Analysis of Triglycerides Using Atmospheric Pressure Chemical Ionization Mass Spectrometry

Wm. Craig Byrdwell* and Edward A. Emken

FQS, NCAUR, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: Atmospheric pressure chemical ionization (APCI) mass spectrometry was investigated as a new method for analysis of a mixture of triglycerides separated by reverse-phase high-performance liquid chromatography. A mixture of homogeneous (monoacid) triglyceride standards containing fatty acids with zero to three double bonds was analyzed to demonstrate the quality of mass spectra obtained by using the APCI interface. The mass spectra showed that minimal fragmentation occurs, resulting primarily in diglyceride $[M - RCOO]^+$ ions and $[M + 1]^+$ protonated molecular ions. The degree of unsaturation within the acyl chains had a marked effect on the proportion of diglyceride ions vs. the $[M + 1]^+$ ions formed in the APCI source. The mass spectra of triglycerides containing fatty acids with two or three double bonds showed predominantly protonated triglyceride ions, with diglyceride peaks representing 13 to 25% of the base peak. The triglycerides containing singly unsaturated fatty acids gave diglyceride ions as the base peak, and $[M + 1]^+$ ions with an intensity 20 to 28% that of the base peak. Only diglyceride ions were observable in the spectra of triglycerides containing saturated fatty acids.

Lipids 30, 173–175 (1995).

Separated (1–6), fractionated (7–9) and unseparated (10–20) mixtures of triglycerides (TG) have been analyzed using direct inlet introduction (1–4,6), desorption chemical ionization (9,19, 20), direct insertion probe introduction (7,8,10–14), electrospray (18) and other (5,15–17) mass spectrometric methods. The use of electron impact ionization methods for analysis of TG generally results in spectra containing low-molecular weight fragments, with no quasimolecular ions present. Spectra of TGs obtained using desorption chemical ionization methods show increased amounts of quasi-molecular ions, while retaining moderate fragmentation, which can be useful for structural assignment. Desorption chemical ionization, however, is not amenable to use for direct detection in a high-performance liquid chromatography (HPLC) flow system. Direct insertion probe methods can provide spectra containing minimal fragmentation, with primarily diglyceride and TG ions. These spectra may readily allow identification of a number of molecular species of TG. However, direct probe introduction is also not amenable to direct detection in a flow system. Direct inlet introduction allows direct detection in an HPLC system and also produces spectra that contain sizeable quasimolecular ions and minimal fragmentation. It has been demonstrated to be an effective interface for TG analysis (1–4,6). Electrospray ionization (ESI) has been used for analysis of a mixture of TGs infused *via* syringe pump (18). Spectra obtained using ESI contained only quasimolecular ions, with no fragmentation. This lack of fragmentation can result in ambiguity in structural assignments for TG of identical molecular weight. Tandem mass spectrometry/mass spectrometry (MS/MS) could provide the additional structural information necessary to uniquely identify TG species. Collision-induced dissociation can also be used to produce lower molecular weight ions. The flow rates used for syringe pump infusion are very low compared to those in a conventional HPLC flow system. The necessity of an ionic buffer in the effluent for ESI will affect the types of columns and solvents which may be used. Alternatively, a sheath liquid may be used to provide the necessary ionic buffer. In either case, the ESI–MS detectability is based on the ability to ionize the neutral TG in solution. So, while preliminary results of use of ESI for detection of nonpolar TG are encouraging, further data will be necessary for a thorough comparison of ionization methods in an HPLC system.

Atmospheric pressure chemical ionization (APCI), like ESI, is an atmospheric pressure ionization method (21). However, unlike ESI, APCI uses a corona needle discharge to impart charge onto vaporized molecules which are sprayed from a capillary inlet. These are swept into the high vacuum region of the mass spectrometer through a capillary bleed. Also unlike ESI, APCI requires no buffers in solution in order to produce efficient fragmentation. This lack of need of a buffer allows neutral, nonpolar molecules to be analyzed as easily as more polar molecules which are ionizable in solution. An APCI interface allows direct introduction of HPLC column effluent at a rate of up to 2 mL/min.

