Rapid Gas-Liquid Chromatographic Procedure for the Analysis of Methyl Esters of Long Chain Fatty Acids[1, 2]

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Abstract

A gas-liquid chromatographic procedure permitting rapid analysis of the major fatty acids commonly found in vegetable oils was evaluated with standards of known composition and found to be both accurate and precise. A standard containing 20% by weight of each of the methyl esters of palmitic, stearic, oleic, linoleic, and linolenic acid was analyzed in 2.7 min with errors of 1.6, 0.7, 3.3, 0.8, and 3.1%, respectively. Six procedures, requiring from 2.7 to 25 min for the elution of methyl linolenate, were used in an evaluation of the effects of the operational parameters, carrier gas flow rate and column temperature, on the precision and accuracy of gas-liquid chromatographic analysis of fatty acid methyl esters. The most rapid procedure, obtained with a column temperature of 235C and a helium flow rate of 110 ml/min was found to be comparable in accuracy and precision to the other procedures and gave quantitative results with National Heart Institute type fatty acid standards KA, KB, and KD, that agreed with stated composition with a relative error of less than 2% for major components (10% or more of total mixture) and less than 6% for minor components (less than 10% of total mixture).

Introduction

GAS-LIQUID CHROMATOGRAPHY (GLC) has been used extensively for quantitative determination of component fatty acids in vegetable oils. The standard GLC procedures require a retention time of 15 to 25 min for linolenic acid (4–6,11). Recent interest in genetic studies of fatty acids has created the need for a rapid analytical procedure. The development and successful use of a rapid GLC procedure has been reported for the analyses of oil from individual corn kernels (9).

This study was undertaken to determine the effects of column temperature and carrier gas flow rate on the accuracy and precision of GLC analyses of the fatty acids of seed oils. Standards of known composition were used to evaluate the procedure.

Materials and Methods

A GLC standard reference mixture containing 20% by weight of each of the methyl esters of palmitic, stearic, oleic, linoleic, and linolenic acid, the fatty acids of major importance in the oil of agronomic crops, was obtained from The Hormel Institute, Austin, Minnesota. Analyses were also performed on National Heart Institute type fatty acid standards KA, KB, and KD, obtained from Applied Science Laboratories, State College, Pennsylvania.

Analyses were made on an F and M Model 700 dual column gas chromatograph equipped with flame ionization detectors. The detector signal was attenuated between 1×10^{-9} and 2×10^{-8} amps, and recorded with a Honeywell Electronik 16 recorder. Fatty acid composition was determined by triangulation (peak height times width at half height) and area normalization of resulting peaks. During the latter part of this study peak areas were also measured with an Infotronics Model No. CRS-11HSB digital integrator. Four chromatograms of the Hormel standard were obtained at three column temperatures (180C, 200C, 235C), each at two helium flow rates (55 and 110 ml/min). Injection port and detector temperatures were held at 265C and 290C, respectively. All samples were injected with a Hamilton No. 7101 1.0 μl syringe without the use of solvent. The syringe was routinely cleaned between injections by flushing with petroleum ether (Skelly F). Residual solvent produced the solvent peaks appearing in Figs. 1 and 2. The sample size varied from 0.01 to 0.03 μl and recorder chart speed from 0.5 to 3.0 in./min, depending on column temperature and helium flow rate. Coiled copper columns, 2.3 m $\times$ 4.8 mm. I.D., were packed with 15%, by weight, of stabilized diethylene glycol succinate (Analabs, Inc., Hamden, Conn.) coated on Anakrom AB 70/80 mesh solid support. Peak retention times are uncorrected and vary slightly, depending upon age of columns. The data were analyzed as a split plot design with helium flow rates as whole plots and column temperatures as subplots.

