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Two-column gas–liquid chromatography of fatty acid methyl esters

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SUMMARY Fatty acid methyl esters were separated into fractions according to chain length on a nonpolar gas–liquid chromatographic column. These fractions were collected and rechromatographed on a polar column. Temperature programming was used in both cases. Data are given for the accuracy of the double procedure applied to a synthetic mixture.

KEY WORDS two-stage · gas–liquid chromatography · fatty acid methyl esters · quantification · accuracy

ALTHOUGH THE DETERMINATION of fatty acid composition by gas–liquid chromatography on polar stationary phases is now accepted as routine, the method still suffers from inaccuracies due to the overlapping of certain components. We have adopted a two-stage procedure in which the mixture is initially separated on a nonpolar column into individual chain length fractions which are collected at the effluent port. Each fraction is rechromatographed on a polar column to determine fatty acids of different degrees of unsaturation. The procedure is in use in several laboratories, but few data on its accuracy are available. As a further refinement, we have programmed the temperature in order to give sharp peaks for all components in each run and to increase the speed of analysis. Satisfactory results for a synthetic mixture containing 16–24 carbons and 0–6 double bonds suggest the potential usefulness of the procedure with more complex natural mixtures.

Procedure. The instrument used was a model 400 biomedical gas chromatograph (F & M Scientific Corporation, Avondale, Pa.) equipped with hydrogen flame ionization detector and an effluent stream splitter. Fatty acid standards, as the methyl esters, were obtained from the National Heart Institute and the Hormel Institute.

Separation according to chain length was done on a 4 ft × 3 mm I.D. glass column packed with 3.8% General Electric SE-30 silicone gum on 80–100 mesh Diatoport S. The temperature was held at 170°C for 5 min after injection of the sample, then raised to 220°C at 4°C/min.

A preliminary chromatogram obtained with the effluent port closed and a helium carrier gas flow rate of 78 ml/min revealed that no overlapping of components of different chain length took place, and yielded the retention time range of the fractions of each chain length. The program delay mentioned above was useful in causing a wider spread between the C_{18} and C_{20} fractions, in particular, the 18:0 and 20:5 esters. The retention data thus obtained were used as a guide for proper timing in introducing collection vessels into the effluent stream, and esters of each chain length from an injected 500 μg sample were subsequently collected at a helium split ratio of approximately 14:1 (Fig. 1).

The collection trap described by James (1) was modified by lining a 5.75 inch glass pipette (Clay-Adams Inc., New York, N. Y., A-2880B) with methanol-wetted, chloroform-extracted glass wool and dipping the pipette tip into a conical 3 ml centrifuge tube containing 0.1 ml of methanol. One end of a Teflon collection tube (F & M Scientific Corp.) was introduced into the glass pipette and the other, equipped with a compression fitting, was held tightly to the effluent port during the collection process.

Collected esters were recovered by rinsing the Teflon tube and glass wool with 1 ml each of chloroform–methanol 2:1 (v/v). Remaining traces of rinsing solution were forced from the collection pipette into the centrifuge tube by means of a pipette bulb. Solvent was evaporated at room temperature under a stream of dry nitrogen and the walls of the tube were washed with 0.5 ml of hexane. The solvent was evaporated and the residue was redissolved in about 10 μl of hexane.

Separation according to number of double bonds was done on a 4 ft × 3 mm I.D. glass column containing 5%

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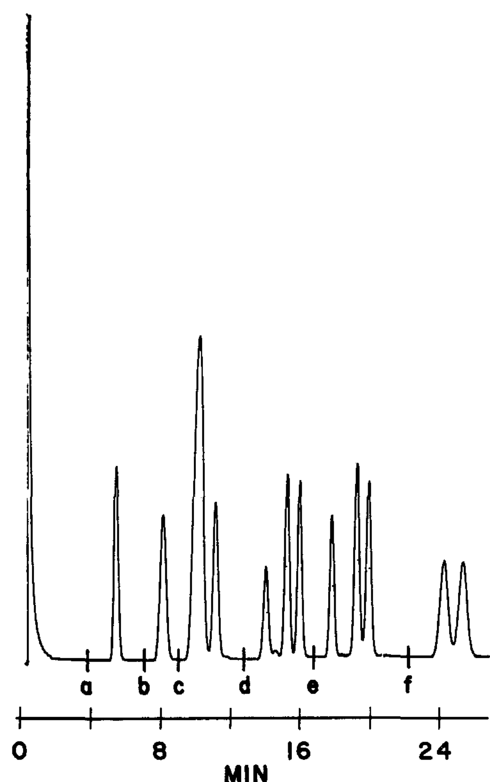

FIG. 1. Gas-liquid chromatographic separation of fatty acid mixture on SE-30 column. Collection vessels were introduced in effluent stream at positions marked on base line. Fractions collected: *a–b*, C_{16}; *b–c*, C_{17}; *c–d*, C_{18}; *d–e*, C_{20}; *e–f*, C_{22}; *f*–end, C_{24}.