Presented here is the first report of an APCI interface for the direct mass spectrometric analysis of TG standards. We demonstrate that, because of minimal fragmentation, APCI is

*To whom correspondence should be addressed at FQS, USDA, ARS, NCAUR, 1815 N. University St., Peoria, IL 61604.

Abbreviations: APCI, atmospheric pressure chemical ionization; DG, diglyceride; ELSD, evaporative light-scattering detector; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; MS, mass spectrometry; RIC, reconstructed ion chromatogram; RP-HPLC, reverse-phase high-performance liquid chromatography; TG, triglyceride.

an effective method for identification of TG species separated by reverse phase HPLC (RP-HPLC).

MATERIALS AND METHODS

TG. Individual TG standards were obtained from Nu-Chek-Prep (Elysian, MN). A TG mixture was made by dissolving 25–37 mg of each TG (liquid or solid) in 5 mL of tetrahydrofuran, for a total concentration of 57.0 mg/mL. The following homogeneous (monoacid) TG were included in the mixture: tripalmitin (tri 16:0, PPP), tripalmitolein (tri 16:1, PoPoPo), tristearin (tri 18:0, SSS), triolein (tri 18:1, OOO), trilinolein (tri 18:2, LLL), trilinolenin (tri 18:3, LnLnLn), triarachidin (tri 20:0, AAA), trigadolein (tri 20:1, GGG) and trierucin (tri 22:1, EEE).

Chromatography. All solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Solvents were HPLC grade or the highest available quality and were used without further purification. The HPLC pump was an LDC 4100 MS (Thermo Separation Products, Schaumburg, IL) quaternary pump system with membrane degasser. The columns used were an Adsorbosphere C18 (Alltech Associates, Deerfield, IL), 25 cm × 4.6 mm, 5 μm (12% carbon load) in series with an Adsorbosphere UHS C18 25 cm × 4.6 mm, 10 μm (30% carbon load). A gradient solvent program with propionitrile (PrCN) and hexane (Hex) was used to accomplish the separations. The gradient used was as follows: initial 88% PrCN, 12% Hex; linear from 10 to 15 min to 75% PrCN, 25% Hex, held until 25 min; linear from 25 to 30 min to 50% PrCN, 50% Hex, held until 40 min. The flow rate was 1 mL/min. The flow was split so that ~600 μL/min went to an evaporative light-scattering detector (ELSD) and ~400 μL/min went to the APCI interface. 5 μL of the TG mixture was injected, which resulted in ~114 μg of total TG mixture (10 to 15 μg of each component) going to the APCI interface. High signal-to-noise ratios in the mass spectra indicate that usable spectra may be obtainable using perhaps a hundred-fold less sample. However, the total ion chromatographic signal-to-noise ratio is much lower, so smaller samples necessitate greater reliance upon the ELSD chromatogram.

The ELSD was an ELSD MKIII (Varex, Burtonsville, MD). The drift tube was set at 140°C, the gas flow was 2.0 standard liters per minute. High-purity N_2 was used as the nebulizer gas.

MS. A Finnigan MAT (San Jose, CA) SSQ 710C fitted with an APCI source was used to acquire mass spectral data. The vaporizer was operated at 240°C; the capillary heater was operated at 250°C. The corona voltage was set at 5 μA. High-purity nitrogen was used for the sheath and auxiliary gases, which were set to 55 psi and 5 mL/min, respectively. Spectra were obtained from 400 to 1100 amu, with a scan time of 2.5 s. Preliminary data showed no substantial ions below 400 amu. The total ion chromatogram was processed using the data system's automatic seven-point smoothing and peak label functions. A peak threshold of 1% intensity was applied to the mass spectra.

