso*-C_5	*n*-C_5
Hog farm	13	14	0.9	3.1	0.70	<0.50
Refuse incinerator	3.6	5.7	3.2	1.8	0.90	<0.50
Rendering plant	26	20	2.4	3.2	1.0	0.52

CONCLUSION

This study of the GC determination of lower fatty acids in air gave the following results. The lower fatty acids were absorbed on glass beads prior to GC without the need for concentration. The recovery from the glass beads was more than 90%. Air samples could be determined quantitatively as TMS esters with a sensitivity about 5–10-fold higher than that for the free fatty acids by taking contamination with and removal of moisture into consideration. The detection limits were 0.5–1.0 ppb (at 0°C and 1 atm) when 200 l of air were sampled. This GC method is therefore considered to be very useful for the determination of fatty acids in air.

ACKNOWLEDGEMENTS

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