esters. Good separations can be obtained with much less material than is required with standard techniques of column chromatography.

Most indicators used in paper chromatography of lipids may be applied in thin-layer chromatography as well. In addition, corrosive spot-test reagents, such as sulfuric acid, can be used to char nearly all organic compounds. Thin-layer chromatograms usually yield spots which are much smaller and more distinct than those observed in cellulose- or glass-fiber paper chromatography.

It is possible to separate up to 10 mg. of a mixture of lipids on one plate. The various fractions can be eluted for identification or further fractionation if nondestructive indicators are used for their localization. The solvent systems utilized in this microtechnique can also be applied on columns of silicic acid if larger amounts are required.

Thin-layer chromatography has been applied to test a variety of seed oils for the occurrence of epoxy and hydroxy acids, and some striking results were obtained (5, 6).

Summary

Adsorption chromatography on thin layers of silicic acid or alumina provides a new and highly efficient analytical tool for the rapid separation of lipids according to classes of compounds.

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A Study of Octadecenoic Acids by Gas-Liquid Partition Chromatography and Infrared Spectrophotometry

F. L. KAUFFMAN and G. D. LEE Swift and Company, Chicago, Illinois

NUMEROUS AUTHORS (1,2,3,6,9) have reported the use of gas-liquid partition chromatography (G.L.P.C.) for the separation of the methyl esters of fatty acids. Techniques were generally used with conventional columns packed with high boiling greases or with polyesters. Such closely related compounds as methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate have been readily separated and determined by this technique. Lipsky, Lovelock, and Landowne (4) and Lipsky, Landowne, and Lovelock (5) reported the use of capillary columns for the separation of the methyl esters of oleic and elaidic acids.

Infrared analysis for *trans*-octadecenoic acid is a standard method (8). Good quantitative results of the *trans*-isomers are possible by infrared analyses, and such analyses are done routinely by many laboratories.

This laboratory would like to report the separation of geometric isomers of octadecenoic acid methyl esters from commercially hydrogenated oils by means of G.L.P.C., using a capillary column coated with Apiezon L. Comparisons with a conventional column packed with a polyester and with *trans*-isomer values from infrared spectrophotometry are also given.

Experimental

A Barber-Colman Model 10 gas chromatograph with an ionization detection system was used for all G.L.P.C. analyses.

A 6-ft. glass column 0.25 in. in inside diameter, containing 60–100 mesh Chromosorb W coated with 20% succinic acid-diethylene glycol polyester, was used for the regular packed column G.L.P.C. Temperatures employed were: column 180°C., detector cell 233°C., flash heater 234°C. The argon carrier gas pressure was 20 p.s.i. with an outlet flow of 155 ml./min. The ionization voltage applied to the cell electrodes was 900 volts, and a radium D source was used in the cell. All experimental conditions were maintained constant throughout the analysis. The sample was dissolved in n-hexane to make a 1% solution, from which a sample of 2.0 microliters was applied to the column, using a 10-microliter syringe. The recorder sensitivity range was 3×10^{-8} amps.

For capillary column chromatography a 100-ft. stainless steel column 0.010 in. in inside diameter, coated with Apiezon L, was used. Temperatures employed were: column 180°C., detector cell 230°C., flash heater 228°C. The ionization voltage applied to the cell electrodes was 1,250 volts, and a radium D source was used in the cell. The argon carrier gas pressure was 40 p.s.i. with a stream-splitting arrangement used in injecting the sample so that only a small percentage of the sample went through the capillary column. The remainder was vented to the atmosphere, using a scavenging flow arrangement. No sample dilution was used with the capillary column. A 1.0-microliter sample was used in this manner.

For infrared analyses a Perkin-Elmer Model 21 infrared spectrophotometer was used.

Results and Discussion

A sample of commercial vegetable oil (mixed cottonseed and soybean) was hydrogenated to an iodine value of 82 by using hydrogen at 5 p.s.i. pressure at 180–200°C. with 0.17% nickel catalyst. This oil was

