Influences of Operating Conditions on Determination of Fatty Acid Methyl Esters by Gas Chromatography[1]

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ABSTRACT

The compositions of standard mixtures of methyl laurate, myristate, palmitate and stearate were determined by gas liquid chromatography, varying the operating conditions such as sample size, column temperature and flow rate of the carrier gas. The influences of these operating conditions on analytical values were examined. The sample size had a significant influence on the analytical values. The degree of influence was dependent on the column temperature and the composition of the sample. It was also observed that the flow rate of the carrier gas affected the analytical values.

INTRODUCTION

A gas chromatograph with a flame ionization detector is widely used as a reliable instrument for the determination of the composition of mixtures of fatty acid methyl esters. However the accuracy of the analysis is not always satisfactory (1). It is important to know the sensitivity of the detector and the influences of operating conditions of gas liquid chromatography (GLC) on the analytical values for accurate analysis.

Several studies (2-5) have been published of the sensitivity of the flame ionization detector for oxygenated compounds. For example, Ackman (3-5) reported that the greater the ratio of oxygen to carbon in the molecule, the smaller the weight response, and that the effect of oxygen on the weight response was negligible if the carbon number in the molecule was more than eight. Although there are many variables of operating conditions that might have significant influences on the responses of fatty acid methyl esters, sufficient information for practical purpose has not been published.

In this study experiments were carried out by two laboratories independently to obtain the fundamental information on the influences of some operating conditions, using a gas chromatograph with a flame ionization detector and an electronic integrator.

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TABLE I

Reproducibility of Total Integration Count[a,b]

Sample size, μl	0.5	1.0	1.5	2.0
Average total count, x10^5	6.54	12.73	18.25	24.15
Coefficient of variation, %	3.45	3.10	2.50	3.20

[a]Sample: 20% (w/w) fatty acid methyl esters in $CHCl_3$.
[b]Column: 10%-SE 30/Chromosorb W, 4 mm x 1 m, 180 C.

TABLE II

Composition of Standard Samples

Sample	C_{12}-Me, %	C_{14}-Me, %	C_{16}-Me, %	C_{18}-Me, %
I	24.39	39.55	19.69	16.37
II	7.37	55.80	13.43	23.40
III	8.83	22.31	48.33	20.53

EXPERIMENTAL PROCEDURES

Instruments

The types of gas chromatographs and integrators used for this work were as follows: GLC–Laboratory A, Hitachi-063-0050(FID); Laboratory B, Shimazu-4APTF

