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REPORT

GAS CHROMATOGRAPHY OF C_{18}-SATURATED AND UNSATURATED FATTY ACID METHYL ESTERS

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Using diethylene glycol succinate polyester as the stationary liquid phase of gas chromatography, the influence of various factors on the separation of methyl stearate and oleate peaks was investigated with respect to both a thermal conductivity detector and hydrogen flame ionization detector. Our experiments produced the following results. The degree of polymerization of the polyesters influenced the column efficiency only slightly over a molecular weight range of 2,700 to 5,400. In contrast, the influence of the technique for supporting the polyesters on the solid supporters was considerable, and the type of supporter used had the greatest influence upon column efficiency. When the column length was increased, the number of theoretical plates increased but the degree of resolution hardly increased at all.

In addition, the relative responses in the flame ionization detector were investigated for methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids. When their contents were large, the relative responses indicated a very close correlation between the percentage of peak areas and weight percent. However, when their contents were small, there was a tendency for the percentage of peak areas of methyl stearate and oleate to be higher than the known weight percent, while the percentage of peak areas of methyl linoleate and linolenate tended to be lower than the known weight percent.

1. FOREWORD

The process of analyzing fatty acid methyl esters by gas chromatography has undergone very rapid advances, to the extent that it has become an indispensable process in the analysis of oil fat. It has already been adopted as a Tentative Method$^{1)}$ of A.O.C.S., and is in very common use, but a number of problems still remain as far as the selection of the column to be used and the operating conditions is concerned. As the stationary liquid phase of the column, polyesters are in most common use, and although many are available on the market, being polymeric, it is difficult to obtain a uniform product, with the result that differences arise in efficiency, and in practise, when this type of column is used, incomplete separation of methyl stearate and methyl oleate has been encountered, which fact has also been cautioned against in the A.O.C.S. method. While there are many reference materials$^{2)-4),6)}$ on the synthesis of polyesters and column preparation techniques, because of the variances in their respective conditions, no relative comparison of efficiency can be made.

On the point of quantitative analysis, relative response is also an important factor, and while many reports$^{5)}$, both theoretical and experimental, exist as far as thermal conductivity detectors (hereafter abbreviated as TCD) are concerned, it is possible to see considerable differences$^{6),7)}$ with respect to methyl linoleate and methyl linolenate, whereas insofar as flame ionization detectors (hereafter abbreviated as FID) are concerned, there are reports$^{8)-11)}$ on saturated acid methyl esters but very few on unsaturated acid methyl esters.

With the complete separation of methyl stearate and methyl oleate in mind, and using diethylene glycol succinate polyester most commonly recommended in the A.O.C.S. method as the stationary liquid phase, the authors undertook a series of experiments to ascertain the influence of such factors as the degree of polymerization of the polyester, column preparation technique, column temperature, type of supporter used, degree of granulation, concentration of the stationary liquid phase, and column length, on the number of theoretical plates of methyl palmitate and on the separation of methyl stearate and methyl oleate, in order to investigate and evaluate the efficiency of the column itself. Additionally, the relative responses of the methyl esters of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were obtained and examined with respect to FID.

2. EXPERIMENT

2.1 Synthesis of Polyesters (Stationary Liquid Phase)

This was performed in accordance with the method put forward by Carothers12) et al. That is, diethylene glycol (for chemical use) amounting to 5% more than its equivalent quantity was added to succinate (extra high-grade reagent), and this was allowed to react for 3 hours under normal pressure while N_2 was blown in at 180°C. Then, under a reduced pressure of 2 mm Hg, this was heated to 230°C under a N_2 gas stream, obtaining products of varying degrees of polymerization by regulating the duration of heating at 230°C. Dissolving the resultant product in 5 parts of chloroform, and adding 4 parts of ether, this was left overnight at room temperature (approximately 20°C) to undergo fractional precipitation, taking the undissolved portion with the low molecular section removed as the polyester.

The molecular weight was determined with a Mechrolab Vapor Pressure Osmometor Model 301A using chloroform as the solvent.

