sparger design, size and location of baffles, etc. The reaction kinetic parameters should be similar in all the cases since essentially the same reactions occur everywhere. As seen in Table III, these parameters are quite similar. However, owing to slight differences in the impurities present in the different cases, which do influence the reactions in a complex way, some deviations can be expected to be present.

REFERENCES

1. Chakravarti, T., S. Bhatia and D.N. Saraf, JAOCS 59:157 (1982).
2. Hashimoto, K., K. Muroyama and S. Nagata, Ibid. 48:291 (1971).
3. Beveridge, G.S.G. and R.S. Schechter, Optimization: Theory and Practice, McGraw-Hill, New York, 1970.

[Received April 20, 1982]

M. W. Dong and J.L. DiCesare. Improved Separation of Natural Oil Triglycerides Using Columns Packed with 3-um Particles. J. Amer. Oil Chem. Soc. 60, 788-791, 1983.

Improved Separation of Natural Oil Triglycerides by Liquid Chromatography Using Columns Packed with 3-µm Particles

M.W. DONG and J.L. DiCESARE, Chromatography Division, Perkin-Elmer Corporation, Norwalk, CT 06856

ABSTRACT

Very high-resolution separations of triglycerides in various natural oils have been demonstrated by liquid chromatography using short columns packed with 3-µm alkyl bonded-phase particles. Analysis times range from 8 to 16 min without prior sample clean-up. The primary detector used was a refractive index detector having low dispersive characteristics. Both the high efficiency of the columns and the selectivity of the 3-µm packing material contribute to the separation of several critical pairs of triglycerides. A substantial reduction in analysis time was also achieved. An ultraviolet detector operated at 220 nm was used to illustrate an alternative detection approach.

INTRODUCTION

The use of high-performance liquid chromatography (LC) for the separation of individual triglycerides present in fats and oils has been increasing in recent years. These analyses are important in the natural oil industry for process and product quality control purposes. Also, at the research/development level, detailed triglyceride data might facilitate the understanding of triglyceride biosynthesis and deposition in plants and animal cells (1).

The LC system most commonly employed in triglyceride analysis consists of an alkyl bonded-phase column and a refractive index detector. Although aqueous mobile phases are generally used with these columns, due to the lipophilicity of triglycerides, water cannot be used in the mobile phase for this particular application. Therefore the mobile phases generally employed consist of mixtures of acetone and acetonitrile, and occasionally tetrahydrofuran, methylene chloride or hexane. The conspicuous absence of water in the mobile phase, prompted the term nonaqueous reversed-phase or NARP to describe the above system.

El-Handy et al. (1-2) and Plattner (3) have reported quite extensively on column and mobile phase selectivities in triglyceride separations. Jansen (4) has studied the effect of low temperature and Lie Ken and Jie (5) have investigated several quantitative aspects of triglyceride analysis. Parris (6) and Payne-Wahl et al. (7) have shown the utility of an infrared detector and gradient elution in triglyceride separation. The latter group has also demonstrated the capability of analyzing free acids, mono-, di- and triglycerides in a single assay (7). Various natural oils have been studied in some detail using NARP chromatography, including oils from palm (1,9), olive (4,10), peanut butter (8), soybean (1,3,7), coconut (2,9), corn (1), rapeseed (9), and cocoa butter (9).

Recent advances in column and instrument technology have significantly enhanced LC performance in recent years (11,12). We have previously reported the separation on many important food constituents in 1-3 min using short, small-particle columns (13,14). The aim of this study is to demonstrate the utility of this improved LC system in triglyceride separations.

EXPERIMENTAL

Reagents

Triglyceride standards were of the highest purity grade purchased from Supelco, Inc. (Bellefonte, PA) and Sigma Chemical Co. (St. Louis, MO). HPLC-grade acetonitrile, acetone and tetrahydrofuran (THF) were obtained from Fisher Scientific (Pittsburgh, PA). Natural oil samples were purchased at the open market in Connecticut. The palm olein sample was obtained from sources within the industry.