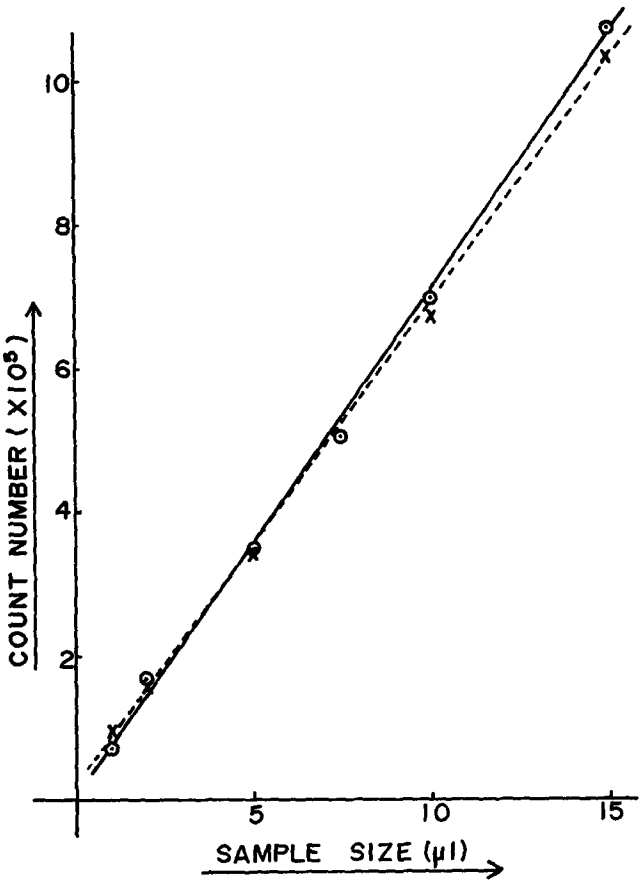

FIG. 1. Relationship between sample size and integration value. Column:20%-DEGS/Chromosorb W, 4 mm x 2 m. Sample: 10% - C_{14} - Me/$CHCl_3$. —O——O— Laboratory A; --X---X- Laboratory B.

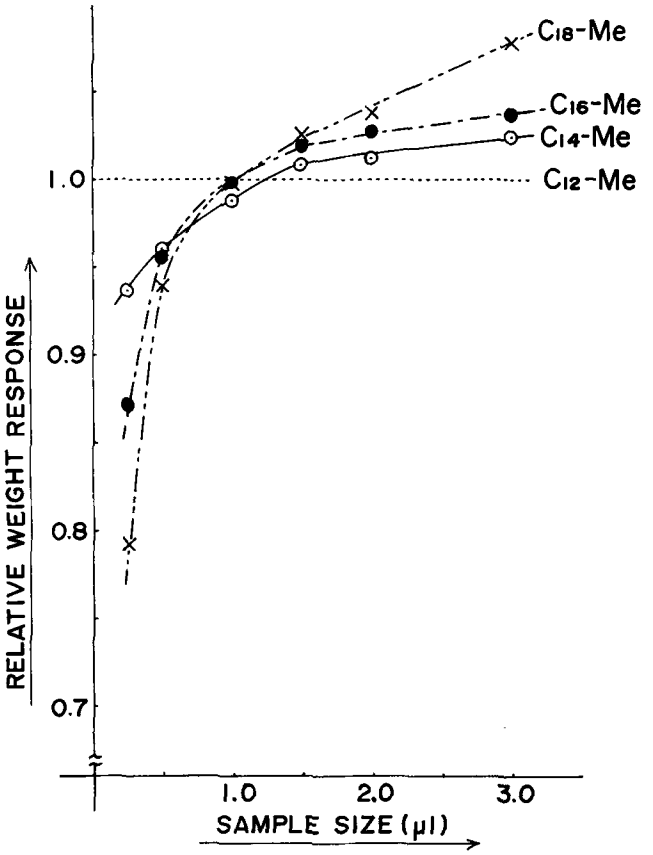

FIG. 2. Influence of sample size on weight response of methyl esters at a column temperature of 155 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m. Attenuation: 8 x 10. Sample II: 20%/$CHCl_3$.