2.2 Preparation of the Column Stationary Liquid Phase

As the stationary phase supporters, the 5 types of commercially sold supporters shown in Table 1 were used directly.

Table 1. Types of Solid Supporters

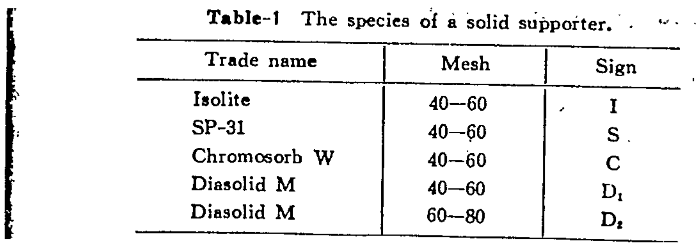


Table-1 The species of a solid supporter.

Trade name	Mesh	Sign
Isolite	40—60	I
SP-31	40—60	S
Chromosorb W	40—60	C
Diasolid M	40—60	D_1
Diasolid M	60—80	D_2

To each of the supporters, the respective weight percent of polyester (10% chloroform solution) was added, and after thoroughly mixing, the chloroform was evaporated at room temperature to yield the stationary phase.

Filling the column with the stationary phase, this was used after heating for 8 hours at 230°C while passing H_2 at 100 ml/min.

2.3 Fatty Acid Methyl Esters

Methyl palmitate: After re-crystallizing commercial palmitic acid with 90% ethanol, it was esterified with methanol (1% sulphuric acid), and repeating distillation at reduced pressure, the distilled fraction at bp_2 146-147°C was used.

Methyl stearate: After re-crystallizing commercial stearic acid with 95% ethanol, it was esterified with methanol (1% sulphuric acid), and repeating distillation at reduced pressure, the distilled fraction at bp_2 165-166°C was used.

Methyl palmitate and methyl stearate both indicated a single peak under gas chromatography.

Methyl oleate: Fatty acid methyl ester of camellia-oil was fractionated by the urea adduction process and by repeated distillation at reduced pressure, and the distilled fraction which showed a single peak under gas chromatography at bp_2 163-164°C was used. No absorption was evident at 10.3 u of the infra-red absorption spectrum (liquid film: NaCl), nor were any trans-isomers present.

Methyl linoleate: Fatty acid methyl ester of safflower oil was obtained as a supplement from the filtrate from which saturated acids and oleic acid were completely removed by the urea adduction process, and then by distillation under reduced pressure, the distilled fraction at bp_2 162-163°C was used. The co-functional dienoic acid content by ultra-violet absorption spectrum was 0.3%, but no absorption was evident at 10.3 u of the infra-red absorption spectrum (liquid film: NaCl), nor were any trans-isomers present. Under gas chromatography, an almost symmetrically balanced single peak was revealed, indicating no shoulders or such due to co-functional dienoic acid.

Methyl linolenate: De-bromiding, esterifying and distilling under reduced pressure the ether-insoluble bromide compound obtained from linseed oil fatty acid, and fractionating by repeating the urea adduction process, it was refined by aluminium column chromatography. The co-functional dienoic acid by ultra-violet absorption spectrum was 1%, while co-functional trienoic acid was 0%. A very slight absorption was indicated at 10.3 u of the infrared absorption spectrum (liquid film: NaCl), but the main peak under gas chromatography was almost symmetrically balanced and no shoulders or such due to isomers could be seen, so that the influence of isomers was disregarded. The composition determined by gas chromatography was 0.2% oleic acid, 1.1% linoleic acid, and 98.7% linolenic acid, and corrections were made to the calculation of relative responses.

2.4 Gas Chromatography

Two types of apparatus were employed under the following respective conditions.

Shimazu GC-1B, TCD detector, stainless U-shaped column of 6 mm inner diameter, carrier gas H_2, sensitivity 2 mV full scale.

Hitachi KGL-2B, FID detector, stainless W-shaped column of 4 mm inner diameter, carrier gas N_2, hydrogen, air 1.2 kg/cm^2, diminishing rate x 10^2, x 3.