Columns

The LC columns used in this study were Perkin-Elmer HS-3 high-speed columns packed with 3-µm C_{18} bonded-phase particles. Dimensions of the columns are 100 X 4.6 mm id with a column void-volume of ca. 0.8 mL and efficiencies in the range of 13,000-15,000 theoretical plates measured under optimized conditions. Details on important column characteristics are available elsewhere (11,15). Because of the low column void-volume and high-efficiency, the resultant peak volumes are typically only 25-100 µL. Therefore, extra-column band broadening from injector, connecting tubing and detector must be minimized to preserve column performance (12). Several Perkin-Elmer HS-5 C_{18} columns (125 X 4.6 mm id) packed with 5-µm particles were also used; however, due to differences in packing selectivity, separation of critical triglyceride pairs were, in general, not satisfactory with these columns.

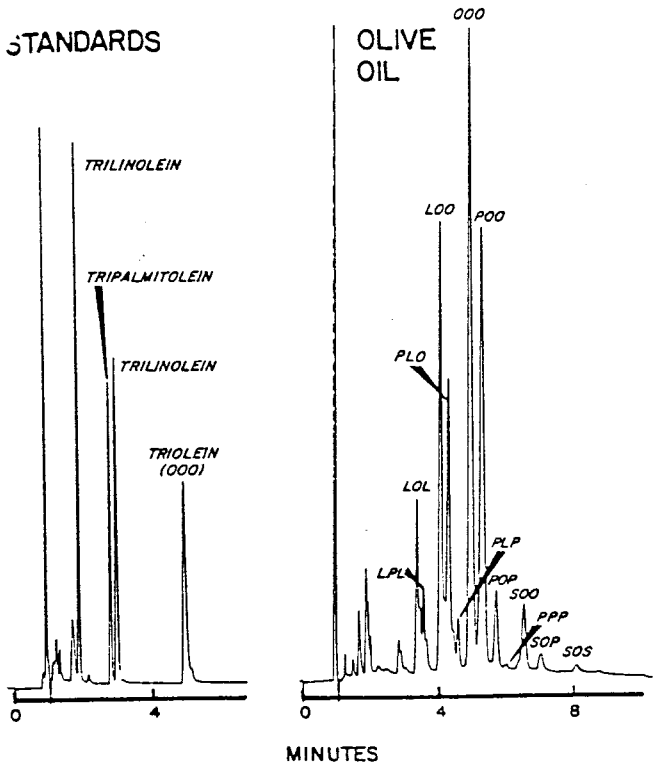

FIG. 1. Separation of triglycerides in a standard mixture and in olive oil. Column: two 100 X 4.6 mm id columns in series, 3-μm C_{18} bonded-phase packing. Mobile phase: 7:3 acetone acetonitrile at 2.5 mL/min; 26 MPa (3800 psi) inlet pressure. Refractive index detector. Key: P=palmitic acid, S=stearic acid, O=oleic acid, L=linoleic acid; 000 triolein, etc. Sample: 5 μL of a 5%-olive oil sample in acetone.

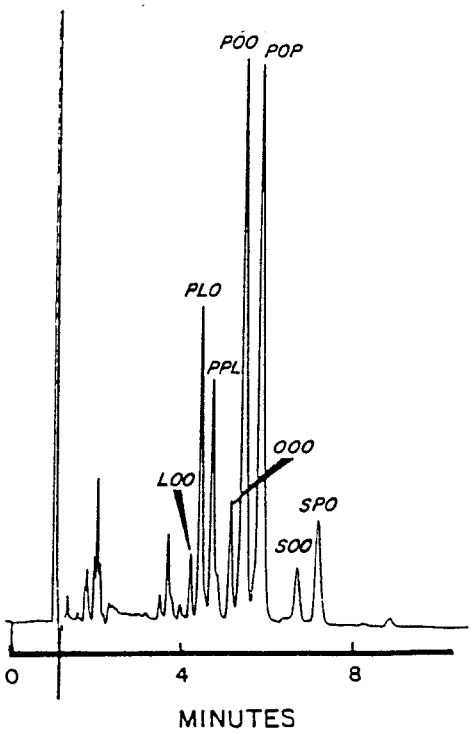

FIG. 2. Separation of triglycerides in palm olein. Column: conditions and key to peak identification as in Figure 1.

