High Performance Size Exclusion Chromatography of Monomer, Dimer and Trimer Mixtures

C.N. Christopoulou[a] and **E.G. Perkins**[b,*]
[a]Colgate-Palmolive Co., 909 River Rd., Piscataway, NJ 08854, and [b]University of Illinois, Department of Food Science, 1208 West Pennsylvania Ave., Urbana, IL 61801

A high performance size exclusion chromatographic method was developed for the separation of monomer, dimer and trimer fatty acids in thermally oxidized or used fats and oils. The system was composed of two styrene/divinylbenzene copolymer columns with toluene as the mobile phase and refractometry as the mode of detection. The internal standardization method was used for quantitation with monostearin as the internal standard. Quantitation of monomer, dimer and trimer content in various samples correlated well with results obtained by GLC analysis and gravimetically by size exclusion chromatography.

Although many analytical methods have been used for the determination of the monomer, dimer and higher polymer content of oxidized fats and oils, the technique of size exclusion chromatography (SEC) may be considered as the most promising. Bartosiewics (1) reported the determination of monomer and dimer acids with cross-linked polystyrene beads but did not attempt quantitative analysis. Chang (2) used a porous styrene/divinylbenzene gel to determine small amounts of fatty acid dimers in tall oil. Hase and Harva (3) separated the monomer acid methyl esters from dimers and higher oligomers using a modified dextran gel, Sephadex LH-20. Inoue et al. (4) accomplished a GPC resolution of the methyl esters up to and including tetramer acids in 24 hr and obtained evidence of the presence of pentamer and higher oligomers. Using LH-20 and Bio-beads SX-1, Perkins et al. (5) separated both fatty acid and triglyceride polymers from heated corn oil. Aitzetmuller (6,7) indicated that SEC can be used as a measure and indication of the extent of heating and polymerization of heated fats and oil. Harris (8) achieved SEC separation of monomer, dimer and trimer acids within three hr and quantitation was possible with the use of heptanoic acid as internal standard. El-Hamdy (9), using Bio-Beads SX-1 and SX-2, fractionated thermally oxidized olive oil into six fractions with molecular weights ranging from 300 to more than 10,300 daltons. Schulte (10), using Bio-Beads SX-2 as separation material and dichloromethane as eluent, was able to separate polymerized triglyceride mixtures. Perrin et al. (11) used a gel permeation chromatographic system proposed by Ottaviani (12) for the separation of dimer from various oxidized oils, and quantitation was obtained by internal standardization with naphthalene as the internal standard.

Several high performance size exclusion chromatographic (HPSEC) systems have been reported for dimer isolation and quantitation. Christopoulou (13) defined a high performance chromatographic system consisting of a Bio-beads SX-4 column and tetrahydrofuran as eluting solovent which was used for the isolation of dimers from thermally oxidized olive oil. Perrin et al. (14) reported a HPSEC method for the fractionation of heated oils using high performance columns of styrene/divinylbenzene and tetrahydrofuran as eluent. Kupranycz et al. (15) used a HPSEC system similar to that of Perrin et al. (14) to determine dimer in thermally oxidized butterfat and vegetable oil samples. In the present study a simple, rapid and quantitative method for the determination of monomers, dimer, and trimer in oxidized oils is presented.

EXPERIMENTAL

High performance size exclusion chromatography (HPSEC). The HPSEC system used for the determination of monomer, dimer and trimer has been described previously and used for the quantitative analysis of mono-, di- and triglycerides (16). Components were separated on two series connected columns 25 cm × cm i.d., packed with spherical, styrene/divinylbenzene copolymer beads of an average particle size of five μm with the Lichrogel PS_4 column placed before the Lichrogel PS_1 column (E.M. Science, Gibbstown, New Jersey). Elution was carried out isocratically using toluene at a solvent rate of 0.5 ml/min. Refractometry was the mode of detection.

Other chromatographic solvents used during HPSEC studies were tetrahydrofuran and dichloromethane (A.C.S. grade, MBS Manufacturing Chemists, Inc., Cincinnati, Ohio). All eluents as well as samples were precleaned by passing them through a filter (<2 microns). Samples concentration was 10–15 mg/ml in toluene.