The inlet temperature for both apparatus was set at 300°C, and the test sample quantity was regulated to produce a recorder response of 75-100, while the carrier gas flow volume was regulated so that the retention time (R_V) of methyl palmitate would be 5-6 minutes.

2.5 Efficiency Test

Using an ether solution of methyl palmitate, methyl stearate and methyl oleate combined in equal parts as the test material, a 1.5 m GC-1B

Fig.

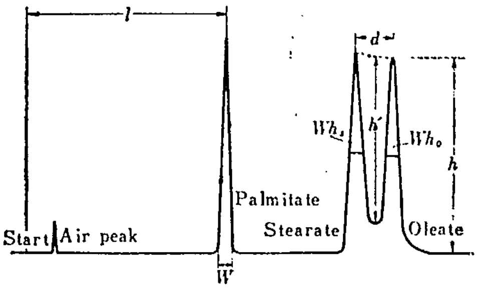


Fig.-1 Terms used in calculating of the number of theoretical plates (n), the resolution (R), and the percent resolution (R_u).

column and a 2 m KGL-2B column were filled with their respective stationary phases, and performing analysis 5 to 7 times with the same column, the number of theoretical plates (n) of methyl palmitate and the resolution (R) and percent resolution (R_u) of methyl stearate and methyl oleate were obtained and compared. By the resolution alone, it is sometimes difficult to express the degree of separation fully, and so it was supplemented with percent resolution which takes the depth of the trough into account.

From Fig. 1, these were obtained using the following equations:

$$n = \left(\frac{4l}{w}\right)^2$$

$$R = d - (Wh_s + Wh_o)$$

$$R_u = \frac{h'}{h} x 100$$

2.6 Relative Response

After accurately weighing the methyl esters of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, and formulating 5 different mixtures, analysis was performed 5-7 times each with respect to FID, and the area % and relative mass response were determined by the half-width method.

3. RESULTS AND CONSIDERATIONS

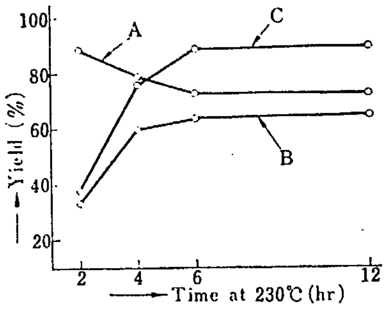
3.1 Influence of the Degree of Polymerization of the Polyester

Heating at 230°C under a reduced pressure of 2 mm Hg, different products were obtained after 2 hours, 4 hours, 6 hours and 12 hours heating, and the yield rate of polyesters whose solvents were fractionated is shown in Fig. 2.

Using stationary phases where 20 wt % of these polyesters were supported on supporter C, efficiency tests were performed with TCD, the results of which are shown in Table 2.

On the synthesis of polyesters, a number of reports has thus far been published, but there is hardly any which touches upon the degree of polymerization or the mean molecular weight of the polyester used, only Lugay et al[13) mentioning merely that the molecular weight of the polyester used in analyzing rice-bran oil was approximately 4000.

In this study, the variation in heating time at 230°C under reduced pressure, without a catalyzer, produced only a slight effect within the molecular weight range of 3000-5000 for a 2-12 hour interval, when the low molecular section was removed by fractional precipitation. The molecular weight of the resultant high molecular section was seen to increase with time, as did the yield rate, but they seemed to generally level off after 6 hours. Even after 2 hours, the efficiency was good, but problems arise in respect of the life of the column and yield rate, while after 12 hours the efficiency showed only a slight decrease, with the result that 4-6 hours is thought to be most appropriate.



A : Product/raw material × 100
B : Polyester/raw material × 100
C : Polyester/product × 100

Fig.-2 Yields of Polyesters.

Table 2. Influence of the Degree of Poly-

Table-2 The influences of the degree of polymerization of polyesters.