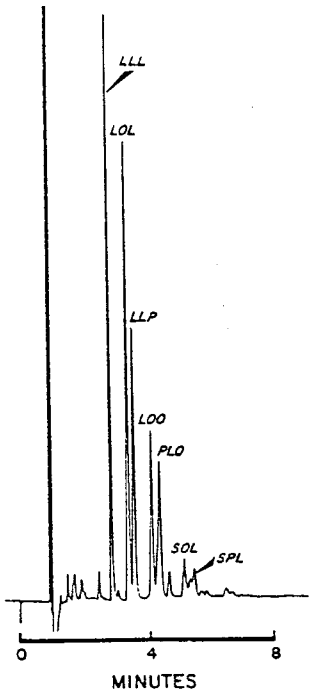

FIG. 3. Separation of triglycerides in corn oil. Column, conditions and key to peak identification as in Figure 1.

Instruments

The LC system used was a Perkin-Elmer Series 4 liquid chromatograph equipped with a Model 7125S syringe-loading injector valve. This injector has an internal bypass loop to prevent pressure surges created during the injection process (16). All connecting tubing in the sample flow path was 1/16-in od and 0.007-in (0.18 mm) id. Two detectors were used: a modified Perkin-Elmer Model LC-25 refractive index detector having an 8-μL flow cell and 0.007-in id inlet tubing, and a Perkin-Elmer model LC-85 variable wavelength ultraviolet-visible (UV/vis) detector (190–600 nm) having a 2.4-μL flow cell and fast response time (290 ms full scale). A Perkin-Elmer Sigma 15 chromatography data station was used for data handling.

RESULTS AND DISCUSSION

Figure 1 shows the separation of a synthetic mixture of triglyceride standards and an olive oil sample using two 3-μm C_{18} columns connected in series with NARP conditions. The mobile phase used was 7:3 acetone/acetonitrile at a flow rate of 2.5 mL/min. Twelve triglycerides were tentatively identified. These identifications were based on comparing retention times of genuine standard materials and by elution orders of olive oil triglycerides obtained from the published literature (1,4,10). A 5% solution (v/v) of olive oil in acetone was injected directly without any prior sample clean-up. To our knowledge, the separation obtained in Figure 1 represents the highest resolution of olive oil triglycerides reported thus far (1,4,10). The analysis time of 8 min is also significantly faster than any other published methods. Attempts to duplicate this separation on several 5-μm C_{18} columns packed with different packing materials were not successful. This leads us to believe that both the efficiency and selectivity of the

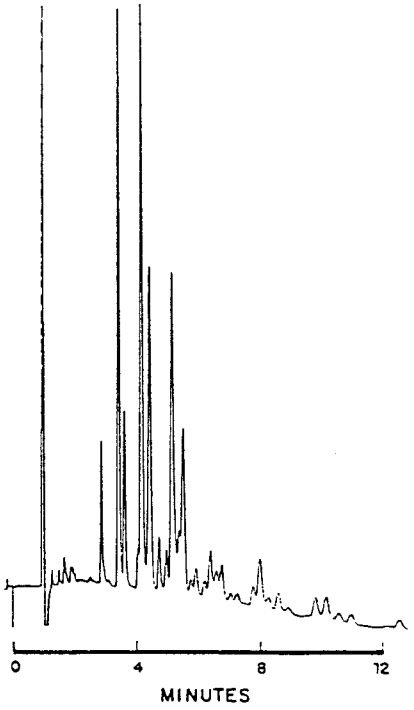

FIG. 4. Separation of triglycerides in peanut oil. Column and conditions as in Figure 1.

