High Performance Liquid Chromatography of Fatty Methyl Esters: Analytical Separations

C.R. SCHOLFIELD, Northern Regional Research Laboratory,[1] Peoria, Illinois 61604

ABSTRACT

Fatty methyl esters are separated on the basis of unsaturation and chain length on an analytical scale by high performance liquid chromatography with a C18/Corasil column and aqueous acetonitrile solvent. Analysis by this method includes polymerized and oxidized esters which may not be detected by gas chromatography.

INTRODUCTION

Many liquid chromatographic separations of fatty methyl esters have been described. They usually have operated near atmospheric pressure and have required several hr for elution of samples. Since the development of gas liquid chromatography (GLC), they generally have been used for small-scale preparative work rather than analysis. Development of commercial high performance liquid chromatographic (HPLC) equipment using narrow columns with small particle size packing for greater efficiency of separation and higher pressures to pump the liquid phase through these columns has made possible rapid separations on an analytical, as well as preparative, scale.

Ramachandran, et al., (1) described HPLC of fatty methyl esters using aqueous methanol as the mobile phase. Their separations were run at 35-60 C to decrease solvent viscosity and improve resolution. We have found aqueous acetonitrile to be more selective than methanol and to give narrower peaks probably because of its lower viscosity.

Although GLC remains the preferred routine analytical method for fatty methyl ester mixtures, HPLC has the advantage, not only of including polymerized and oxidized esters which would not be detected in a GLC analysis, but also of easier scale-up for small preparative samples.

[1]ARS, USDA.

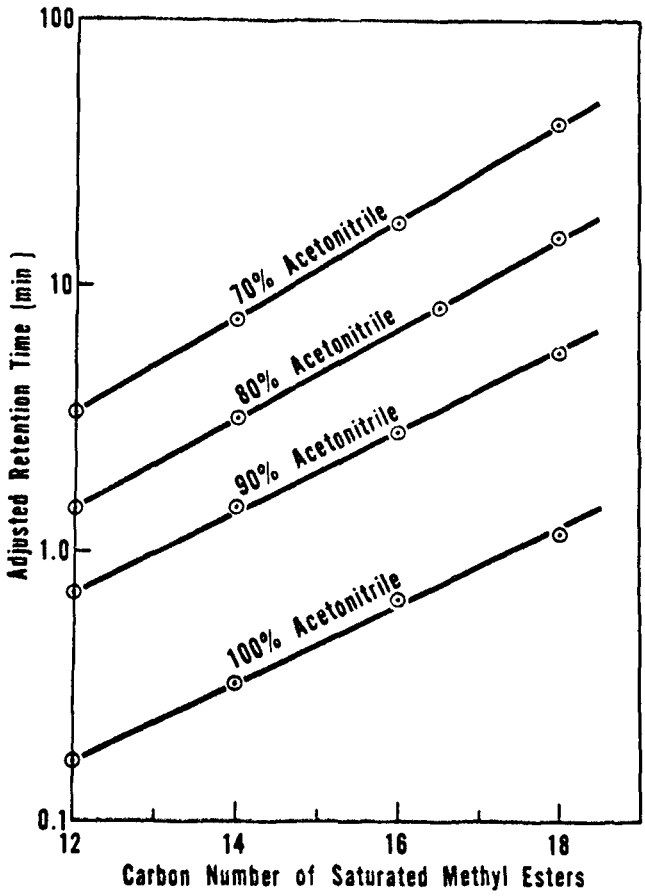

FIG. 1. Relation between carbon number and log adjusted retention time for saturated methyl esters in high performance liquid chromatography (HPLC) with acetonitrile water mixtures.

EXPERIMENTAL PROCEDURES

Chromatograms were run on a Waters Associates ALC-202 instrument with a 2 ft x 1/8 in. outside diameter stainless steel C18/Corasil column supplied by Waters and with a differential refractometer detector. The solvent was distilled Union Carbide acetonitrile with varying amounts of water. This use of C18/Corasil, a commercial packing with 18 carbon hydrocarbon bonded to silica on a solid glass bead core, and acetonitrile is analogous to our previous use of hexane and acetonitrile in countercurrent distribution for similar separations (2). However, because of the small amount of hydrocarbon on the support, better separations were obtained when water was added to the acetonitrile to increase distribution coefficients and retention times.