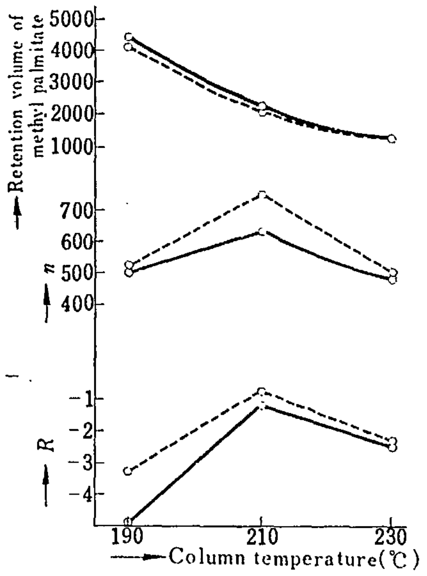
Polyester	Heating time at 230°C (hr)	*n*	*R*	Mean molecular weight
P_1	2	480	−2.8	2300
P_2	4	490	−2.6	3000
P_3	6	480	−2.5	5300
P_4	12	460	−4.9	5400

3.2 Influence of Column Preparation Technique

Dividing polyester P_2 into two parts, and using 2 stationary phases A and B where these were supported separately on supporter C from the same lot, an efficiency test was performed with TCD, the result of which is shown in Table 3 along with the result of an FID test where two columns C and D were filled separately with the same stationary phase in which P_2 was supported on supporter D_2.

Table-3 The influences of column preparation.

	Retention volume	n	R	$R_н$
A		452	−2.2	
B		499	−2.5	
C	783	1400	−0.6	86
D	734	1411	−0.4	86



--- P_1 polyester ...... P_3 polyester

Fig. 3 The influence of the column temperature.

er

re

The difference between A and B reflects the effect of technique (341) used in supporting the stationary liquid phase, while the difference between C and D reflects the effect of filling technique, and although both are commonly said to require some knack, as far as the effect on efficiency is concerned, the process of supporting the liquid phase (for example solvent quantity, drying, etc.) seems to be more problematical than the technique of filling the column, and thus requires further investigation.

3.3 Influence of Column Temperature

Using columns in which 20 wt % of P_1 and P_3 was supported on supporter C, comparisons were made with TCD for column temperatures of 190, 210 and 230°C. The temperature of the detector was set 30°C higher than the column.

The results are shown in Fig. 3.

The retention volume decreased as the temperature rose, with a resultant reduction in analysis time, but from the aspect of efficiency, it would seem that the vicinity of 210°C is best. At any of the temperatures the heating time was long, and P_3, whose mean molecular weight is high, is superior to P_1.

3.4 Influence of the Supporter

Together with the stationary liquid phase, the selection of the supporter has a great influence on column efficiency. On the 5 commercial supporters, 20% of the same polyester was supported, and upon comparing their efficiency with respect to both TCD and FID, the results which are shown in Table 4 were obtained.

Table 4. Influence of the Solid Supporters

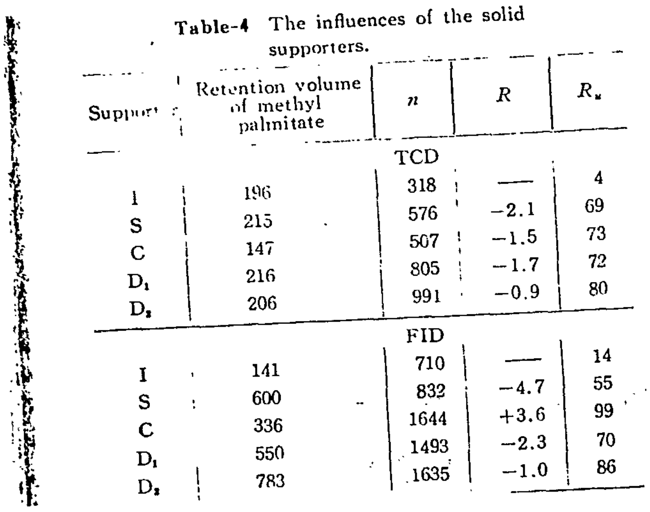


Table-4 The influences of the solid supporters.