Results

Averages of four chromatograms obtained from each of six analytical procedures are given in Table I. All procedures resulted in values over 20% for stearic and oleic acids and under 20% for linolenic acid. The greatest deviation was the consistent underestimation of linolenic acid. The average for all procedures (24 chromatograms) obtained with the Hormel standard gave an error of 0.0, 1.0, 3.8, 0.7, and 5.4% for palmitic, stearic, oleic, linoleic, and linolenic acid, respectively. Typical chromatograms with peak retention times for each of the procedures

TABLE I

Average Fatty Acid Composition of Four Chromatograms of Hormel Standard Analyzed at Three Column Temperatures, Each at Two Helium Flow Rates

Procedure No.	Column temp	Helium flow rate	Fatty acid composition (%)				
			16:0	18:0	18:1	18:2	18:3
	C	ml/min					
1	180	55	19.59	20.07	20.71	20.23	19.41
2	180	110	20.25	20.23	20.46	20.07	19.00
3	200	55	20.12	20.07	20.57	20.40	18.85
4	200	110	19.82	20.53	20.60	20.03	19.03
5	235	55	20.52	20.19	21.58	19.87	17.85
6	235	110	19.67	20.14	20.66	20.16	19.38
Average			20.00	20.20	20.76	20.13	18.92
Standard error			0.21	0.16	0.13	0.12	0.21
C. V. (%)			2.12	1.63	1.24	1.15	2.21

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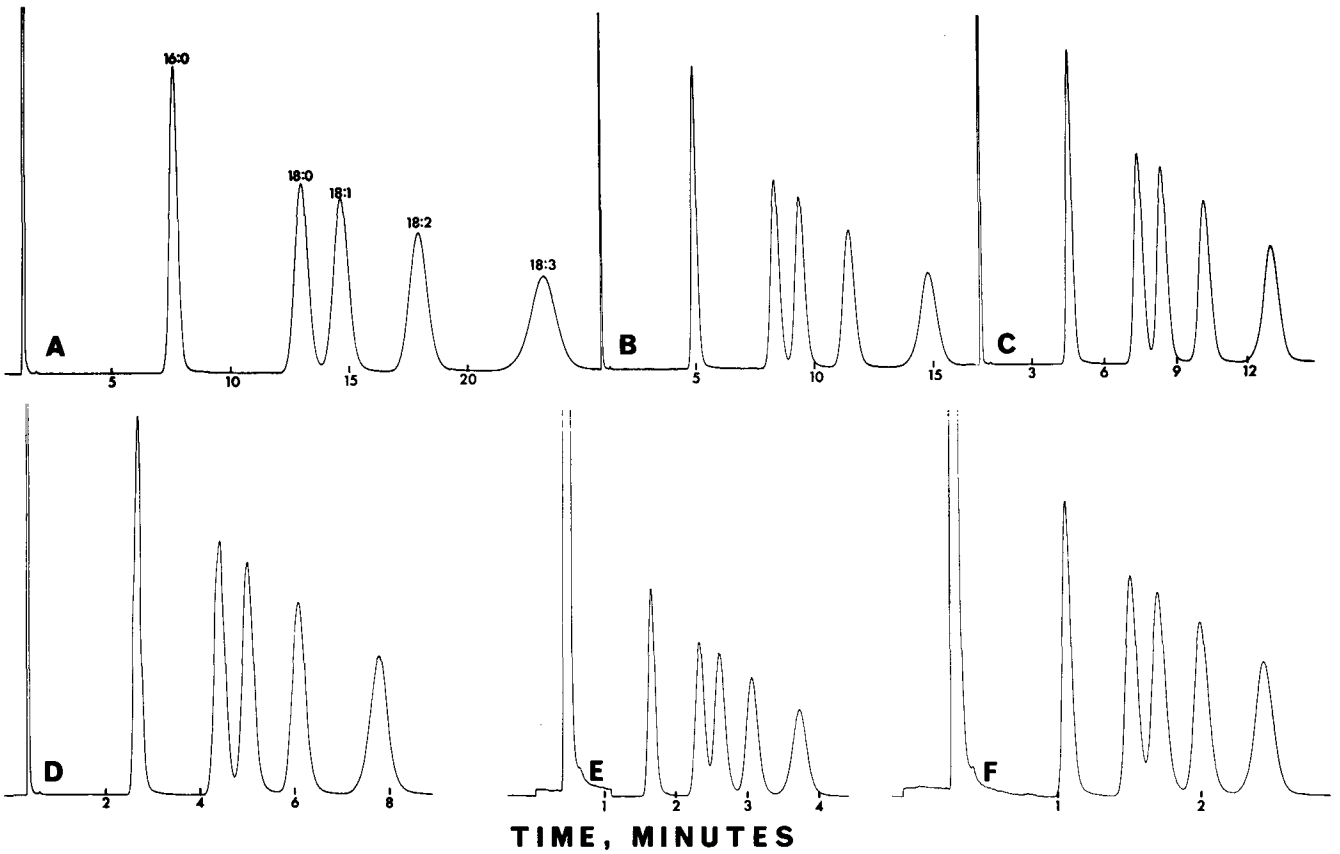