RESULTS AND DISCUSSION

The reconstructed ion chromatogram (RIC) of the separated TG mixture is presented in Figure 1. The chromatogram obtained from the ELSD is similar to the RIC and is not presented here. Representative mass spectra of monoacid TG containing 3, 2, 1 and 0 sites of unsaturation in the acyl chains are shown in Figure 2. The mass spectra of the TG showed very little fragmentation. The most significant fragments present in these spectra arose from the protonated molecular ion $[TG + 1]^+$ and the diglyceride (DG) ion $[M - RCOO]^+$, or $[DG]^+$. The TG that contained two or more sites of unsaturation (LnLnLn and LLL) in the fatty acyl chains gave a $[TG + 1]^+$ base peak. The $[DG]^+$ peak of those TG had an intensity of approximately 20 to 35% that of the base peak. Those TG containing less than two sites of unsaturation in each acyl chain showed a base peak arising from the DG ion. TG that contained one site of unsaturation in each of the acyl chains (PoPoPo, OOO, GGG, EEE) showed a $[DG]^+$ base peak, with a $[TG + 1]^+$ peak intensity which was 15 to 20% that of the base peak. Completely saturated TG produced no detectable $[TG + 1]^+$ peak. The spectra of these TG consisted solely of $[DG]^+$ fragments. Spectra of most TG showed minor peaks representing the addition of water to the DG and/or TG ion, $[DG + 18]^+$ and/or $[TG + 1 + 18]^+$. Adduct formation of some TG with the propionitrile solvent was also observed with the appearance of $[TG + 1 + 55]^+$ ions.

The variation with respect to molecular ion and fragment ion abundances that we observed in these spectra due to varying degrees of unsaturation has been observed using other ionization methods (1,2,8,11,19). This variation in ion intensities has necessitated the use of response factors, or calibration factors, for quantitation. Quantitation from spectra obtained with APCI will likewise require the use of calibration factors. For qualitative analysis, however, APCI spectra allowed direct identification of numerous TG species.

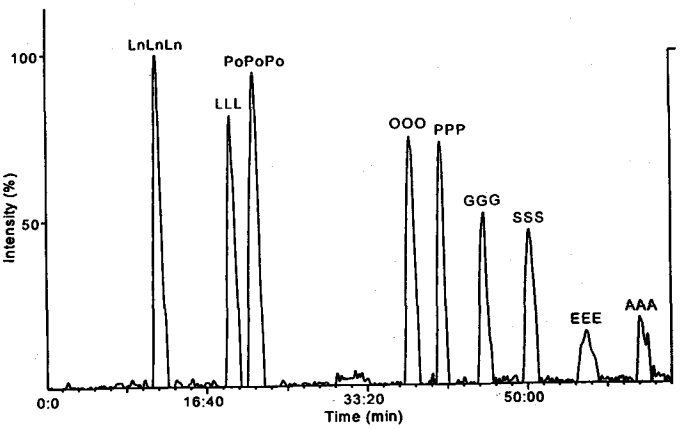

FIG. 1. Total ion chromatogram of triglyceride mixture. Order of elution: trilinolenin (LnLnLn), trilinolein (LLL), tripalmitolein (PoPoPo), triolein (OOO), tripalmitin (PPP), trigadolein (GGG), tristearin (SSS), trierucin (EEE), triarachidin (AAA).