Gas liquid chromatography (GLC). Analytical GLC separations of monomer, dimer and trimer were carried out on an HP 5710-A Programmable Gas Chromatograph (Hewlett-Packard Company, Avondale, Pennsylvania). The following column and conditions were used for the analysis. Column: 3% OV-1 (dimethyl silicone) on 80/100 Supelcoport, 1 ft × 2 mm i.d., glass. Chromatographic conditions: Initial temp and time 120°C, temperature rate of 8°C/min to final temperature of 350°C with a hold time of 20 min. Injector and Detector (FID) temperature at 360°C and nitrogen carrier gas flow at 60 ml/min.

Size Exclusion Chromatography (SEC). The preparative SEC system consisted of a Tracor 995 Isochromatographic Pump (Tracor, Inc., Austin, Texas); a Rheodyne 7120 syringe loading sample injector with a 100-μl loop (Rheodyne, Berkeley, California), and a Waters Model 401 Differential Refractometer (Waters Associates, Framingham, Massachusetts). Chromatograms were recorded and peak areas determined using an HP 3390 A Integrator (Hewlett-Packard, Avondale, Pennsylvania).

*To whom correspondence should be addressed.

Separations and analyses were performed on two glass columns, 109 cm × 12.5 mm i.d., connected in series and packed with Bio-Beads SX-2 (Bio-Rad Laboratories, Richmond, California) which is a porous styrene/divinylbenzene copolymer with a molecular exclusion limit of 2,700 daltons. Toluene (A.C.S. grade, Fisher Scientific Co., Fairlawn, New Jersey) was used as the eluent at a constant flow rate of one ml/min. Sample concentration was 50–100 mg/ml in toluene, with 100-μl injection size.

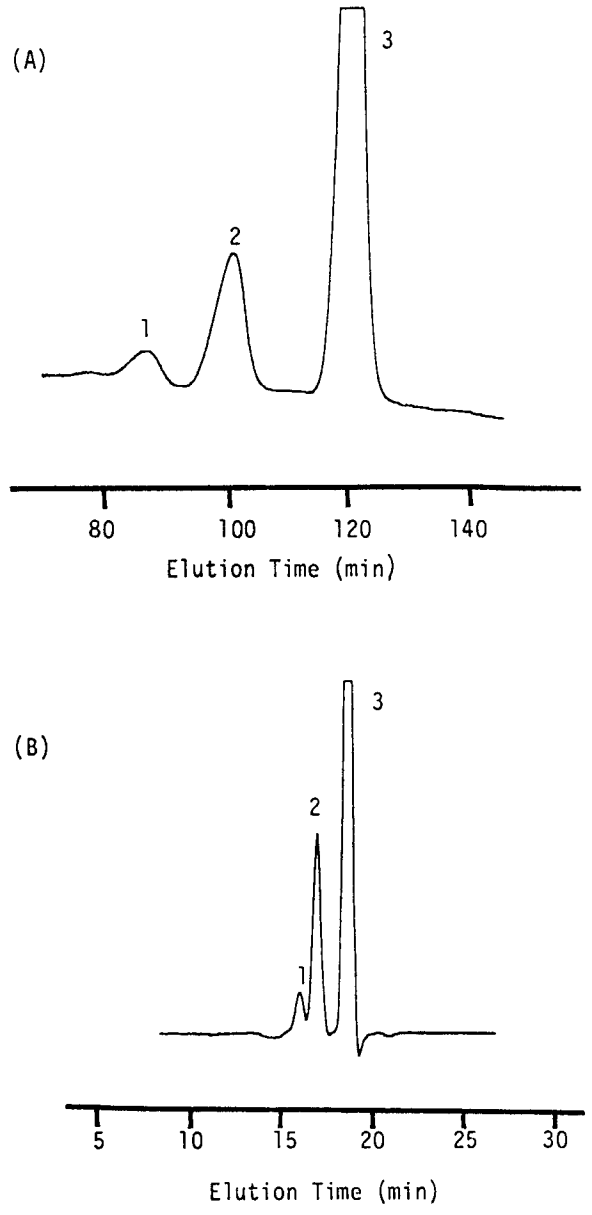

FIG. 1. HPSEC separation of standard mixtures. GPC (A) and HPSEC (B) separation of standard mixtures. For GPC (A) separation: Columns: Two glass 109 cm × 12.5 mm i.d., packed with Bio-Beads SX2, Eluent: Toluene, at a flow rate of 1 ml/min. Detector: Refractive index, Injection volume: 100 μl of 50–100 mg solute/ml toleune. Ambient temperature for HPSEC (B) separation. Columns: Lichrogel PS_4 + Lichrogel PS_1 (each 25 cm × 0.7 cm i.d.). Eluent: Toluene at a flow rate of 0.5 ml/min. Detection: RI. Injection volume: 20 μl. Sample conc: 1.5%. Temp: ambient. Peaks: 1, trimer (Empol. 1014); 2, dimer (Empol. 1010), and 3, monomer (methyl stearate) as the methyl ester.