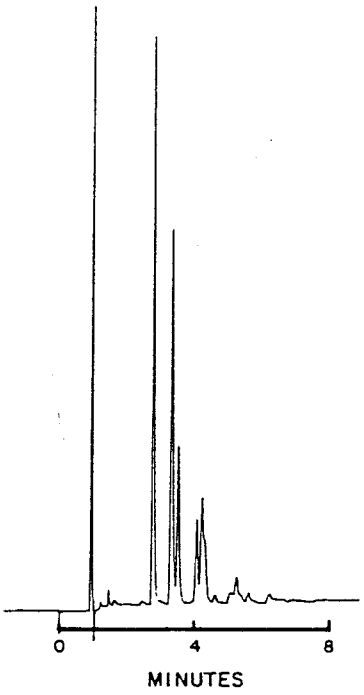

FIG. 6. Separation of triglycerides in sunflower seed oil. Column and conditions as in Figure 1.

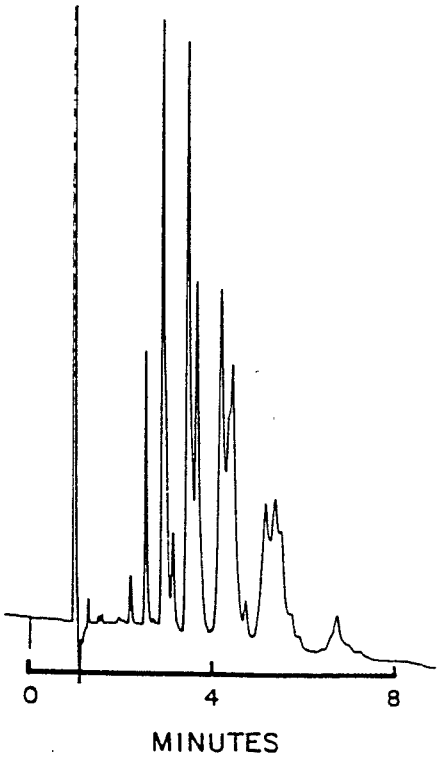

FIG. 5. Separation of triglycerides in vegetable oil. Column and conditions as in Figure 1.

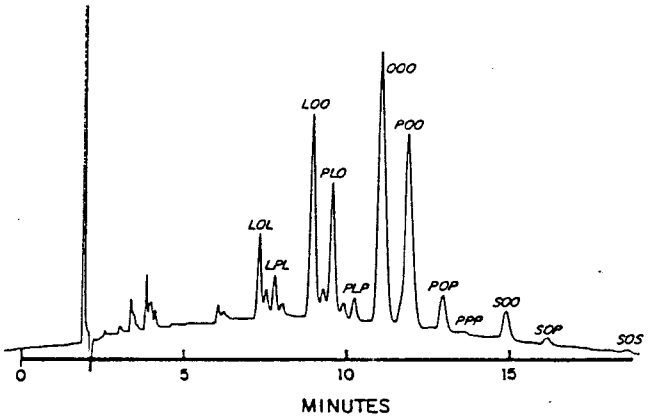

FIG. 7. High-resolution separation of triglycerides in olive oil. Column and mobile phase as in Figure 1, except three columns were connected in series. Mobile phase flow rate and inlet pressure: 1.8 mL/min, 28 MPa (4100 psi). Refractive index detector.

3-μm packings contribute to the resolution of these triglycerides.

Triglycerides in Other Natural Oils

Figures 2–6 show chromatograms of triglycerides in various natural oils, including oils from palm, peanut, corn, vegetable and sunflower seed. LC conditions were identical to that in Figure 1. Resolution between various triglyceride components was also found to be better than those previously published (1,4,8–10) with reduction in analysis times by a factor of 3–10.