Support	Retention volume of methyl palmitate	n	R	R_v
		TCD		
I	196	318	—	4
S	215	576	−2.1	69
C	147	507	−1.5	73
D_1	216	805	−1.7	72
D_2	206	991	−0.9	80
		FID		
I	141	710	—	14
S	600	832	−4.7	55
C	336	1644	+3.6	99
D_1	550	1493	−2.3	70
D_2	783	1635	−1.0	86

With TCD, the efficiency is seen to decrease in the order D_2, C, D_1, while with FID this order is C, D_2, D_1. The use of S and I under the conditions of this experiment is impossible. With D_1 and D_2, the R_V is large and the analysis time becomes long, making them somewhat unsuitable. With any of the supporters, some sort of treatment is already thought to have been done, but nevertheless the effect of the supporter on the efficiency is extremely large and thorough investigations are necessary. The difference between D_1 and D_2 is one of degree of granulation, and the smaller the grain the better the efficiency, but then the problem of longer analysis time arises.

3.5 Influence of the Concentration of the Stationary Liquid Phase

Using stationary phases where 20, 10, 5 and 1 wt % of polyester were supported on supporters C, D_1 and D_2, the efficiency with TCD and FID were compared, the results of which are shown in Table 5.

Because of the large test sample quantity for TCD, with less than 20% for any of the supporters, the number of theoretical plates decreased, and thus the separation of methyl stearate and methyl oleate was impossible. With FID, since the test sample quantity is a few hundredths that of TCD, for C and D_2 even 10% produces considerable separation, albeit somewhat inferior to 20%. The foregoing are results obtained at one constant temperature, and thus for varying temperatures, further investigations are necessary.

Table 5. Influence of the Concentration of the Stationary Liquid Phase in a Supporter

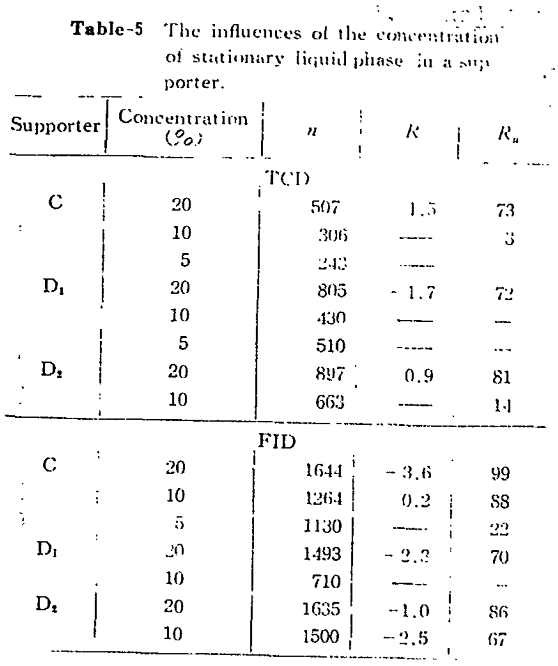


Table-5 The influences of the concentration of stationary liquid phase in a supporter.

Supporter	Concentration (%)	n	R	R_n
		TCD		
C	20	507	1.5	73
	10	306	—	3
	5	243	—	
D_1	20	805	- 1.7	72
	10	430	—	—
	5	510	-----	...
D_2	20	897	0.9	81
	10	663	—	14
		FID		
C	20	1644	- 3.6	99
	10	1264	0.2	88
	5	1130	—	22
D_1	20	1493	- 2.3	70
	10	710	—	...
D_2	20	1635	-1.0	86
	10	1500	-2.5	67

3.6 Influence of Column Length

In the experiments thus far, the object has simply been comparison, so that columns of shorter length than in actual practise were used. With FID, because of the narrow diameter of the column and the small quantity of test material required, complete separation was obtained in certain cases even with 2 m, but a comparison with the use of 3 m is given in Table 6.

Table 6. Influence of Column Length

Table 6 The influences of the column length.

Column length (m)	Retention volume of methyl palmitate	*n*	*R*	R_u
2	336	1644	+3.6	99
3	424	1997	+4.6	99

While the number of theoretical plates increases by lengthening the column, the separability does not increase that much. The increase in separability is caused by the longer retention time, and the separation rate is the same.