Quantitation. The internal standardization method was used in order to determine monomer, dimer and trimer content by either HPSEC or GLC. Correction factors and weight percentage of each component in a sample were calculated as described earlier (16). Commercially available Empol 1014 and Empol 1010 (Emery Industries, Cincinnati, Ohio) as methyl esters as well as methyl stearate (Nu-Chek Prep, Inc., Elysian, Minnesota) were used to calculate correction factors for trimer, dimer and monomer, respectively. Monostearin (Nu-Chek Prep, Inc., Elysian, Minnesota) and dotriacontane (K&K Laboratories, Inc., Hollywood, California) were used as internal standards for HPSEC and GLC, respectively. The SEC determination of monomer, dimer and trimer was gravimetric.

Preparation of samples. The used oxidized soybean oil sample (Sample A) was prepared as described by Pinter (17). Sample B consisted of the reaction mixture from a synthesis of the dehydrodimer of methyl oleate

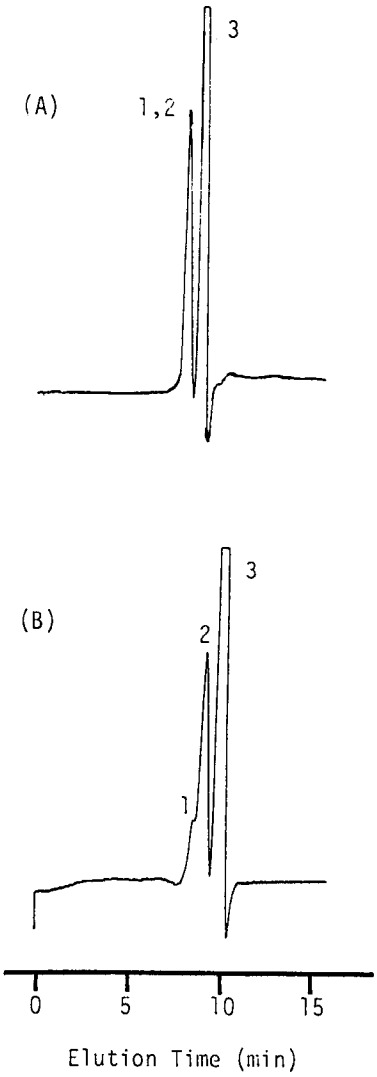

FIG. 2. Effect of gel pore dimensions on the HPSEC separation of standard mixtures. A, Lichrogel PS_1, and B, Lichrogel PS_4 (chromatographic conditions and peak identification as in Fig. 1).

as described by Paschke et al. (18). Both samples A and B were analyzed as methyl esters prepared according to AOCS Official Method Ce 1-62 (19).

RESULTS AND DISCUSSION

HPSEC involves the separation of molecules in solution according to their "effective size," which is closely related to the molecular weight and shape of the molecules. In Figure 1, a typical chromatogram of the separation of a standard mixture of monomer, dimer and trimer with both SEC and HPSEC is presented.

The standard mixture was used to examine the various parameters that affect separation of its components. Calibration curves were determined for each column. The upper molecular weight exclusion limit was 5.10^3 daltons and 2.10^3 daltons for Lichrogel PS_4 and Lichrogel PS_1, respectively. One hundred sixty daltons was the lower exclusion limit for both columns. In Figure 2, the chromatograms of the separation of the standard mixture using either Lichrogel PS_4 or Lichrogel PS_1 column as the stationary phase are presented. In order to achieve separation of dimer and trimer fatty acids, it was necessary to use both columns in series.