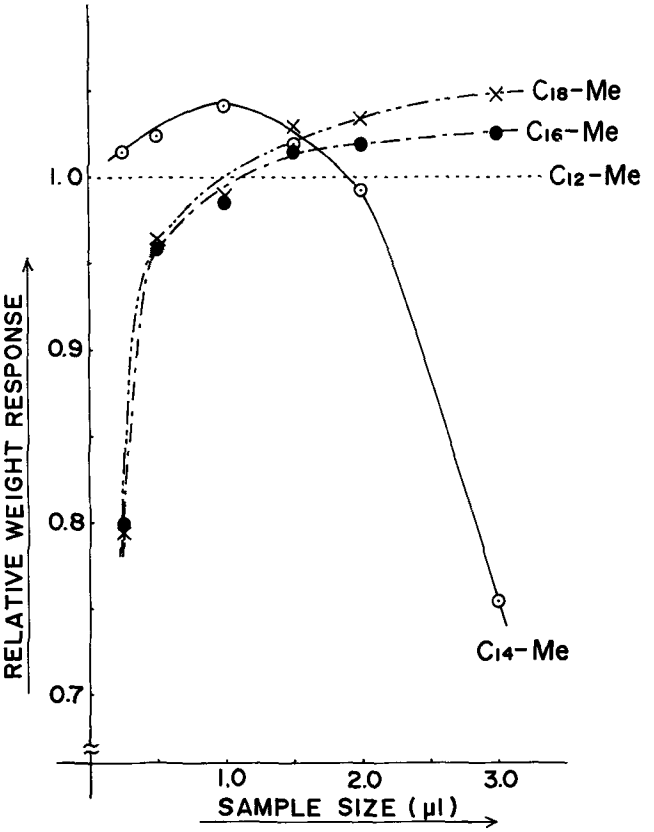

FIG. 4. Influence of sample size on weight response of methyl esters at a column temperature of 170 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m. Attenuation: 8 x 10. Sample II: 20%/$CHCl_3$.

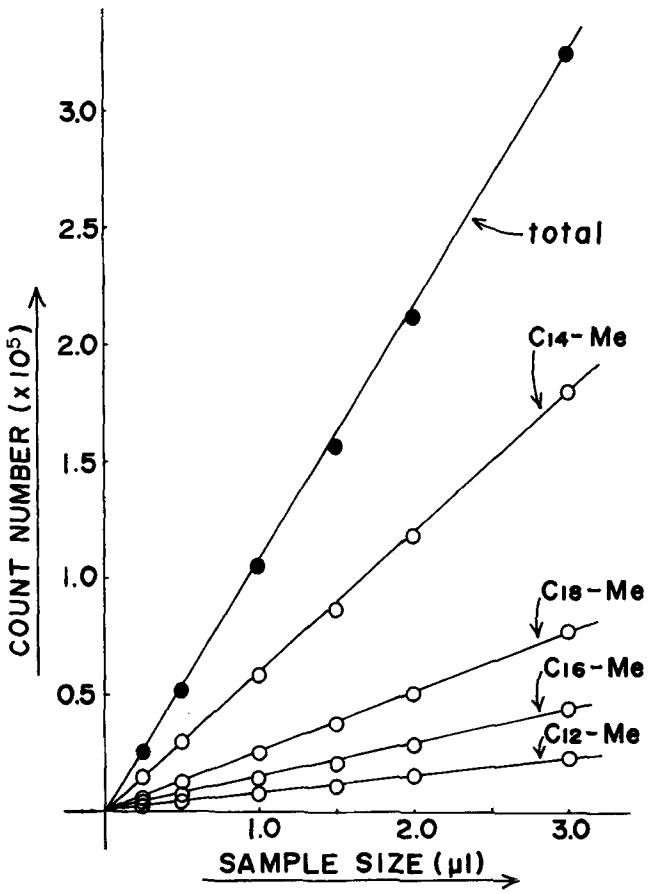

FIG. 3. Relation between sample size and integration count of each component of Sample II at a column temperature of 155 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m.