FIG. 1. Typical chromatograms obtained with a standard mixture of fatty acid methyl esters for six chromatographic procedures. *A*, Procedure 1: 180C column temperature, 55 ml/min helium flow rate, and 0.5 in./min recorder chart speed. *B*, Procedure 2: 180C column temperature, 110 ml/min helium flow rate, and 0.5 in./min recorder chart speed. *C*, Procedure 3: 200C column temperature, 55 ml/min helium flow rate, and 0.5 in./min recorder chart speed. *D*, Procedure 4: 200C column temperature 110 ml/min helium flow rate, and 1 in./min recorder chart speed. *E*, Procedure 5: 235C column temperature, 55 ml/min helium flow rate, and 1.5 in./min recorder chart speed. *F*, Procedure 6: 235C column temperature, 110 ml/min helium flow rate, and 3 in./min recorder chart speed.

are shown in Fig. 1. The results obtained with procedure 6 were comparable to any of the other procedures and sample analysis was completed in 2.7 min. Values obtained by procedure 6 for the National Heart Institute type standards are shown in Table II.

Analysis of variance for each fatty acid of the Hormel standard is shown in Table III. The effect of column temperature was significant for oleic and linolenic acid, and the interaction of column temperature × helium flow rate was significant for all fatty acids except stearic.

Discussion

Methyl esters of palmitic, stearic, oleic, linoleic, and linolenic acid were separated by a rapid GLC procedure in 2.7 min with 1.6, 0.7, 3.3, 0.8, and 3.1% errors, respectively. The development of a rapid procedure was necessary for genetic studies of vegetable oils where the analyses of numerous samples are required to determine inheritance of fatty acids. The accuracy of the rapid procedure (procedure 6) was similar to procedures 1, 2, and 3 which required 25, 16, and 14 min per sample, respectively.

Injection of a uniform small sample was readily accomplished with a Hamilton No. 7101 1.0 μl syringe. The Hamilton No. 7001 1.0 μl syringe under identical conditions gave erratic results, both in sample size and in values obtained for individual fatty acids. Palmitic acid was usually overestimated and consequently the other fatty acids were underestimated. The use of stabilized diethylene glycol succinate permitted extensive operation of columns at temperatures in excess of 200C. Myristic, behenic, and lignoceric acid can be determined by the rapid procedure; however, these acids plus palmitoleic, margaric, and eicosenoic acid are usually present in minor amounts. In the rapid procedure arachidic acid is eluted with linoleic acid and eicosenoic acid with linolenic acid; consequently, this method is of limited value in the analysis of oils containing substantial quantities of either arachidic or eicosenoic acid.

The rapid procedure (procedure 6) gave results comparable to any of the others when used on a

TABLE II

Average of Three Determinations Obtained by Digital Integration of National Heart Institute Type Fatty Acid Standards; Standards Analyzed at a Column Temperature of 230C and a Helium Flow Rate of 110 ml/min

	Standard KA				Standard KB				Standard KD			
Fatty acid	Reported (%)	Found (%)	Error (%)	C.V. (%)	Reported (%)	Found (%)	Error (%)	C.V. (%)	Reported (%)	Found (%)	Error (%)	C.V. (%)
14:0	25.0	24.8	0.80	4.68	4.0	4.2	5.00	6.93	11.8	11.7	0.85	2.26
16:0	10.0	10.0	0.00	5.10	40.0	40.2	0.50	2.59	23.6	23.2	1.69	0.68
16:1									6.9	7.2	4.35	0.98
18:0	65.0	65.2	0.30	2.39	56.0	55.6	0.71	2.31	13.1	13.2	0.76	0.54
18:1									44.6	44.6	0.00	0.86