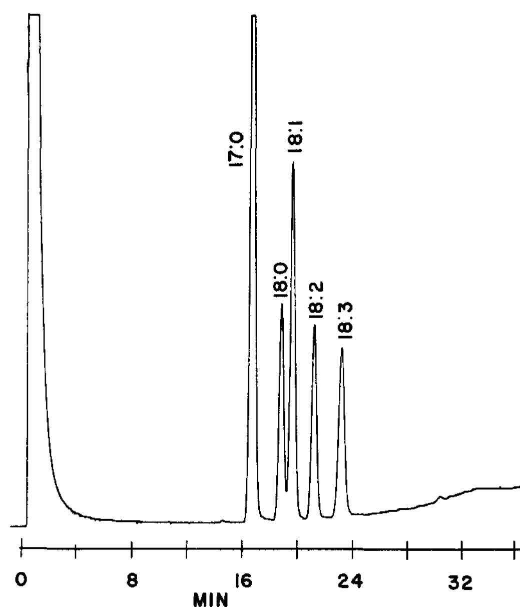

FIG. 2. Gas-liquid chromatogram of collected C_{18} fraction from Fig. 1. Methyl heptadecanoate (17:0) added as internal standard.

diethylene glycol succinate polyester (DEGS) on 80–100 mesh Diatoport S. Helium was used as carrier gas at a flow rate of 78 ml/min with temperature programming from 100° to 210°C at 3°C/min. Usually 1–4 μl of sample solution was injected. Peaks were identified by comparison of their retention times, relative to that of methyl heptadecanoate, with those of the individual standards.

TABLE 1 EVALUATION OF PROCEDURE

Fatty Acid*		Known Wt. Composition	GLC Analysis† SE-30 Column		GLC Analysis† DEGS Column		Calculated Composition‡	Difference	Relative Error
		%	%	SD	*rel.* %	SD	%	%	%
16:0	—	3.2	7.1	0.3	45.9	1.6	3.3	0.1	3
16:1	9	3.9			54.1	0.9	3.8	0.1	3
17:0	—	7.5	7.5	0.2	100.0	—	7.5	—	—
18:0	—	6.8	32.9	0.2	22.0	1.0	7.2	0.4	6
18:1	9	12.4			36.5	0.5	12.0	0.4	3
18:2	9, 12	6.6			20.4	0.9	6.7	0.1	2
18:3	9, 12, 15	7.0			21.1	0.3	6.9	0.1	1
20:0	—	6.9	18.2	0.3	37.7	0.3	6.9	—	—
20:1	11	7.1			41.2	0.4	7.5	0.4	6
20:4	5, 8, 11, 14	0.2							
20:5	5, 8, 11, 14, 17	4.4			21.1	0.5	3.8	0.6	14
22:0	—	6.7	20.6	0.2	35.6	1.8	7.3	0.6	9
22:1	13	7.7			39.0	1.3	8.0	0.3	4
22:6	4, 7, 10, 13, 16, 19	5.2			25.4	0.9	5.2	—	—
24:0	—	7.0	13.7	0.2	49.3	1.5	6.8	0.2	3
24:1	15	7.5			50.8	1.6	7.0	0.5	7

* Fatty acids are expressed as numbers describing in order the chain length, number of double bonds, and position of unsaturation (2).
† Composition is given in terms of area percentage. Area was determined by multiplication of peak height by width at half-height.
‡ Composition was calculated by multiplication of the percentage of the component within a chain-length fraction by the percentage of that fraction in the total mixture.

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Fig. 2 shows individual members and the lack of extraneous material in the C_{18} fraction. Chromatograms of the C_{20}, C_{22}, and C_{24} fractions revealed traces of shorter chain length components probably originating through contamination in the effluent splitter.

The precision and accuracy of results in the analysis of a 16-component synthetic mixture carried 5 times through the entire procedure can be seen in Table 1. Reproducibility of $\pm 1\%$ was observed in every instance on the SE-30 column and in most cases on the DEGS column. Any inequalities in the collection technique and subsequent handling of the sample for injection would influence adversely the precision of the DEGS figures. The largest discrepancy (relative error) between true composition values and calculated values was 14% while most figures differed by 6% or less, which does not exceed the maximum considered acceptable for fatty acid quantitative analysis in single-column work with no collection step involved (3). Because the recorder pen barely moved off the base line in the analysis of 0.2% eicosatetraenoate, this level was probably near the lower limit of detection under conditions described here.

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