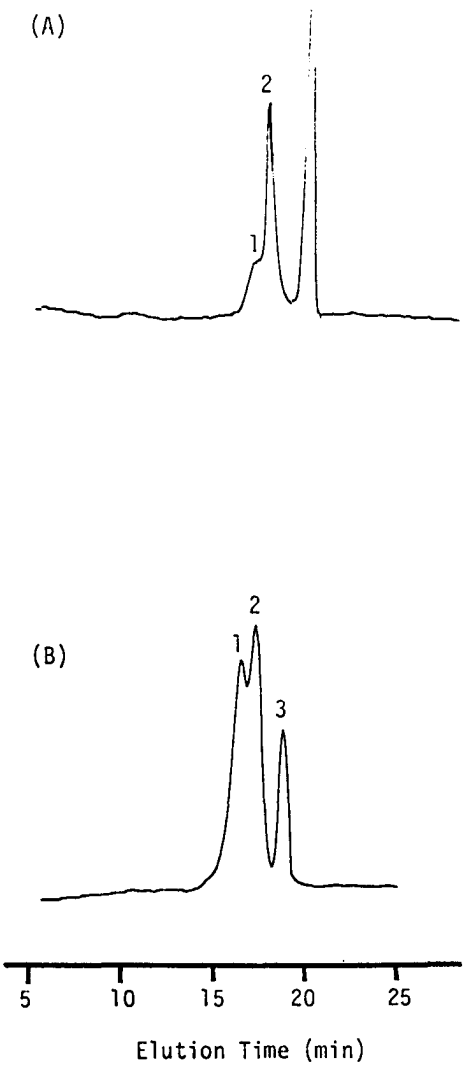

FIG. 3. Effect of solvent on the HPSEC separation of standard mixture. A, CH_2Cl_2, and B, THF. (Chromatographic conditions and peak identification as in Fig. 1).

The effect of solvent type on the separation of the components of the standard mixture was also examined. Many solvents of varying degrees of polarity and compatible with SEC were examined as potential swelling agents and eluting solvents. The separations obtained when either tetrahydrofuran or methylene chloride were used as eluents are shown in Figure 3. The more nonpolar solvent results in less swelling of the gels, which is reflected in a decrease of the fractionation range of the gel. The decrease in the fractionation range resulted in reduced resolution for the diner and trimer peaks. In general, all solvents with a solvent strength parameter ($\varepsilon°$) greater than that of toluene ($\varepsilon° = 0.29$) resulted in incomplete resolution of dimer and trimer peaks (20).

In order to determine the optimum flow rate in terms of resolution and analysis time, separations of the standard mixture at various flow rates were also evaluated. In Figure 4, the chromatograms of the standard mixture determined at flow rates 0.3 and 1.0 ml/min of toluene are presented. At flow rates higher than 0.5 ml/min the resolution between dimer-trimer is decreased, whereas at flow rates smaller than 0.5 ml/min the resolution of dimer-trimer was not superior to that obtained at 0.5 ml/min; thus, the flow rate of 0.5 ml/min was considered optimum.

Quantitation of monomer, dimer and trimer mixtures was also of primary interest. Monostearin was used as internal standard and is well resolved from the monomer peak, as indicated in Figure 5. In Figure 6, the GLC separation of the standard mixture and the internal standard, dotriacontane, is presented. Correction factors (16) calculated for the components of the standard mixture from either HPSEC or GLC data are presented in Tables 1 and 2. In both, HPSEC and GLC correction factors increased with molecular weight and exhibited small standard deviation for these components. Response correction factors in GLC have been shown to increase in proportion to molecular weight and are proportional to the carbon number and at percent carbon in the molecule (21,22). Response correction factors in HPSEC and GPC obtained by refractometry have not been studied, and they also are usually determined with the aid of standards. (Recent results obtained in our laboratory have indicated that the differences in response as one goes from monomer to trimer appears to be related to color of the components which increases with molecular weight. This effect appears related to detector design since a differential refractometer from another manufacturer showed little differences in response as did an infrared detector.) There does appear, however, to be a relationship between the response factor and molecular weight as indicated in the Loventz-Lorenz equation describing molar refraction as a function of refractive index, density and molecular weight (23). These corrections factors were used for quantitation of trimer, dimer and monomer in standard and authentic samples of various reaction mixtures and heated and oxidized fats and oils. In Figure 7, the HPSEC chromatograms of a heated oxidized soybean oil and the reaction mixture of the dehydrodi-

