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Notes on Methodology

Quantitative gas-liquid chromatography of fatty acid methyl esters with the thermal conductivity detector*

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► Thermal conductivity detectors (katharometers) are widely used in gas-liquid chromatography (GLC). In the analysis of fatty acid methyl esters, composition is often estimated from the peak area response when a katharometer is used (1, 2, 3). However, peak area is not a simple function of concentration expressed in mole per cent or weight per cent (4). Messner *et al.* (5) recently demonstrated that the relative peak area, area per mole of component divided by area per mole of standard, is a function of molecular weight and structure. In the present investigation, the relative katharometer response for a number of fatty acid methyl esters in two homologous series has been calculated by the method of least squares from experimental data. These correction factors were used in the analysis of known mixtures and in the recalculation of published experimental data on known mixtures in order to demonstrate their value in quantitative GLC analysis. Calculated correction factors were compared with a theoretical relationship, employing molecular weights.

Methyl butyrate, octanoate, and decanoate were purchased.[1] Other methyl esters were prepared in this laboratory by fractional distillation and low temperature crystallization. Methyl linolenate was further purified by chromatography on silicic acid. The purity of *cis*- and *trans*-unsaturated esters was demonstrated by infrared spectra. All samples were over 99% pure on the basis of GLC examination.

An Aerograph A-90-C gas chromatograph[2] with a Wheelco 1 millivolt recorder[3] and a 5-foot column containing G.E. SF-96 silicone resin and Chromosorb in a 1:3 ratio[2] was used. A silicone resin was selected to avoid the possibility of losses by reactions such as esterification. The temperature of the column and katharometer varied from 208° to 228°, and filament current from 200 to 250 milliamps. for different runs. Helium was the carrier gas. Peak areas were measured with a Wheelco Type A electronic integrator[3] with a maximum counting rate of 2500 cpm. The chart was run at 180 inches/hour so that the counts per peak ranged from about 60 for methyl butyrate to about 2500 for methyl behenate. These conditions were chosen in order to minimize errors in measurement of peak areas (6).

Known mixtures of the methyl esters were prepared and chromatographed. The area of each peak was compared with the area of a standard peak. Methyl palmitate, the internal standard, was assigned a calibration factor of 100. Esters of C_{14}- and C_{18}-fatty acids were compared with a methyl decanoate standard to ensure against any overlapping of peaks. Calibration data are summarized in Tables 1 and 2. The method of least squares (7) gave the following equation for relative molar response in the saturated methyl ester series:

$$R = 24.68 + 5.79\,N - 0.075\,N^2$$

where R is the relative molar response compared with methyl palmitate and N is the number of carbon atoms in the fatty acid. Values for relative response obtained from this equation were multiplied by 1.019, a factor that corrected the calculated response of methyl palmitate to 100. The effect of *cis* double bonds in the C_{18} series was calculated by the method of least squares from the experimental data in Table 2:

$$R' = 92.84 - 1.865\,D - 0.175\,D^2$$

where R' is the relative mass response and D is the number of *cis* double bonds. Values obtained for relative mass response from this equation were multiplied by the factor 1.04 in order to obtain an equivalent relative response for methyl stearate in the saturated and unsaturated series. The effect on relative response by a *trans* double bond did not correspond to the effects obtained with *cis* compounds.

Calibration factors were shown to improve the accuracy of GLC analysis. When two known methyl

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[1] Eastman Kodak Co., Rochester, N. Y.

[2] Wilkens Instrument and Research, Inc., Walnut Creek, Calif.

[3] Barber-Colman Co., Rockford, Ill.

TABLE 1. RELATIVE RESPONSE OF SATURATED METHYL ESTERS IN THE KATHAROMETER DETECTOR

Ester	Relative Response				
	Experimental Mass*	Experimental Molar	Calculated† Mass	Calculated† Molar	Theoretical‡ Molar
Acetate (7)§	128 ±7	35	133.8	36.6	42.2
Butyrate (11)	121 ±14	46	125.9	47.5	52.3
Hexanoate			120.1	57.8	61.4
Octanoate (23)	114 ±9	67	115.3	67.5	70.0
Decanoate (12)	108 ±6	74	111.1	76.5	78.0
Laurate (17)	105 ±5	83	107.2	85.0	85.6
Myristate (11)	102.6 ±3.9	92	103.5	92.8	93.0
Palmitate			100.0	100.0	100.0
Stearate (10)	93.7 ±5.1	103	96.6	106.6	106.8
Arachidate (16)	91.0 ±6.0	110	93.2	112.6	113.4
Behenate (23)	88.3 ±5.0	116	90.0	118.0	119.8

* Relative mass response ± standard deviation of observation.
† Calculated from equation: $R = 24.68 + 5.79\ N - 0.075\ N^2$.
‡ Calculated from relationship: $(M_1/M_2)^{2/3}$.
§ Figures in parentheses represent the number of experiments.

ester mixtures were examined by GLC (Table 3), the percentage composition calculated from uncorrected areas was elevated for low molecular weight esters and decreased for high molecular weight esters. These errors were corrected by the relative response factors. Although many quantitative studies are concerned only with higher molecular weight esters, for which differences in relative response are not large (Table 1), GLC analyses of known higher molecular weight ester mixtures reported in the literature (2, 3) were improved by their recalculation with the relative response factors obtained in this study (Table 4). The percentage composition obtained for the lower molecular weight esters was decreased, while the percentage composition obtained for the higher molecular weight and unsaturated esters was increased by the calibration factors. The data of Herb *et al.* (2) were obtained with the katharometer at 235° to 240°, and an unspecified filament current while Craig and Murty (3) operated their detector at 205° with filament currents between 160 and 200 milliamps. Since the GLC analyses described in these two studies (Table 4) were improved, even though the instruments and operating conditions were different from the present investigation, the calibration factors reported in this investigation appear to have a general application.