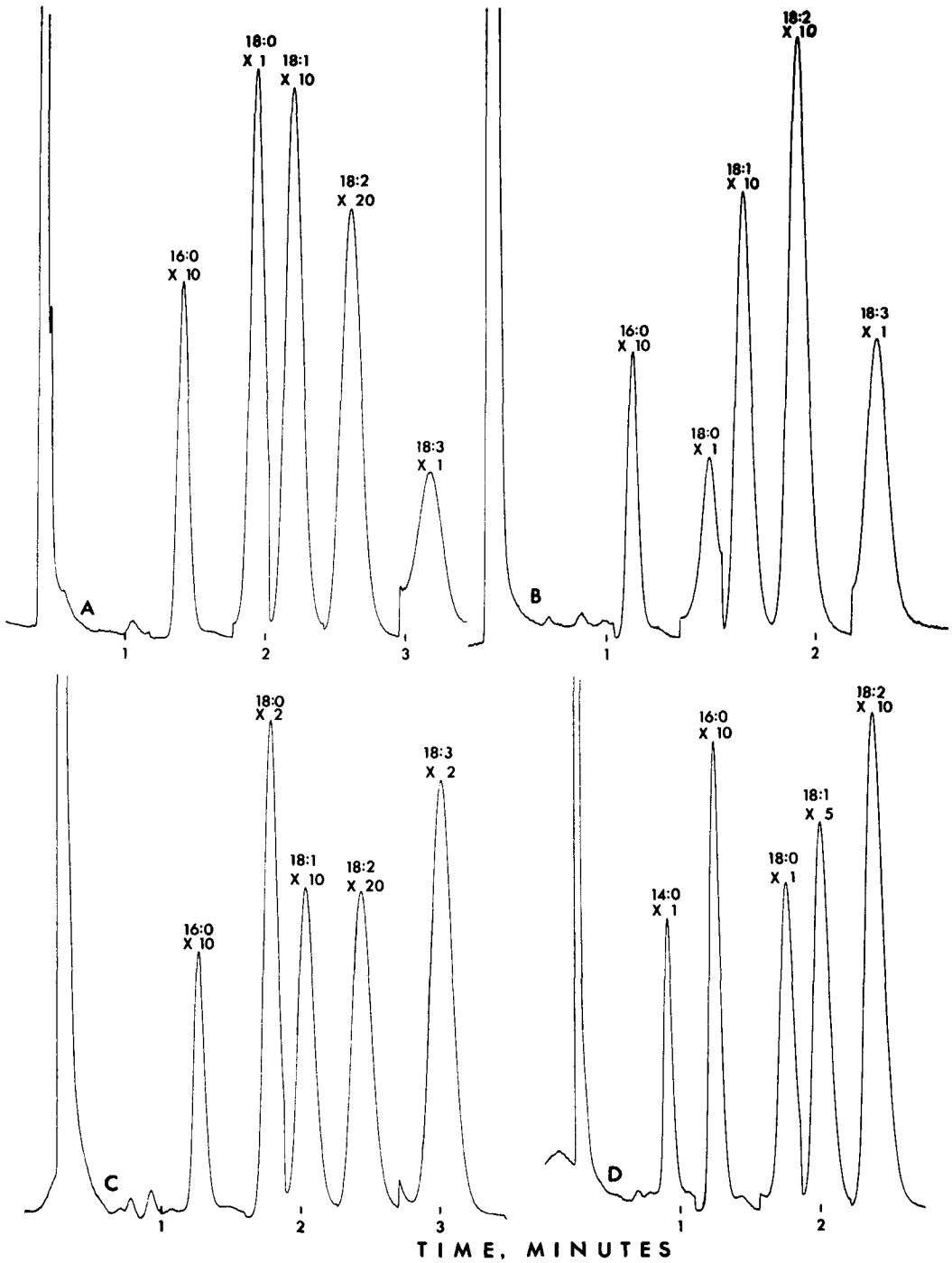

FIG. 2. Typical chromatograms of four common oils analyzed by a rapid analytical procedure (235C column temperature, 110 ml/min helium flow rate, and 3 in./min recorder chart speed). Attenuation is shown for each peak area. *A*, Commercial corn oil. *B*, Single seed of Ga. 246E sorghum. *C*, Single seed of Clark 63 soybean. *D*, Single seed of Empire WR 61 cotton.