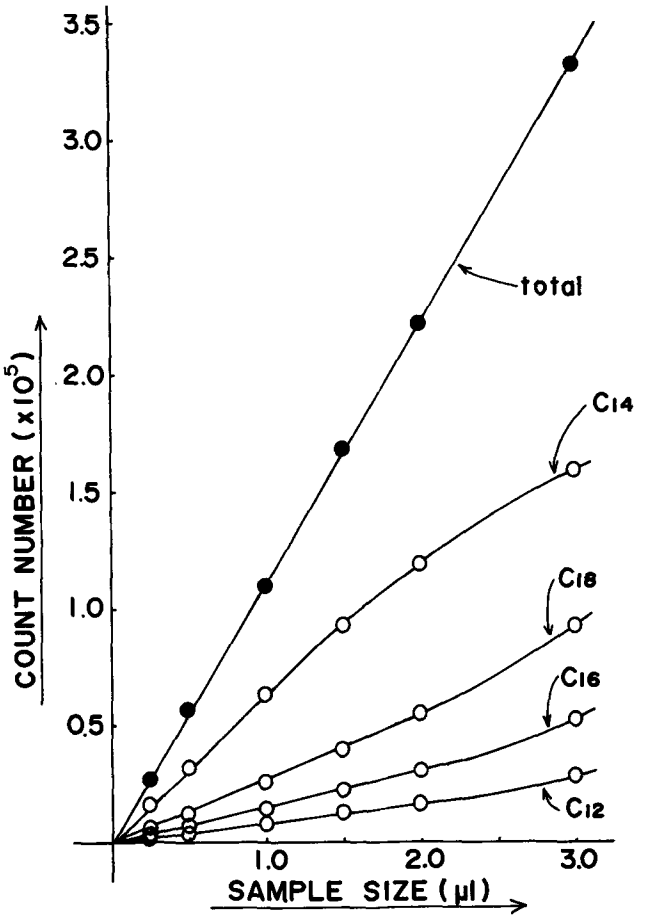

FIG. 5. Relationship between sample size and integration count of each component of Sample II at a column temperature of 170 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m.

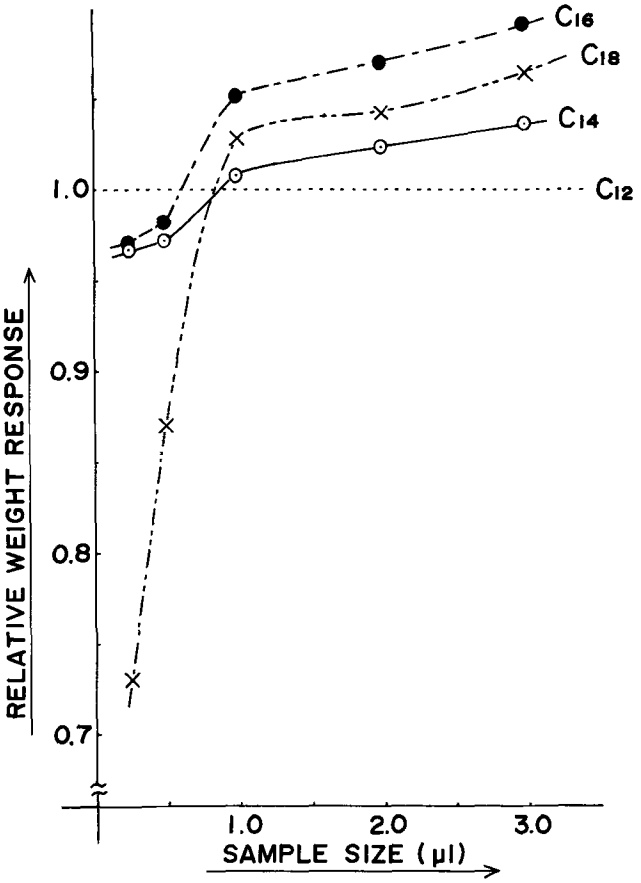

FIG. 6. Influence of sample size on weight response of methyl esters at a column temperature of 155 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m. Attenuation: 8 x 10. Sample III: 20%/$CHCl_3$.

