Gas Chromatography–Fourier Transform Infrared Spectrometry of Fatty Acids: New Applications with a Direct Deposition Interface

E. Sémon[a,*], S. Ferary[b], J. Auger[b], and J.L. Le Quéré[a]

[a]Laboratoire de Recherches sur les Arômes, INRA, 21034 Dijon-Cedex, France, and [b]IBEAS URA CNRS 1298 and SAVIT, Université F. Rabelais, 37200 Tours, France

ABSTRACT: Infrared spectroscopy is a suitable spectroscopic method to differentiate geometric *Z* and *E* isomers of unsaturated compounds. A direct-deposition Fourier transform infrared spectrometer (FTIR), coupled to a gas chromatograph, was used successfully to analyze with a high sensitivity traces of C18:1 fatty acid methyl ester (FAME) isomers. It could also conclusively distinguish between isomers of conjugated diunsaturated FAME. The achievable sensitivity of this direct-deposition device makes possible accurate FAME mixture analyses that are not currently attainable with the more conventional light-pipe interface.
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KEY WORDS: Conjugated fatty acid methyl esters, Fourier transform infrared spectrometry, gas chromatography, GC–FTIR, geometric isomers, *trans* isomers.

Coupled gas chromatography–mass spectrometry (GC–MS) is widely used for analyses of volatile compound mixtures from oils and fats. Excellent separation of fatty acid methyl esters (FAME) is achieved by high-resolution gas chromatography, and their identification is generally obtained by MS. However, this technique suffers from a lack of specificity for the accurate identification of unsaturated compounds. Double bond locations in unsaturated FAME may be deduced after studying mass spectra of corresponding derivatives (1), but configuration of the olefinic centers cannot be inferred.

Infrared spectroscopy is a dedicated spectroscopic technique used in the identification of *Z* or *E* configurations of olefinic isomeric compounds. Three different types of interfaces between the gas chromatograph and the Fourier transform infrared (FTIR) spectrometer are available. The most common one is a gas cell (so-called light-pipe) through which the eluate passes (2) and where vapor phase spectra are collected. This technique has achieved a certain popularity in analyses of flavor compounds (3,4) and was successfully applied in the analyses of unsaturated FAME (5). Thus, it made possible the distinction of the size of the ring (5 or 6 carbon atoms), in unsaturated cyclic fatty acids formed in frying oils, by differentiating *cis* double bonds in a five-membered ring from those in a six-membered ring (6). The method was also employed to characterize geometrical isomers of eicosapentaenoic and docosahexaenoic acids, found in liver lipids of rats that were fed high levels of geometrical isomers of linolenic acid (7). The technique was also used more recently in the definitive characterization of the mono- and diunsaturated cyclic fatty acids found, respectively, in heated sunflower and linseed oils (8,9). The main drawback of the light pipe cell lies in its poor sensitivity (few ng) compared to MS.

Sensitivity to the same level as that of MS was reached with a matrix isolation–FTIR (MI–FTIR) interface. With this device, the GC effluent, mixed with a small quantity of argon, is cryotrapped on a rotating disk that is maintained at 11 K under vacuum (10). The resulting solid argon matrix spectra are obtained in reflection mode. Hydrogenated soybean oil and margarines have been studied with such a spectrometer, and determination of octadecadienoic acid methyl esters has been reported (11). These spectra with excellent signal-to-noise ratios, indicating the high sensitivity of the technique, display narrower IR band widths than the vapor-phase IR spectra owing to collection of the compounds in the argon matrix.

On the basis of the pioneering work of Bourne *et al.* (12), a direct-deposition GC–FTIR interface has been recently developed by Bio-Rad (Cambridge, MA). The GC effluent is deposited onto a step-by-step moving zinc selenide (ZnSe) window, which is held at 77 K (liquid nitrogen) under vacuum. Spots of compounds are collected in the condensed phase, and spectra are acquired in the traditional transmittance mode. Post-run scanning of the spots can be realized, as they are trapped on the window, to increase signal-to-noise ratios (12). Direct-deposition GC–FTIR has proved its usefulness for pesticides and pharmaceutical samples (12), environmental contaminants (13), identification of trace plant substances (14), and for 1,2-propanediol quantitation in Acyclovir cream formulations (15).