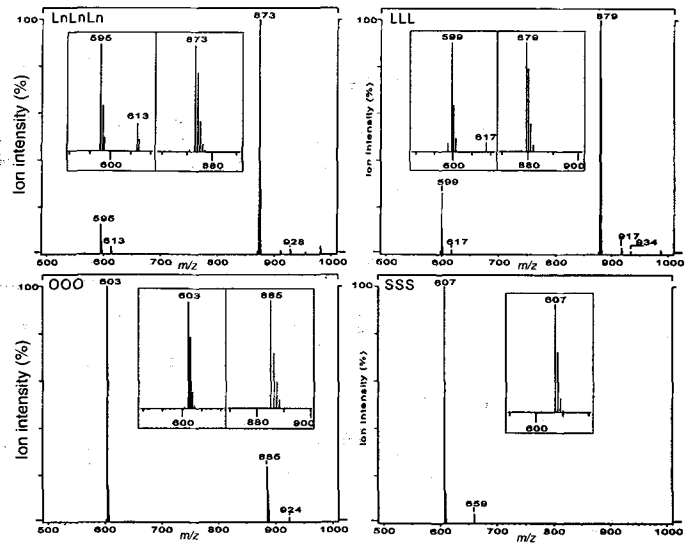

FIG. 2. Mass spectra of LnLnLn, LLL, OOO and SSS. See Figure 1 for abbreviations.

These results clearly show the utility of APCI as an ionization method for TG analysis. The compatibility of APCI with a nonpolar chromatographic solvent system allows an expanded range of molecules to be analyzed, compared to the other widely used atmospheric pressure ionization method, ESI. While the APCI source did not give molecular ions as does ESI for trisaturated TG, the simple fragmentation pattern of all species allowed identification by the DG fragments.

REFERENCES

1. Kuksis, A., Marai, L., and Myher, J.J. (1983) *J. Chromatogr. 273*, 43–66.
2. Myher, J.J., Kuksis, A., Marai, L., and Manganaro, F. (1984) *J. Chromatogr. 283*, 289–301.
3. Kuksis, A., Myher, J.J., and Marai, L. (1985) *J. Am. Oil Chem. Soc. 62*, 762–767.
4. Kuksis, A., and Myher, J.J. (1986) *J. Chromatogr. 379*, 57–90.
5. Kallio, H., Laakso, P., Huopalahti, R., Linko, R.R., and Olsman, P. (1989) *Anal. Chem. 61*, 698–700.
6. Kuksis, A., Marai, L., and Myher, J.J. (1991) *Lipids 26*, 240–246.
7. Merritt Jr., C., Vajdi, M., Kayser, S.G., Halliday, J.W., and Bazinet, M.L. (1982) *J. Am. Oil Chem. Soc. 59*, 422–432.
8. Rezanka, T., Husek, P., and Podojil, M. (1986) *J. Chromatogr. 355*, 265–271.
9. Singleton, J.A., and Pattee, H.E. (1987) *J. Am. Oil Chem. Soc. 64*, 534–538.
10. Barber, M., Merren, T.O., and Kelly, W. (1964) *Tetrahedron Lett.*, 1063–1067.
11. Hites, R.A. (1970) *Anal. Chem. 42*, 1736–1740.
12. Aasen, A.J., Lauer, W.M., and Holman, R.T. (1973) *Lipids 5*, 869–877.
13. Lauer, W.M., Aasen, A.J., Graff, G., and Holman, R.T. (1973) *Lipids 5*, 861–868.
14. Murata, T., and Takahashi, S. (1977) *Anal. Chem. 49*, 728–731.
15. Lehmann, W.D., and Kessler, M. (1983) *Biomed. Mass Spectrom. 10*, 220–226.
16. Barber, M., Tetler, L.W., Bell, D., Ashcroft, A.E., Brown, R.S., and Moore, C. (1987) *Org. Mass Spectrom. 22*, 647–650.
17. Showell, J.S., Fales, H.M., and Sokoloski, E.A. (1989) *Org. Mass Spectrom. 24*, 632–636.
18. Duffin, K.L., Henion, J.D., and Shieh, J.J. (1991) *Anal. Chem. 63*, 1781–1788.
19. Mares, P., Rezanka, T., and Novak, M. (1991) *J. Chromatogr. 568*, 1–10.
20. Rezanka, T., and Mares, P. (1991) *J. Chromatogr. 542*, 145–159.
21. Covey, T.R., Lee, E.D., Bruins, A.P., and Henion, J.D. (1986) *Anal. Chem. 58*, 1451A–1461A.

[Received on September 27, 1994, and in revised form December 16, 1994; Revision accepted on December 28, 1994]