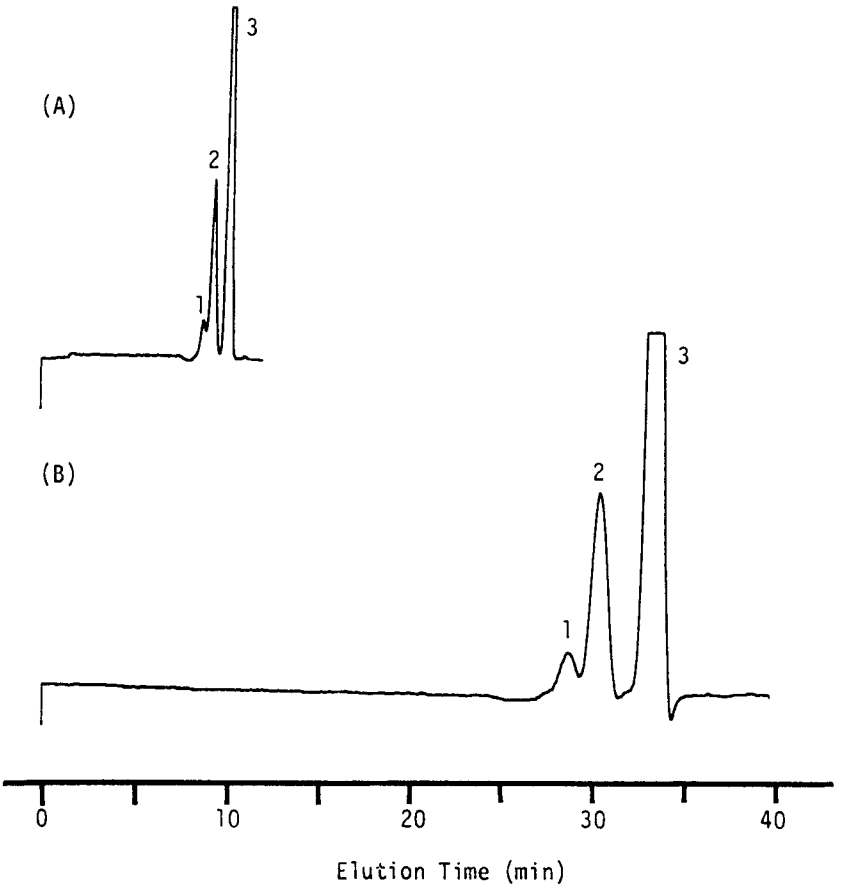

FIG. 4. Effect of solvent flow rate on the HPSEC separation of standard mixture. A, 1 ml/min, and B, 0.3 ml/min. (Chromatographic conditions and peak identification as in Fig. 1).

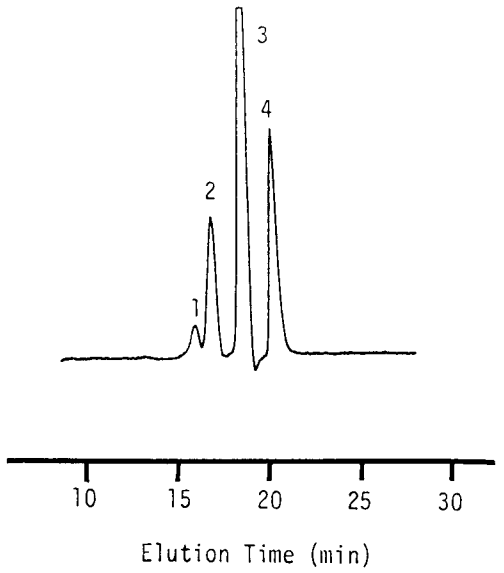

FIG. 5. HPSEC separation of standard mixture and internal standard. [Conditions and peaks identification as in Fig. 1; Peak 4, monostearin (internal standard)].

TABLE 1

HPSEC Correction Factors for Trimer, Dimer and Monomer Mixtures[a,b]

Mixture no.	Correction factors		
	Trimer	Dimer	Monomer
1	8.88	2.89	0.62
2	9.49	2.77	0.61
3	8.92	2.83	0.65
X ± std dev	9.07±0.34	2.83±0.06	0.63±0.02

[a]HPSEC conditions as in Fig. 1.
[b]Correction factors calculated as described in (16). Average of four observations.

TABLE 2

GLC Correction Factors for Trimer, Dimer and Monomer Mixtures[a,b]

Mixture no.	Correction factors		
	Trimer	Dimer	Monomer
1	1.93	1.45	1.05
2	1.84	1.46	1.02
3	1.91	1.43	1.04
X ± std dev	1.89±0.05	1.45±0.02	1.04±0.02

[a]GLC conditions as described in experimental section.
[b]Correction factors calculated as described in (16). Average of four observations.