TABLE 2. RELATIVE RESPONSE OF C_{18} UNSATURATED METHYL ESTERS IN THE KATHAROMETER DETECTOR

Ester	Relative Response	
	Experimental Mass*	Calculated† Mass
Stearate (10)‡	93.7 ± 5.1	96.6
Oleate (26)	88.2 ± 6.2	94.5
Linoleate (25)	91.0 ± 6.6	92.0
Linolenate (11)	84.8 ± 5.6	89.2
Elaidate (22)	93.2 ± 6.8	

* Relative mass response ± standard deviation of observation.
† Calculated from the equation: $R' = 92.84 - 1.865\ D - 0.175\ D^2$.
‡ Figures in parentheses represent the number of experiments.

TABLE 3. EFFECT OF RELATIVE RESPONSE ON THE COMPOSITION OF KNOWN METHYL ESTER MIXTURES DETERMINED BY GAS-LIQUID CHROMATOGRAPHY

Ester	Composition (Weight Per Cent)				
	Known	Found*	Per Cent Error	Corrected†	Per Cent Error
Butyrate	4.6	5.9	28.3	4.7	2.2
Octanoate	9.9	11.5	16.2	10.0	1.0
Laurate	10.5	11.3	7.6	10.5	0
Palmitate	25.2	24.7	2.0	24.7	2.0
Arachidate	49.8	46.6	6.4	50.0	0.4
Decanoate	11.0	12.2	10.9	10.6	3.6
Myristate	22.8	24.6	7.9	23.0	0.9
Stearate	20.5	20.7	1.0	20.7	1.0
Behenate	45.7	42.5	7.0	45.7	0

* Calculated from uncorrected areas.
† Corrected for relative response.

Hinkle and Johnsen (8) calculated from empirical data that the molar response in a katharometer is proportional to the vapor density raised to the two-thirds power. Since the density of a gas is very nearly proportional to molecular weight, it follows that:

$$\frac{R_1}{R_2} = \left(\frac{M_1}{M_2}\right)^{2/3}.$$

TABLE 4. EFFECT OF RELATIVE RESPONSE ON THE COMPOSITION OF KNOWN METHYL ESTER MIXTURES DETERMINED BY GAS-LIQUID CHROMATOGRAPHY AND REPORTED IN THE LITERATURE

Ester	Composition (Weight Per Cent)				
	Known	Found*	Per Cent Error	Corrected†	Per Cent Error
Palmitate‡	24.9	27.0	8.4	25.9	3.6
Stearate	25.0	25.3	1.2	25.1	0.4
Oleate	25.0	24.8	0.8	25.1	0.4
Linoleate	25.1	22.9	8.8	23.9	4.4
Myristate‡	5.9	6.4	8.5	5.9	0.0
Palmitate	11.8	13.8	16.9	13.0	10.2
Stearate	19.1	20.9	9.4	20.4	6.8
Oleate	23.3	23.8	2.1	23.8	2.1
Linoleate	13.3	12.5	6.0	12.9	3.0
Linolenate	15.3	12.9	15.7	13.7	10.5
Erucate	11.2	9.6	14.3	10.3	8.0
Palmitate§	6.0	6.8	13.3	6.3	5.0
Stearate	5.2	5.1	1.9	4.9	5.8
Oleate	19.1	20.0	4.5	19.6	2.6
Linoleate	58.7	58.6	0.3	59.1	0.7
Linolenate	11.0	9.6	12.7	10.0	9.1
Palmitate§	5.5	6.4	16.4	5.9	7.3
Stearate	5.6	6.0	7.1	5.7	1.8
Oleate	19.7	21.0	6.6	20.3	3.0
Linoleate	10.5	10.1	3.8	10.1	3.8
Linolenate	58.7	56.5	3.8	58.0	1.2

* Calculated from uncorrected areas.
† Corrected for relative response.
‡ Analysis reported by Herb *et al.* (2).
§ Analysis reported by Craig and Murty (3).

Relative molar responses for saturated methyl esters were calculated from this relationship (Table 1) and found in close agreement with experimental values.

The same molecular weight relationship is obtained if it is assumed that the relative katharometer response in a homologous series is proportional to the collision frequency between molecules of the sample gas in the detector, and that differences in heat transfer per collision are not large for compounds in a homologous series. The collision frequency of a gas is proportional to the average area, r^2, where r is the radius of the molecule and equal to:

$$\left(\frac{3M}{4\pi d}\right)^{1/3}$$

and *d* is the density in the solid state (9). Since densities for long-chain fatty acid esters are nearly equal, the relative frequency of collision for equal molar quantities of two sample gases is proportional to:

$$\left(\frac{M_1}{M_2}\right)^{2/3}.$$

Thus differences in relative response for a homologous series may be caused by differences in molar volume and therefore collision frequency. The anomalous relative response found in the unsaturated C_{18} homologous series with the introduction of a *trans* double bond (Table 2) can then be explained by the negligible effect of a *trans* double bond on molar volume. Deviations for low molecular weight compounds in the homologous series (Table 1) can result from the assumptions made in approximating molar volume or significant differences in heat transfer per collision with the sensing element.

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