Triglyceride Analysis Performed With Higher Resolution and With UV Detection

Figure 7 shows the separation of olive oil triglycerides using three 3-μm columns connected in series. The efficiency of this column system exceeds 40,000 theoretical plates measured under optimized conditions. The mobile phase was unchanged and the flow rate was reduced to 1.8 mL/min. Compared to Figure 1, a dramatic improve-

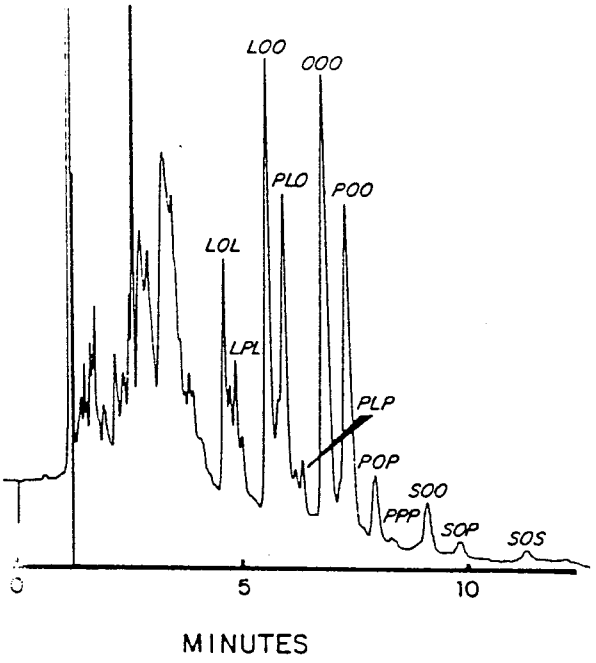

FIG. 8. Separation of triglycerides in olive oil using UV detection. Column as in Figure 1. Mobile phase: 3:7 tetrahydrofuran acetonitrile at 2.0 mL/min; 22 MPa (3200 psi) inlet pressure. UV detector at 220 nm.

ent in resolution was observed. The analysis time, however, was increased by a factor of two.

Figure 8 shows the separation of the same sample of olive oil using a different mobile phase (3:7, THF acetonitrile) with UV detection at 220 nm. Compared to the separation in Figure 1 using an acetone/acetonitrile mobile phase and refractive index detection, the chromatogram in Figure 8 appears remarkably similar for triglycerides of high retention times. However, peaks corresponding to the lower retention times appear to be enhanced, presumably due to the higher molar absorptivity of these species. The use of UV detection for triglyceride separations is of some interest because of its compatibility with gradient elution. This aspect of the study is currently being investigated.

REFERENCES

1. El-Handy, A.H., and E.G. Perkins, JAOCS 58:867(1981).
2. El-Handy, A.H., and E.G. Perkins, JAOCS 58:49(1981).
3. Plattner, R.D., JAOCS 58:638(1981).
4. Jensen, G.W., J. Chromatogr. 204:407(1981).
5. Jie, M.S., and F. Lie Ken, Ibid. 192:457(1980).
6. Parris, N.A., J. Chromatogr. Sci. 17:54(1979).
7. Payne-Wahl, K., G.F. Spencer, R.D. Plattner and R.O. Butterfield, J. Chromatogr. 209:61(1981).
8. Bezard, J.A., and M.A. Ouedraogo, Ibid. 196:279(1980).
9. Peterson, B.O. Podlaha and B. Toregard, JAOCS 58:1005 (1981).
10. Anon. Supelco Reporter 2:1(1980).
11. DiCesare, J.L., M.W. Dong and L.S. Ettre, Chromatographia 14:257(1981).
12. DiCesare, J.L., M.W. Dong, and J.G. Atwood, J. Chromatogr. 217:369(1981).
13. Dong, M.W., and J.L. DiCesare, Presentation at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1982, Paper No. 667.
14. Dong, M.W., and J.L. DiCesare, Food Technol., 37:58(1983).
15. DiCesare, J.L., M.W. Dong and F.L. Vandemark, Lab. 13:52 (1981).
16. DiCesare, J.L., M.W. Dong and J.R. Gant, Chromatographia, 15:595(1982).

[Received August 23, 1982]