Methanol gave similar separations with slightly longer retention times for the same water content but with smaller separation factors and broader peaks.

Addition of water to acetonitrile increased the retention times of saturated esters to a greater extent than those of unsaturated esters. Figure 1 shows that, for these saturated esters, there is a linear relationship between log adjusted retention time and carbon number like that in GLC. Injections of water or 100% acetonitrile were used to determine the elution time of a nonretained compound. Nickell and Privett (3) have shown a similar relationship for triglycerides in reversed-phase partition chromatography.

Resolution and theoretical plates decrease as solvent flow rate is increased from 0.3-1.0 ml/min. Based upon the above results and upon separations of linolenate, linoleate, and

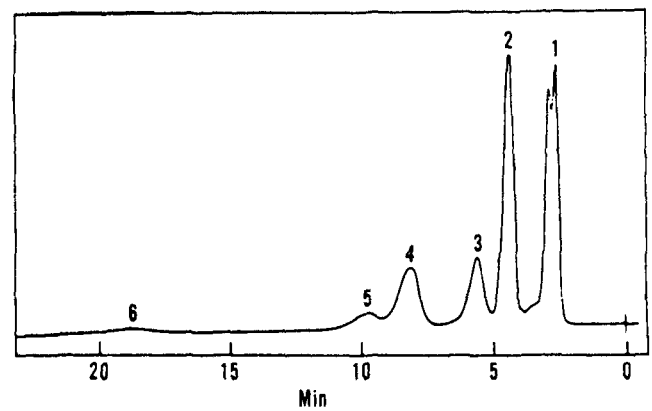

FIG. 2. High performance liquid chromatography (HPLC) of 0.20 μliter linseed esters with 0.5 ml/min 80:20 acetonitrile-water. Sample is 20 μliter of 1% linseed methyl esters in solvent mixture. Peaks are (1) oxidized or polymerized material, (2) methyl linolenate, (3) methyl linoleate, (4) methyl oleate, (5) methyl palmitate, and (6) methyl stearate.

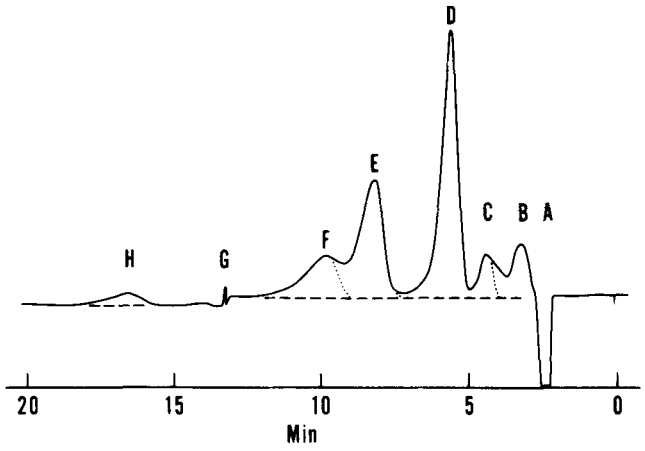

FIG. 3. High performance liquid chromatography (HPLC) of methyl esters of liquid hydrogenated vegetable oil. 30 μliter methyl esters diluted with 1 ml acetonitrile and 10 μliter of solution injected. Solvent is 80% acetonitrile and 0.5 ml/min. A. Acetonitrile; B. Unidentified, probably oxidized or polymerized material; C. Triene; C. Diene; E. Monoene; F. Palmitate; G. Solvent flow increased to 1 ml/min refractometer sensitivity increased 2X; and H. Stearate.

oleate with acetonitrile containing various amounts of water, 80 volume percent acetonitrile and 0.5 ml/min solvent flow were chosen to give good separations of unsaturated esters without an unreasonably long retention time for stearate. Figure 2 shows a typical separation of linseed methyl esters. In these esters which had been stored some time in a freezer, HPLC shows a large peak, probably oxidized or polymerized esters, near the position for nonretained compounds. In GLC, the presence of this component is shown only by a few small peaks of volatile material. Samples were injected as 5-20 μliter aliquots of solutions which contained 5-40 μliter of each major component with 100 μliter acetonitrile. Samples containing more than ca. 0.5 μliter of each component gave poorer resolution.