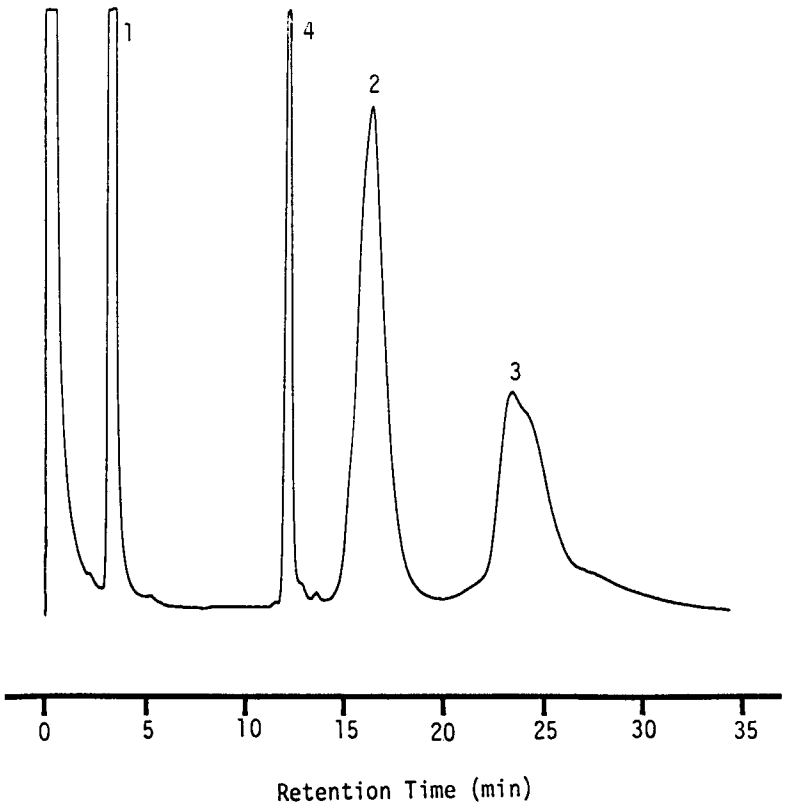

FIG. 6. GLC separation of standard mixture. (Conditions as described in experimental section). Peaks: 1, monomer (methyl stearate); 2, dimer (Empol. 1010); 3, trimer (Empol. 1014), and 4, dotriacontane (internal standard).

TABLE 3

Quantitative Analysis of Oxidized Soybean Oil (sample A) and Dehydrodimer of Methyl Oleate Reaction Mixture (sample B)[a,b]

Sample	Component	HPSEC[c]	GLC[d]	GPC[e]
A	Trimer	6.95	6.47	7.86
	Dimer	15.23	16.90	16.53
	Monomer	77.82	76.63	76.61
B	Trimer	8.56	9.33	8.37
	Dimer	17.90	18.88	17.34
	Monomer	73.44	73.79	74.29

[a]Samples A and B prepared as described in experimental section.
[b]Average of four observations.
[c]HPSEC conditions as in Fig. 1.
[d]GLC conditions as described in experimental section.
[e]GPC conditions as described in experimental section.

