# Quantitative GLC Determination of Methyl Linolenate in the Presence of Conjugated Dienes[1]

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## ABSTRACT

Methyl linolenate and *cis,trans* conjugated isomers of methyl linoleate are not resolved by gas liquid chromatography because they have the same retention time on polar columns used for separation of methyl ester mixtures. A new column of intermediate polarity made from a mixture of OV-17 and OV-225 separated these critical pairs and enabled quantitative determination of methyl linolenate in partially hydrogenated or conjugated esters.

Selective hydrogenation of polyunsaturated fatty acids with copper catalysts is accompanied by formation of conjugated diene isomers (1-3). Homogeneous hydrogenation with $Fe(CO)_5$ (4) and Pt-Sn complexes (5) also produce conjugated isomers. Because some of these isomers have the same retention time as methyl linolenate on polar columns during gas liquid chromatography (GLC) of methyl ester mixtures, it has not been possible to estimate methyl linolenate by GLC (6,7). Usually methyl linolenate is determined spectrophotometrically by the alkali isomerization method (8), which is slow and cumbersome. A similar separation problem arises during GLC analysis of selectively conjugated polyunsaturated fatty acid esters (9). The alkali isomerization method is not applicable to selectively conjugated esters which contain large amounts of preconjugated fatty esters. I wish to report on a chromatographic substrate capable of resolving these critical pairs.

Separation of methyl linolenate from conjugated diene was accomplished on a Varian Aerograph model 1860 GC instrument with dual flame ionization detectors equipped with stainless steel columns (10 ft x 1/8 in.; packed with a 50:50 mixture of 10% OV-17 on Supelcoport 80/100 mesh and 10% OV-225 on Supelcoport 80/100 mesh). The analysis was performed at a column temperature of 210 C with a nitrogen gas flow of 20 ml/min. The detectors were operated at 230 C while the injectors were held at 220 C. The analog signal from the detector was fed to a Modcomp realtime computer programmed to integrate areas under the curves, calculate the composition, and return a formatted report via a teletype immediately following completion of the chromatogram. Some typical chromatograms are shown in Figure 1. The percentage of linolenate determined by this method was compared with data from the alkali isomerization method, as shown in Table I.

Methyl linolenate and *cis,trans* conjugated diene have the same retention time on polar columns. A survey was made of substrates with intermediate polarity. Both OV-17 and OV-225 resolved these critical pairs partially on 5 ft

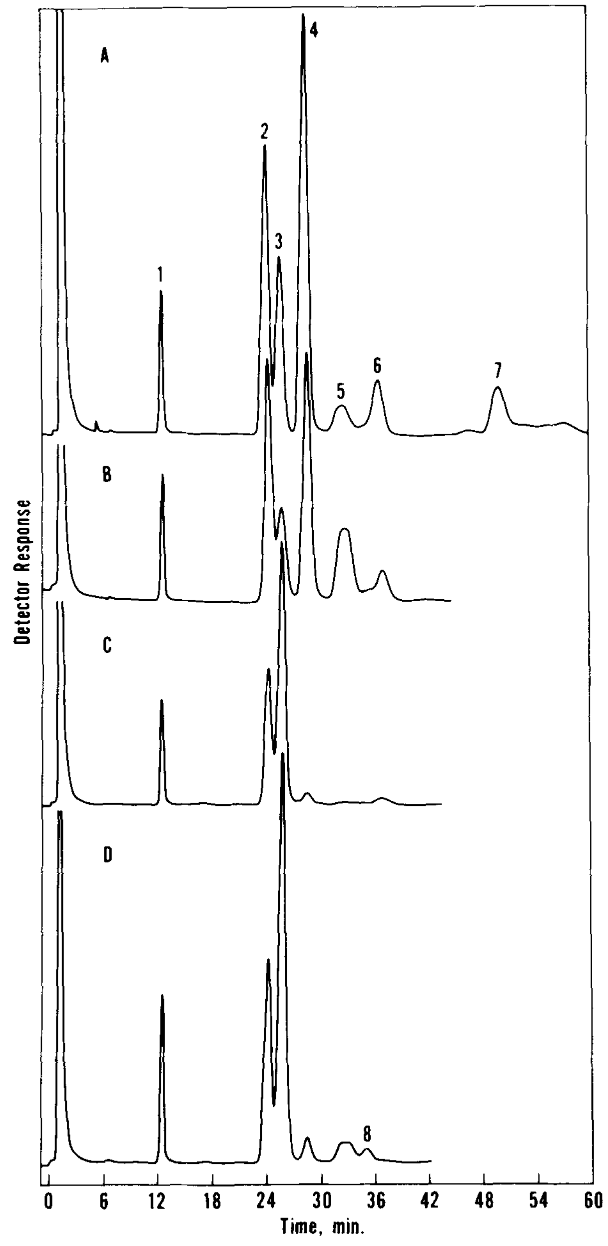

FIG. 1. Separation of fatty acid methyl esters on a 50:50 mixture of OV-17 on 80/100 Supelcoport and OV-225 on 80/100 Supelcoport. Column: 10 ft x 1/8 in. O.D. stainless steel. Temperature: 210 C. $N_2$ gas flow 20 ml/min. Methyl esters separated: (1) palmitate, (2) stearate and octadecenoates, (3) octadecadienoates, (4) octadecatrienoates, (5) *cis,trans* conjugated octadecadienoate, (6) *trans,trans* conjugated octadecadienoate, (7) α-eleostearate, and (8) diene-conjugated trienoate. Chromatograms (A) partially hydrogenated methyl ester mixture of linseed and tung oil, (B) partially hydrogenated methyl ester mixture of linseed oil and alkali conjugated safflower oil, (C) partially hydrogenated soybean oil methyl esters, and (D) partially conjugated methyl esters of soybean oil.

TABLE I

Comparison of Linolenate Determination by GLC and Alkali Isomerization

| Me ester sample | % Me linolenate by GLC | % Me linolenate by Alkali isomerization |
|---|---|---|
| Soybean oil | 8.3 | 8.3 |
| Copper-hydrogenated soybean oil | 3.1 | 3.2 |
| Copper-hydrogenated soybean oil | 0.9 | 0.8 |
| Linseed oil and alkali-isomerized safflower oil-copper-hydrogenated | 30.2 | 31.6 |
| Linseed oil and alkali-isomerized safflower oil-copper-hydrogenated | 26.8 | 27.4 |

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[1] Presented at the AOCS meeting, San Francisco, April, 1979.

columns. A better separation was observed with a 50-50 mixture of these substrates. Similar improvement had been observed for the separation of aldehydes from aldehyde esters (10). A 10 ft column packed with this mixture gives near base line separations as shown in Figure 1. Unfortunately, stearate, monoene, and diene are not well separated in this system. Therefore, to obtain total fatty acid composition, it also is necessary to analyze the sample on a polar column.

The quantitative data shown in Table I indicate excellent correlation between alkali isomerization values and the new GLC method. The present method has the advantage of being faster and less tedious than the alkali isomerization method.

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[Received October 6, 1978]