Good separations, like that in Figure 2, have been obtained with soybean, safflower, corn, and olive esters. Elaidate and *cis*-15 octadecenoate are eluted after oleate and are partly separated from it. Conjugated *trans,trans* octadecadienoate is eluted after and well separated from unconjugated linoleate; *cis,trans* conjugated octadecadienoate is separated only slightly from linoleate, and the conjugated *cis,cis* ester lies between its *cis,trans* and *trans, trans* conjugated isomers. Laurate is separated from lauroleate, myristate from myristoleate, and palmitate from palmitoleate.

Area percent of the peaks corresponds ca. to wt percent compositions, but some work indicates accuracy is increased by correcting for difference in refractive index of the esters. Table I shows results for a quantitative sample. For these results, N_D^{20} for 80% acetonitrile was measured with a dipping refractometer as 1.34614. Refractive indices for the unsaturated esters are taken from Gouw and Vlugter (5). Values for the saturated esters were obtained by extrapolating the equation of Craig (6) to 20 C, although the esters actually would be solid at this temperature. Correction factors are $(N_{18:0}-N_{solvent})/(N_{ester}-N_{solvent})$. Since oleate and plamitate were not separated well enough for individual integration, a correction factor was based upon the given composition of the sample. These values for correction factors only indicate the magnitude which might be expected. Additional work will be necessary to choose the best set of values.

TABLE I

Analysis of Gas Liquid Chromatographic Reference Standard AOCS 15-A (4) (Nu Chek Prep) by HPLC[a]

Ester	Wt (%)	Area (%)	Correction factor	Corrected (%)
18:3	3.0	3.2	0.782	2.9
18:2	50.0	51.6	0.849	49.3
18:1 + 16:0	41.0	41.4	0.940	43.7
18:0	3.0	1.8	1.000	2.0
20:0	3.0	2.0	0.977	2.1

[a]HPLC = high performance liquid chromatography.

TABLE II

Analysis of Methyl Esters of Commercial Liquid Hydrogenated Oil by HPLC and Comparison with Gas Chromatographic Values

Ester	Area (%)	Correction factor	Corrected (%)	Gas chromatograph[a] (%)
18:3	5.3	0.782	4.6	4.8
18:2	45.8	0.849	43.1	41.8
18:1	29.7	0.923	30.4	38.0
16:0	15.1	1.035	17.3	10.9
18:0	4.1	1.000	4.6	4.4

[a]Liquid Oil B of ref. 6.

Application of HPLC to analysis of a liquid commercially hydrogenated oil is shown in Figure 3 and Table II. The oil is Liquid Oil B in a previous publication (7). As shown in the figure, a small amount of unidentified material, probably oxidized or polymerized esters, is eluted before linolenate. Application of correction factors calculated as described above brings better agreement with the published gas chromatographic values. The greatest difference is in monoene and palmitate where the HPLC separation may place some *trans* monoenes in the palmitate peak. Other differences may be caused by variation of the tentative HPLC correction factors from their best values and difference between published gas chromatographic area percent and HPLC wt percent.

Work is currently in progress on scaling up these separations for small-scale preparative use.

REFERENCES

1. Ramachandran, S., P. Pei, and R.S. Henly. Paper 31G presented at 15th Eastern Analytical Symposium and National Meeting of the Society for Applied Spectroscopy, New York, N.Y., November, 1973.
2. Scholfield, C.R., J. Nowakowska, and H.J. Dutton, JAOCS 37:27 (1960).
3. Nickell, E.C., and O.S. Privett, Separ. Sci. 2:307 (1967).
4. "Official and Tentative Methods of the American Oil Chemists' Society," Vol. I and II, Third Edition, AOCS, Champaign, Ill., 1964 (revised to 1972).
5. Gouw, T.H., and J.C. Vlugter, JAOCS 41:426 (1964).
6. Craig, B.M., Can. J. Chem. 31:499 (1953).
7. Scholfield, C.R., V.L. Davison, and H.J. Dutton, JAOCS 44:648 (1967).

[Received April 12, 1974]