Determination of Fatty Acid Composition by Gas Chromatography: I. Analysis with Use of Thermal Conductivity Detector[1]

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ABSTRACT

For the purpose of establishing a standard method for the gas chromatographic determination of fatty acid composition, a collaborative study team has carried out replicate analyses of specified samples using gas chromatographs equipped with thermal conductivity detectors and has examined the entire set of experimental data by a statistical method. From the results of the four collaborative works it was found that deviation of analytical values from exact composition and interlaboratory scattering of data may be considerably decreased by the following means: (a) enlarging the size of narrow peaks (less than 5 mm at a half height) or peaks with low height by adjusting the attenuator range or chart speed; (b) correcting the analytical values by using correction factors determined from analysis of known mixtures having composition similar to that of an unknown sample.

INTRODUCTION

With the purpose of establishing the standard method for the determination of fatty acid composition by gas chromatography, the Gas Chromatography Committee was organized in the Japan Oil Chemists' Society. Since 1967, the group has investigated many problems of fatty acid analysis by gas chromatography.

A large number of reports on the gas chromatographic analysis of fatty acid methyl esters have been published. However there are only a few reports (1) on collaborative analysis of the esters using a gas chromatograph. This paper describes some results of collaborative works undertaken by the Committee to obtain fundamental data to specify the standard method.

The collaborators were industrial, independent, university or government laboratories of fat and oil chemistry.

COLLABORATIVE WORK I

Procedures

The object of the first experiment was to obtain general information concerning the relation between operating conditions of a gas chromatograph and deviation from real value or scattering of the data obtained. For this purpose standard samples A, B, C and D consisting of mixtures of methyl laurate, methyl myristate, methyl palmitate and methyl stearate were prepared. The methyl esters used were at least 99.5% pure.

Use of any type of gas chromatograph with a thermal conductivity detector was permitted. As details were not specified, each collaborator could choose the optimum conditions for the analysis of each sample. Analyses were carried out using ethyleneglycol succinate or diethyleneglycol succinate columns operating at 150-210 C. The chromatograms were obtained without changing attenuator range or chart speed during each analysis. Sample size injected was 0.5-4.0 μl. Peak areas were determined by two different methods. One consisted of drawing lines tangent to the sides of the peak and intersecting the base line, and calculating the area of the resulting triangle by multiplying the height by one-half the base. The other consisted of multiplying the peak height by the width at half height. When the data obtained by both peak area determinations were compared statistically, no significant difference was observed. Therefore, throughout this paper, only the results obtained by the former are presented except for the fourth collaborative work. The percentage content of each component was calculated from the ratio of the area of each peak to the sum of the areas of all peaks. Correction factors were not used. The collaborators were not informed of the exact composition of the standard samples and were requested to present their data for each sample using duplicate analyses. The two trials were carried out on different days.

Results and Discussion

Table I shows averages and coefficients of variation (CV) calculated from the results of the analyses of samples A, B, C and D by 19 collaborators. It was found that the following two types of components in the samples had a tendency to give more widely scattered data: the component that eluates first, such as methyl laurate in samples A and C, and the component that is a minor one in a sample, such as methyl stearate in sample B. The former gives a peak with a narrow width and the latter a peak with a low height, and it is difficult to determine the precise area of

[1]Presented at the JOCS-AOCS Joint Meeting, Los Angeles, April 1972.

TABLE I

Averages and Coefficients of Variation of Analytical Values Obtained in Collaborative Work I, II and III

% and CV	Collaborative work	Sample A		B		C			D			
		$C_{12:0}$	$C_{18:0}$	$C_{12:0}$	$C_{18:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$
$\bar{x}$, %	I	32.7	67.3	91.6	8.4	10.7	30.3	58.9	20.9	31.1	29.6	18.4
	II	32.2	66.8	93.2	6.8	11.0	30.5	58.5	20.9	31.1	29.4	18.7
	III	—	—	91.0	9.0	10.7	30.8	58.5	—	—	—	—
CV	I	5.84	2.84	1.52	16.54	8.72	3.66	2.46	5.64	3.22	3.45	3.73
	II	2.72	1.36	1.01	13.70	6.91	3.75	1.93	5.70	3.38	3.39	3.91
	III	—	—	0.49	4.93	5.79	1.78	1.46	—	—	—	—
Known value, %		30.00	70.00	90.01	9.99	10.18	29.91	59.91	20.00	30.00	30.00	20.00

TABLE II

Correction Factors Determined by Analyzing Samples E and G

Laboratory[a]	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]
1E	0.912	0.952	0.984	1.075	1.091
1G	0.912	0.963	0.980	1.070	1.074
3E	0.983	0.973	0.969	1.029	1.059
3G	0.942	0.980	0.990	1.026	1.082
4E	0.941	0.962	0.973	1.075	1.059
4G	0.890	1.000	1.054	1.087	0.948
5E	0.922	0.967	0.981	1.059	1.085
5G	0.993	0.983	0.980	1.007	1.051
6E	0.898	0.967	0.988	1.059	1.098
6G	0.935	0.958	0.990	1.042	1.107
7E	—	—	—	—	—
7G	0.966	0.980	1.000	1.007	1.082
8E	0.967	0.973	0.988	1.029	1.053
8G	—	—	—	—	—
Known composition of sample, %					
E	17.70	17.80	25.20	21.40	17.90
G	14.50	33.90	9.70	27.40	14.50

[a]E and G show set of correction factors determined by analyzing samples E and G, respectively.

[b]Methyl elaidate.

either peak. The scattering of the data was presumed to be due to difficulty in a precise determination of a peak area.

The components of lower molecular weight, such as methyl laurate, gave higher values than the known content, while those of higher molecular weight, such as methyl stearate, gave lower values. This tendency was presumed to depend on the difference of the instrument response, the molecular weight difference of esters, etc.