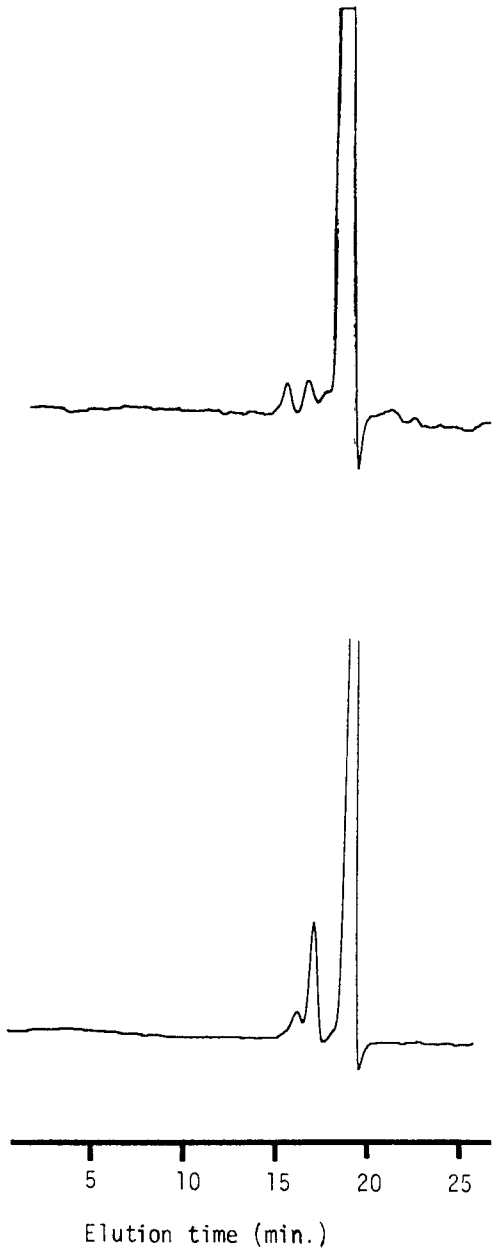

FIG. 7. HPSEC chromatograms of oxidized soybean oil (sample A) and the dehydrodimer at methyl oleate reaction mixture (sample B). (Chromatographic conditions and peaks identification as in Figure 1).

mer of methyl oleate are presented. The quantitative results obtained are presented in Table 3. The HPSEC results compare well with the SEC and GLC quantitative analysis of these samples.

The HPSEC method described here has been used for the analysis of a wide variety of samples. It is especially simple and provides good resolution and quantitation of monomer, dimer and trimer mixtures.

ACKNOWLEDGMENT

Partial support from the University of Illinois Agricultural Experimental Station is gratefully acknowledged.

REFERENCES

1. Bartosiewics, R.L., *J. Paint Technol. 39*:28 (1967).
2. Chang, T.L., *Anal. Chem. 40*:989 (1968).
3. Hase, H., and O. Harva, *Kem. Teollisus. 25*:134 (1968).
4. Inoue, H., K. Koniski and N. Tanigushi, *J. Chromatogr. 47*:348 (1970).
5. Perkins, E.G., R. Taubold and A. Hsieh, *J. Am. Oil Chem. Soc. 50*:223 (1972).
6. Aitzetmuller, K., *J. Chromatogr. 74*:355 (1972).
7. Aitzetmuller, K., *Fette, Seifen, Anstrichm. 77*:598 (1972).
8. Harris, W.C., E.P. Crowell and B.B. Burnett, *J. Am. Oil Chem. Soc. 50*:537 (1973).
9. El Hamdy, A., MS Thesis, University of Illinois, Urbana, Illinois, 1979.
10. Schulte, Von T., *Fette, Seifen, Anstrichm. 84*:178 (1982).
11. Perrin, J.L., P. Perfetti, C. Dimitriades and M. Naudet, *Rev. Fr. Corps Gras. 32*:151 (1985).
12. Ottaviani, P., Thesis de doctorate de specialitié, Marseille, France, 1977.
13. Christopoulou, C.N., MS Thesis, University of Illinois, Urbana, Illinois, 1984.
14. Perrin, J.L., F. Federo and A. Prevot, *Rev. Fr. Corps Gras. 32*:131 (1984).
15. Kupranycz, D.B., M.A. Amer and B.E. Baker, *J. Am. Oil Chem. Soc. 63*:332 (1986).
16. Christopoulou, C.N., and E.G. Perkins, *Ibid. 63*:679 (1986).
17. Pinter, S., and E.G. Perkins, *Ibid. 65*:783 (1988).
18. Paschke, R.F., L.E. Peterson, S.A. Harrison and D.H. Wheeler, *Ibid. 41*:56 (1964).
19. *Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1,* 3rd edn., edited by R.O. Walker, AOCS, Champaign, IL 1981, Method Ce 1-62.
20. Snyder, L.R., in *Principles of Adsorption Chromatography,* edited by J.C. Giddings and R.A. Keller, Marcel Dekker, Inc., New York, NY, 1968, pp. 192-199.
21. Ackman, R.G., and J.C. Sipos, *J. Chromatogr. 16*:298 (1964).
22. Scanlon, J.T., and D.E. Willis, *J. Chrom. Sci. 23*:333 (1985).
23. Physical Methods of Organic Chemistry part II, in *Techniques of Organic Chemistry Vol. 1,* edited by A. Weissberger, Interscience Publishers, Inc., N.Y., 1960, pp. 1621 and 1627.

[Received March 2, 1988;
accepted October 21, 1988]