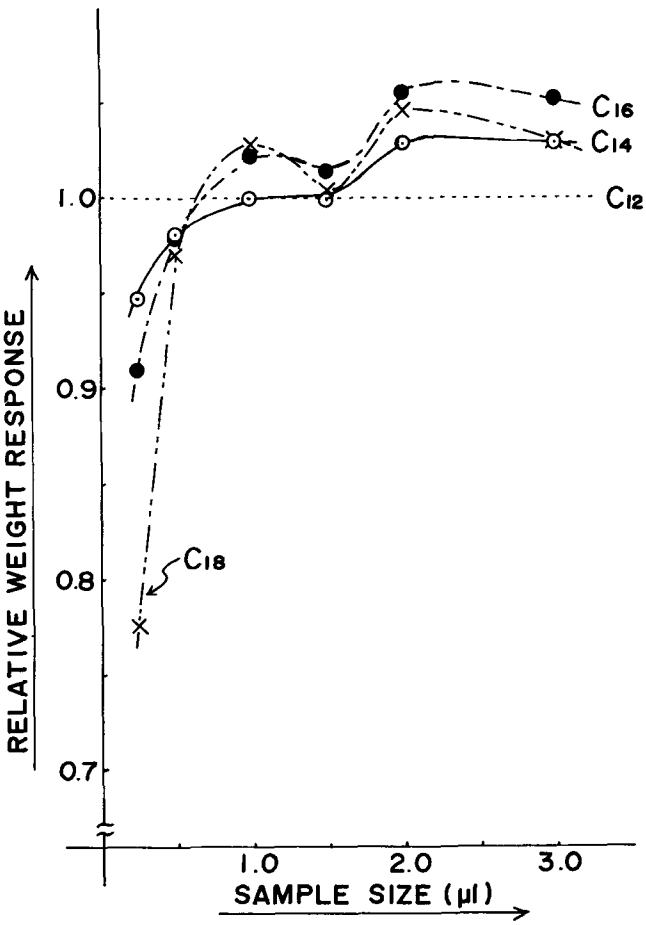

FIG. 7. Influence of sample size on weight response of methyl esters at a column temperature of 170 C. Column: 15%-EGS/Chromosorb W, 4 mm x 2 m. Attenuation: 8 x 10. Sample III: 20%/$CHCl_3$.

(FID); and Integrator—Kent Chromalog II and Infotronics CRS-100.

Micro syringes of 1.0, 5.0 and 10.0 μl capacities were used to inject the sample into the gas chromatograph quantitatively.

Materials

The following materials were packed in 4 mm x 2 m stainless steel tubing and used for GLC columns: 10% SE30/Chromosorb W (60-80 mesh) (Nishio Industry Co., Ltd., Tokyo); 15% EGS/Chromosorb W (60-80 mesh) (Nishio Industry Co., Ltd., Tokyo); and 20%-DEGS/Chromosorb W (60-80 mesh) (Nihon Chromato Industry Co., Ltd., Tokyo).

Methyl esters of lauric, myristic, palmitic and stearic acids were provided by Nippon Oils and Fats Co., Ltd., Tokyo. The purities of these esters were higher than 99.5%. Standard mixtures were prepared by mixing these esters in definite ratios and dissolving these mixtures in chloroform.

Procedures

The reproducibility of total count and the linearity between the sample size and the integration value were checked by injecting a standard sample. The influences of the operating conditions of GLC on the analytical values were examined by analyzing the standard mixtures and varying the operating conditions such as sample size, column temperature and flow rate of the carrier gas. The response of each component in the mixture was compared in terms of relative weight response, which was defined as the ratio of weight response of a component in a mix to that of methyl laurate. The weight response was calculated by dividing the integration count for a component by its known percentage.

The operating conditions of the integrator were fixed throughout the experiments to avoid confusion.

RESULTS AND DISUCSSION

Reproducibility

A 20% (w/w) solution of a standard mixture of four different sample sizes (0.5, 1.0, 1.5 and 2.0 μl) was injected into the gas chromatograph, and the integration count of every peak area was recorded. Five measurements were carried out for each sample size. Average values of the total count numbers and the coefficients of variation obtained in Laboratory A are shown in Table I.

As is evident from the table, the coefficients of variation obtained in the range of sample size examined were of such low values that they were considered insignificant. Similar results were obtained in Laboratory B.

Linearity between Sample Size and Integration Value

The relationship between the sample size and the integration count was examined by using a 10% (w/w) solution of methyl myristate and several columns. Figure 1 shows the results obtained by using a column of 20%-DEGS.

The relation between sample size and integration count was found to be linear in the range of the sample size employed.

Effect of Variation of Sample Size and Column Temperature

The compositions of the standard samples used for these experiments are shown in Table II.