COLLABORATIVE WORK II

Procedures

The object of this work was to investigate whether or not the difficulty in precise measurement of a peak area was responsible for the scattering of analytical values. From the 38 chromatograms obtained in the first experimental series for each sample, one chart was arbitrarily chosen and then four exact duplicate Xerox copies of the chosen charts for the four samples were sent to the collaborators to determine each peak area.

Results and Discussion

The results reported by 20 collaborators were treated statistically, and averages and CV are shown in Table I, together with those of the first experimental series. The CV of the data obtained from the second experimental series were generally as large as those obtained from the first one, and a mode of wide scattering of data of the second experiment was similar to that of the first. Therefore it was considered that the data scattering observed in the first experiment was caused mostly by the differences in the measurement of peak areas.

COLLABORATIVE WORK III

Procedures

In the first and second experiments, larger scattering of the analytical values was observed for the components that gave a peak with a narrow width or a low height. To confirm the reasoning considered from the results of second experiment and to reduce such scattering, the following regulations were added in this work: (a) sensitivity—changed to obtain peak heights of at least one-third of full chart span; (b) chart speed—changed to obtain peak widths at half height of at least 5 mm.

Samples B and C, which had given largely scattered data in the two previous series of experiments, were analyzed collaboratively in this work.

Results and Discussion

The results obtained by nine collaborators were treated in the same way as were the results of the first and second experiments, and the treated results are shown in Table I. Comparing the results of the third experiment with those of the first one (which had been performed without enlarging a peak height or a peak width) showed that the CV of the analytical values of samples B and C were reduced remarkably in the third experiment. It was confirmed that enlarging a peak size by adjusting attenuator range or chart speed was effective on reducing of scattering of analytical values.

COLLABORATIVE WORK IV

Procedures

In the series of experiments described above, the response of each methyl ester was disregarded in the calculation of each sample composition. Researchers such as Horrocks et al. (2), Craig and Murty (3) and Pons and Frampton (4) have reported the use of response correction factors in the works carried out in a single laboratory. In our experiment the effect of correction was examined from the result of the collaborative work.

Standard samples E, F, G and H, compositions of which are shown in Table II or III, were used for the analyses. The composition of sample E was not close to that of sample F, while the composition of sample G was close to that of sample H.

Eight collaborators participated in this work and, as they

TABLE III

Comparison of Corrected and Noncorrected Data

Sample	% and CV		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[a]
F	Known value, %		5.3	10.4	52.6	21.2	10.5
	$\bar{x}$, %	Noncorrected	5.6	10.7	53.3	20.6	9.8
		Corrected (E)[b]	5.2	10.3	52.1	21.8	10.6
	CV	Noncorrected	3.6	1.6	1.2	1.7	3.5
		Corrected (E)[b]	4.0	1.4	1.6	2.2	2.8
H	Known value, %		16.2	32.5	10.9	25.7	14.7
	$\bar{x}$, %	Noncorrected	17.3	32.9	11.0	24.3	14.5
		Corrected (E)[b]	16.3	31.7	10.7	25.5	15.7
		Corrected (G)[c]	16.3	32.1	11.0	25.3	15.3
	CV	Noncorrected	5.1	1.1	3.2	3.9	4.7
		Corrected (E)[b]	7.5	1.0	4.4	3.5	4.5
		Corrected (G)[c]	2.8	0.9	1.9	1.9	1.0

[a]Methyl elaidate.

[b,c]Corrected by set of correction factors obtained from analytical values of samples E and G, respectively.

were not informed of the exact composition of these four samples, the authors calculated two sets of correction factors for every laboratory from the analytical values of E and G reported by the collaborators.

The specified operating conditions concerning peak size control were the same for this experiment as for the third series of experiments. Peak areas were measured by multiplying the peak height by the width at half height.

Results and Discussion

The two sets of correction factors determined by analysis of E and G, which were different in composition, are illustrated in Table II. Throughout five methyl esters, good agreement between the two correction factors for each methyl ester was observed for laboratory 1 (Table II), while considerable differences between them were seen for laboratory 4. Although the two correction factors did not always agree throughout all the components in the sample, it was generally observed that the correction factor became greater with the increase of the number of carbon atoms in the ester. This observation is compatible with the results of the first experiment.

The set of correction factors from sample E was applied to the determination of sample F, the composition of which was not close to that of sample E, and the set obtained from sample G to the determination of sample H, which had a composition similar to that of sample G. The averages and CV of the corrected and noncorrected data for samples F and H are shown in Table III. The CV of the corrected data for sample H were considerably smaller than those of the noncorrected data, while those for sample F were as large. The averages of the corrected analytical values were closer to the exact composition than the noncorrected ones in determination of both samples F and H.

Moreover the two sets of correction factors from samples E and G were applied to a determination of one sample, H, and the resulting two sets of corrected data were compared. In this case only the results reported by the five laboratories that analyzed three samples, E, G and H, using same instrument and column, were treated. Table III shows the averages and CV of the two sets of corrected data, which are abbreviated as corrected (E) and corrected (G), where (E) and (G) show the reference samples used to obtain the correction factors, respectively. The CV of the corrected (G) were considerably smaller than those of the noncorrected, while such an apparent decrease in CV was not observed for the corrected (E).

Comparing the averages of the noncorrected and the two sets of the corrected analytical values with the known composition, both of the corrected values were apparently closer to the actual composition than the noncorrected, and the corrected (G) was the closest. From the results of this experiment, it was found that correction factors of methyl esters of fatty acids varied with laboratory and sample composition in addition to molecular weight of esters. However both the deviation of analytical values from the exact composition and the interlaboratory scattering of the data were considerably decreased by applying the correction factors.

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