3.7 Relative Response

Table 7 shows the results of using mixtures of varying compositional content, with mixture 1 being a mixture of almost equal [illegible] mixture 2 a predominantly saturated acid mixture, mixture 3 a [illegible] unsaturated

Table (342)

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Table 7 Relative responses of flame ionization detector to methyl esters in mixtures of various compositions.

Methyl ester	Konwn weight (%)	Peak area (%)	Deviation (%)	Response per equal weight* (%)
		Mixture 1		
Palmitate	20.20	21.66	−1.46	100
Stearate	19.55	20.91	−1.36	100.6
Oleate	19.60	20.01	−0.41	95.2
Linoleate	20.27	19.63	+0.64	90.3
Linolenate	20.38	17.78	+2.60	81.3
		Mixture 2		
Palmitate	38.56	41.16	−2.60	100
Stearate	33.04	34.35	−1.31	97.4
Oeate	9.49	9.94	−0.45	98.1
Linoleate	9.62	8.38	+1.24	81.6
Linolenate	9.30	6.20	+3.10	62.5
		Mixture 3		
Palmitate	5.16	5.61	−0.45	100
Stearate	5.08	5.87	−0.79	106.4
Oleate	29.83	30.44	−0.61	93.8
Linoleate	29.62	30.19	−0.57	93.7
Linolenate	30.31	27.89	+2.42	84.7
		Mixture 4		
Palmitate	37.30	40.22	−2.92	100
Stearate	32.91	34.46	−1.55	97.1
Oleate	4.33	4.91	−0.58	105.2
Linoleate	4.58	4.19	+0.39	84.8
Linolenate	20.88	16.22	+4.96	72.1
		Mixture 5		
Palmitate	36.56	38.53	−1.97	100
Stearate	32.80	34.59	−1.79	100.1
Oleate	5.62	5.88	−0.26	99.3
Linoleate	4.63	4.20	+0.43	86.1
Linolenate	20.39	16.80	+3.59	78.2

acid mixture, and mixtures 4 and 5 being mixtures in which saturated acid and trienoic acid predominate.

On the relative response of FID with respect to fatty acid methyl esters, there are reports[8)] that the weight % agrees very closely with the peak area %, and yet there are also examples[10)] where they do not strictly agree but show considerable differences, and thus in addition to the theoretical response, errors attributable to the actual measurement of peak areas can be taken into consideration. In the results of this experiment, the relative mass response of the various C_{18} saturated and unsaturated fatty acid methyl esters where methyl palmitate was equated with 100, varied considerably depending on their content in the mixture, as is shown in Fig. 4.

With stearic acid and oleic acid, when the content is small, the peak area % shows a higher value than the weight %, while on the other hand, with linoleic acid and linolenic acid, the peak area % shows a lower value than the weight %. When the content increases, the peak area % and the weight % agree fairly closely in all cases. The reason for this is not

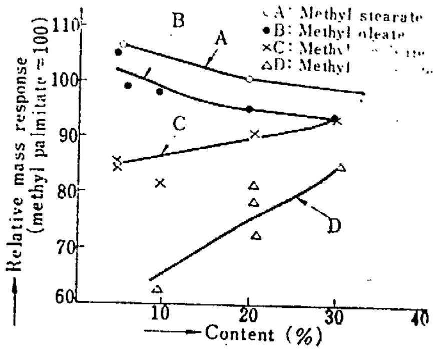

Fig.-4 The relations between relative mass responses and contents of each esters in the mixture.

Fi ponse and

ixture

simply attributable to the relative response alone, but also to the fact that, compared with stearic acid and oleic acid, the retention time of linoleic acid and linolenic acid is quite long. Also to be considered are such factors as changes in unsaturated cohesion including polymerization during the gas chromatography process, or such reactions as the exchange of ester base with the stationary liquid phase, as well as errors in area measurement where minute quantities are involved. In any case, in analyzing test samples with small contents of any unsaturated acid above dienoic acid, the exercise of special considerations is deemed to be in order.

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