known GLC standard. This procedure has been successfully used on small samples of oil (single seeds) from corn, sorghum, soybean, and cotton. Typical chromatograms for these analyses are shown in Fig. 2 for commercial corn oil and for single seeds of Ga. 246E sorghum, Clark 63 soybean, and Empire WR 61 cotton. Attenuation for each peak gave peaks which can be measured with a high degree of accuracy; however, attenuation is unnecessary with the digital integrator. The integrator response is unaffected by attenuation within a given electrometer range. A corn oil sample was used in a comparison of values obtained with the digital integrator and by triangulation of attenuated peaks. The results shown in Table IV, are averages of six chromatograms produced by procedure 6. A sample of commercial corn oil has been used over a period of several months as

TABLE III

Analysis of Variance of Data for Each Fatty Acid of Hormel GLC Standard Analyzed by Three Column Temperatures, Each at Two Helium Flow Rates

Source of variation	d.f.	Mean square				
		16:0	18:0	18:1	18:2	18:3
Replications	3	0.23	0.06	0.03	0.01	0.10
Helium flow rates	1	0.16	0.21	0.85	0.04	1.11
Error (a)	3	0.07	0.29	0.09	0.14	0.46
Column temperatures	2	0.07	0.05	0.78[b]	0.09	0.70[a]
Flow × temperature	2	1.18[a]	0.14	0.48[a]	0.23[a]	1.99[b]
Error (b)	12	0.19	0.08	0.07	0.04	0.12

[a] Significant at the 5% level.
[b] Significant at the 1% level.

TABLE IV

Comparison of Data Obtained with Digital Integrator and by Triangulation of Chromatogram Peaks. Data Obtained from Six Chromatograms of a Corn Oil Sample Analyzed at a Column Temperature of 230C and a Helium Flow Rate of 110 ml/min

Chromatogram No.	Peak triangulation					Digital integration				
	Fatty acid composition (%)					Fat acid composition (%)				
	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
1	12.6(10)[a]	2.1(1)	27.3(10)	56.7(20)	1.3(1)	12.1	2.1	27.2	57.1	1.5
2	12.3(10)	2.2(1)	27.7(10)	56.5(20)	1.3(1)	12.0	2.0	27.2	57.2	1.5
3	12.4(10)	2.2(1)	27.8(20)	56.4(20)	1.3(1)	12.2	2.0	27.2	57.2	1.4
4	12.1(10)	2.2(2)	27.6(20)	56.8(50)	1.3(1)	12.2	2.1	27.2	57.1	1.4
5	12.0(10)	2.1(2)	27.2(20)	57.4(50)	1.3(1)	12.2	2.1	27.2	57.2	1.4
6	12.2(10)	2.2(1)	26.7(10)	57.6(20)	1.3(1)	12.1	2.1	27.2	57.3	1.3
Average	12.3	2.2	27.4	56.9	1.3	12.1	2.1	27.2	57.2	1.4
C. V. (%)	1.78	2.88	1.49	0.86	0.00	0.74	2.88	0.00	0.14	5.53

[a] Attenuation $\times$ 10^{-9}amps.

a check on the chromatographic procedure. An average of 14 chromatograms obtained by procedure 6 has given the following results: 12.5% palmitic, 2.5% stearic, 28.7% oleic, 55.4% linoleic, and 1.0% linolenic. These results agree with those reported for commercial corn oil (3,7,8). Results of the other chromatograms in Fig. 2 are as follows: Ga. 246E sorghum—14.0% palmitic, 1.2% stearic, 31.8% oleic, 50.6% linoleic, and 2.5% linolenic; Clark 63 soybean—11.9% palmitic, 5.8% stearic, 21.8% oleic, 52.8% linoleic, and 7.8% linolenic; Empire WR 61 cotton—1.2% myristic, 28.4% palmitic, 2.6% stearic, 16.9% oleic, and 50.9% linoleic. Since these results are from individual seeds of a particular variety, they may not be identical to reported composition for commercial oils of these crops. However, the results from these analyses are similar to those reported for soybean and cottonseed oils (1,2,10).

Chromatogram B in Fig. 2 was obtained on columns used frequently over a period of several months. These columns gave a retention time of less than 3 min for linolenic acid. The other chromatograms were obtained from freshly prepared columns operated at 240C. These columns gave better separation of stearic and oleic acid but increased the retention time of linolenic acid to 3.3 min.

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