Twenty per cent (w/w) solution of Sample I was analyzed by injecting two sample sizes, one of 0.5 μl and

TABLE III

Analytical Values Obtained by Gas Liquid Chromatography of Different Amounts of Methyl Ester Mixture[a,b]

	C_{12}-Me		C_{14}-Me		C_{16}-Me		C_{18}-Me	
	Content, wt %							
	24.39		39.55		19.69		16.37	
	Sample size, µl							
Laboratory	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.5
Laboratory A	25.98	18.49	40.50	40.76	19.11	22.47	15.59	18.29
Analytical	24.30	19.88	40.41	41.01	19.43	21.52	15.86	17.59
Values, %	24.16	19.44	40.38	40.67	19.46	21.98	16.01	17.91
Average, %	24.81	19.27	40.43	40.89	19.33	21.99	15.82	17.93
CV,[c] %	4.21	3.68	0.15	0.43	1.00	2.16	1.35	1.95
Laboratory B	25.01	21.38	40.88	40.69	19.09	20.99	15.02	16.93
Analytical	25.42	21.69	40.85	40.69	18.92	20.88	14.81	16.94
Values, %	24.66	22.71	41.17	40.77	18.99	20.34	15.17	16.17
Average, %	25.03	21.93	40.97	40.65	19.00	20.74	15.00	16.68
CV[c] %	1.52	3.17	0.43	0.49	0.45	1.68	2.21	2.65

[a]Column: 10%-SE30/Chromosorb W, 4 mm x 1 m, 180 C.
[b]Sample: 20% (w/w) fatty acid methyl esters in $CHCl_3$.
[c]Coefficient of variation.

the other 1.5 µl. Three measurements were performed for each sample size. Each analytical value, the average values and the coefficients of variation are shown in Table III.

The differences between the values obtained by injecting different sizes of the sample were examined statistically for every component. It was found that there were no significant differences between the coefficients of variation, while there were significant differences between the average values of some components.

Figure 2 shows the result obtained by the analysis of Sample II, using the column of 15%-EGS at the column temperature of 155 C. The ordinate of the graph indicates the relative weight response of each component in the sample, which was calculated as mentioned above. When the sample size was 0.25 µl, the weight responses of methyl palmitate and stearate were small compared with that of laurate. The responses of four components were close together from 1 to 2 µl. In this range of sample size, analytical values could be obtained that agreed well with the known percentage. However the response of methyl stearate became considerably larger than that of laurate when the sample size was more than 2 µl.

Figure 3 shows the relationship between sample size and the integration count of each component in Sample II at a column temperature of 155 C. There was good linearity for all components in this sample size range.

Similar experiments were carried out using the same sample and the same column at a column temperature of 170 C.

The result of this experiment is shown in Figure 4. In the range of small sample size, similar tendencies to those at 155 C were observed, i.e., the responses of methyl palmitate and stearate were considerably smaller than that of laurate. Differences between the responses of all components became smaller as the sample size increased. There was a narrow range of the sample size in which the responses of all components were close together. When the sample size was larger than 2 µl, the response of methyl myristate became remarkably smaller than that of laurate.