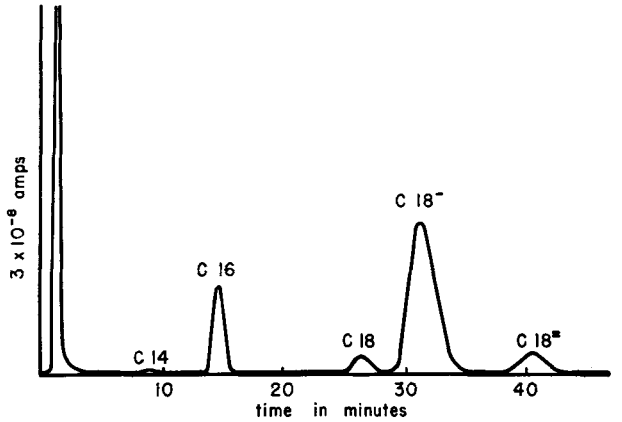

FIG. 1. Chromatogram of the methyl esters of an hydrogenated vegetable oil on a 6-ft. by 0.25-in. packed column of succinic acid-diethylene glycol polyester. Column temperature 180°C., flow rate of argon gas 155 ml./min.

methylated by refluxing with an excess of methanol in the presence of sodium hydroxide. Portions of the methyl esters were analyzed on both the polyester column and the capillary column. Figure 1 shows the chromatogram using the polyester column and Figure 2 that using the capillary column. These chromatograms are typical of the resolution of fatty acid methyl esters obtained in our laboratory in studies of many series and various mixtures. One such sample was a prepared mixture of equal amounts of methyl oleate and methyl elaidate. This mixture was satisfactorily resolved by using the capillary column. Identification was made in each instance by comparison of the retention time with that of the pure methyl ester. Other isomers may also have the same retention time as the standards employed.

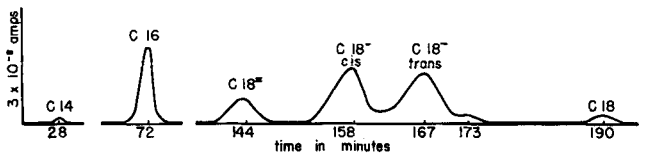

FIG. 2. Chromatogram of the methyl esters of an hydrogenated vegetable oil on a 100-ft. by 0.01-in. capillary column coated with Apiezon L. Column temperature 180°C., argon pressure 40 p.s.i.

It is to be noted that on the polyester column the octadecenoic acids were not separated while on the capillary column separation was good. When using the capillary column, an additional compound not observed on the regular column was eluted after the methyl elaidate. It is possible that this represents a third octadecenoic acid.

Quantitation of the various methyl esters was accomplished by measurement of the areas under the peaks of the curves by the triangulation method. These results, together with the results of the infrared analysis for the *trans*-isomer, are shown in Table I. It is seen that good agreement was obtained among the three methods of analysis. The amount of the single octadecenoic methyl ester component determined on the packed column is equal to the sum of the oleate, elaidate, and unknown components determined on the capillary column.

TABLE I

Fatty Acid Composition of an Hydrogenated Vegetable Oil by Different Techniques

Methyl ester	Percentage composition		
	Infrared	Capillary column	Packed column
Myristate	.....	0.3	0.5
Palmitate	.....	14.8	15.2
Stearate	.....	6.3	5.8
Oleate		30.2	67.0
Elaidate	33.5	32.1	
Unknown		4.7	
Linoleate (isomers?)	.....	11.6	11.5

The specific characteristics of both columns are shown in Table II. The separation factor is calculated according to standard procedures (7). This shows excellent separation of all the known constituents in this sample. The retention time is measured from the time of sample introduction.

TABLE II

Operating Parameters for Packed and Capillary Columns

Methyl ester	Packed column [a]		Capillary column [b]	
	Retention time, min.	Sf [c]	Retention time, min.	Sf [c]
Myristate	8.5	0.57	28	0.39
Palmitate	14.8	1.00	72	1.00
Stearate	26.0	1.76	190	2.64
Oleate	31.2	2.11	158	2.19
Elaidate			167	2.32
Linoleate	40.5	2.74	144	2.00

[a] 6-ft. glass column, 0.25-in. inside diameter, coated with succinic acid-diethylene glycol polyester (Figure 1).
[b] 100-ft. capillary column, 0.010-in. inside diameter, coated with Apiezon L (Figure 2).
[c] Separation factor Sf based on methyl palmitate equal to 1.00.

Summary

The separation of methyl esters of octadecenoic acids by G.L.P.C. on a capillary column is shown for a sample of hydrogenated commercial vegetable oil. Comparisons are made with a regular packed column G.L.P.C., a capillary column G.L.P.C., and with infrared analysis. Good separation of methyl oleate and methyl elaidate was accomplished, using a capillary column. The amount of elaidate found by G.L.P.C. compared well with the *trans*-acid found by infrared analysis. In addition, a small amount of a component was detected that is possibly another isomer of methyl oleate.

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