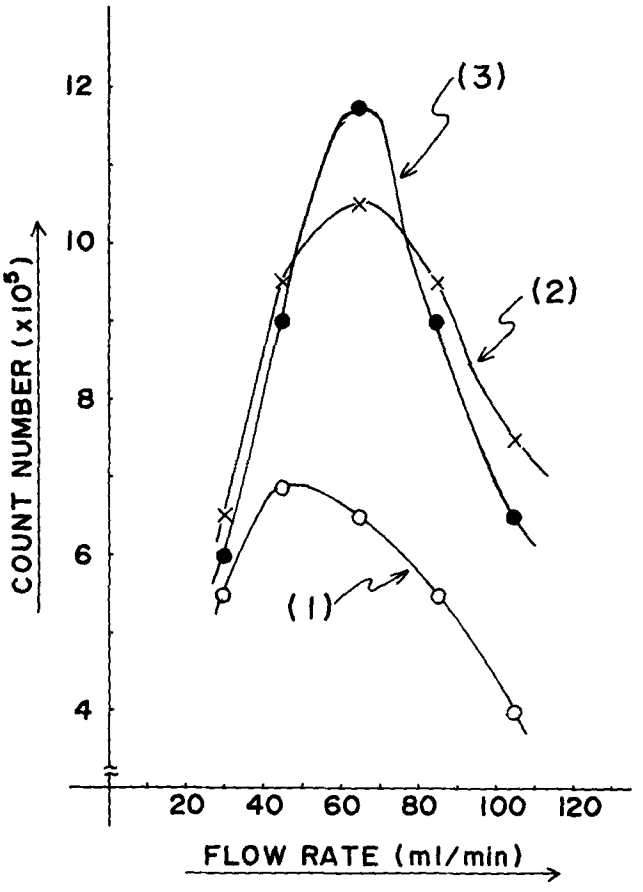

FIG. 8. Influence of carrier gas flow rate. Column: 10%-SE30%Chromosorb W, 4 mm x 2m.

Curve no.	1	2	3
Sample	10%-C_{14}Me-$CHCl_3$	10%-C_{18}-Me/$CHCl_3$	1%-C_{14}-Me/$CHCl_3$
Sample size	1 µl	2 µl	1 µl
Attenuation	6.4×10^2	3.2×10	3.2×10

TABLE IV

Optimum Sample Sizes[a,b]

	Column temperature	
Sample	155 C	170 C
II	1.0-3.0 µl	1.0-1.5 µl
III	1.0-3.0 µl	1.0-2.0 µl

[a]Column: 15%-EGS/Chromosorb W (60-80 mesh), stainless steel 4 mm x 2 m.
[b]Carrier gas: N_2, 40 ml/min.

The relationship between the sample size and the integration count of each component of Sample II at a column temperature of 170 C is shown in Figure 5.

The curve of methyl myristate deviated downward from a straight line in the range of the sample size larger than 1.5 μl, while the curves of the other components deviated upward from straight lines. However no significant deviation was observed in the relation between the sample size and the total count throughout the range examined. The reason for the deviations from straight lines has not been elucidated yet; but for methyl myristate they might be due to the fact that its content was so high and the shape of its peak on the chromatogram was so sharp that it was not integrated completely. Deviation was more prominent at higher column temperature, which supported this reasoning.

The discussion above was supported further by additional experiments using Sample III, which contained a larger amount of methyl palmitate. The results of the experiments at a column temperature of 155 C and 170 C are shown in Figures 6 and 7.

In the range of small sample size, the weight response of methyl stearate was remarkably lower than that of laurate at both column temperatures. However there were no significant differences, among the weight responses of all components in the range of sample size, more than 1 μl at 155 C. Even at 170 C, the response of any component did not vary significantly by increasing the sample size, just as observed in the case of Sample II.

Influence of Flow Rate of Carrier Gas

Three samples—10% solution of methyl myristate, 10% solution of methyl stearate and 1% solution of methyl myristate—were analyzed at various flow rates of the carrier gas. The results are shown in Figure 8.

The flow rate at the maximum integration for the 10% solution of methyl myristate (curve 1) was different from that for the 10% solution of methyl stearate (curve 2). The flow rate at the maximum for the 1% solution of methyl myristate (curve 3) agreed with that for the 10% solution of methyl stearate (curve 2). But the tangents of these three curves at the same flow rate were different. It is believed that, when a mixture of methyl esters is analyzed at a definite flow rate of the carrier gas, the response of each component varies depending on its content and carbon number. However the flow rate of the carrier gas appeared to have some influence on the analytical values; but this has not been examined in detail.

The optimum sample sizes for the 20% solution of the mixture of fatty acid methyl esters under the operating conditions examined are shown in Table IV.

These optimum ranges of sample size should be variable with the other operating conditions or the composition of the sample. Strict care should be taken to the selection of operating conditions for accurate GLC analysis of fatty acid methyl esters.

ACKNOWLEDGMENT

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