In this paper we report application of this technique of cryotrapping–FTIR to characterize unsaturated FAME.

*To whom correspondence should be addressed at Laboratoire de Recherches sur les Arômes, INRA, 17 rue Sully BV 1540, 21034 Dijon-cedex, France. E-mail: semon@arome.dijon.inra.fr.

EXPERIMENTAL PROCEDURES

Methyl elaidate and methyl oleate of gas chromatographic purity were purchased from Merck (Darmstadt, Germany) and were dissolved in purified *n*-hexane.

Conjugated polyunsaturated FAME were prepared from human milk lipids (16) as previously described (17).

All GC–FTIR spectra were collected with a Bio-Rad Digilab FTS 45A spectrometer. This was connected by means of a Digilab Tracer® direct-deposition interface to a Hewlett-Packard HP 5890 series II (Palo Alto, CA) gas chromatograph, which was equipped with a splitless-split injector. GC separation was obtained with a SGE (Ringwood VIC, Australia) fused-silica capillary column (30 m × 0.32 mm i.d.), coated with a BPX 70 stationary bonded phase (0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. GC temperature was programmed from 50 to 150°C at 15°C/min, from 150 to 220°C at 3°C/min, and then held isothermally until the analyses were completed.

The GC column was connected *via* a zero dead-volume connector to a deactivated fused-silica capillary column (50 cm × 0.32 mm i.d.), which was maintained at 250°C. The GC eluates were deposited onto a ZnSe window, which was cooled with liquid nitrogen. The spectrometer was controlled, and data were acquired with an SPC 3200 data system. The spectral resolution was 8 cm^{-1}, and real-time spectra were obtained by co-addition of four scans. A narrow-band (4000–700 cm^{-1}) mercury-cadmium-telluride (MCT) detector was used.

RESULTS AND DISCUSSION

The high sensitivity of the direct-deposition GC–FTIR interface is of great interest to analyze complex mixtures of FAME. These samples, especially those resulting from heat treatment of fats, contain amounts of FAME within a dynamic range that is extensively unbalanced, the most abundant compounds being commonly 10^3-fold more concentrated than the minor ones. The detection of the less concentrated compounds of these samples, i.e., minor *trans* isomers in a complex mixture, is often simply not possible with the light-pipe gas cell.

The best separation of FAME is obtained by using a very polar stationary GC phase (i.e., high percentage of cyanopropyl methylpolysiloxane). With this type of stationary phase, only a thin film thickness (0.25 μm) is available. Concentration of the extracts is then limited to maintain the GC separation of sample components and avoid saturation of the stationary phase with the most abundant compounds. GC–FTIR analyses with the new direct-deposition interface need no extensive preliminary concentration owing to its subnanogram sensitivity.

Figure 1 shows the chromatograms of a test sample solution, prepared with 10 ng of methyl elaidate (*t*9–18:1) and 100 ng of methyl oleate (*c*9–18:1). The first peak, corresponding to the *trans* isomer, is hardly located on the Gram–Schmidt reconstructed total IR chromatogram (bottom) but is well distinguished on the functional group chromatogram (3000–2800 cm^{-1}), where these two peaks are almost baseline resolved (top). Conclusive identification can be achieved by observing the characteristic bands of the respective spectra. The spectrum of the less abundant methyl elaidate (Fig. 2A) exhibits a good signal-to-noise ratio. The band at 969 cm^{-1} corresponds to the CH out-of-plane deformation vibration for the *E*-configuration of the double bond. The *Z*-configuration of the methyl oleate (Fig. 2B) is confirmed with the two bands at 723 cm^{-1} (CH out-of-plane) and 3001 cm^{-1} (=C-H stretch).

Human milk samples were analyzed to assess their *trans* mono- and polyunsaturated fatty acid contents (16). To complete this study on identification of different FAME, these samples were studied by direct-deposition GC–FTIR. This technique appeared crucial to discriminate between diunsaturated

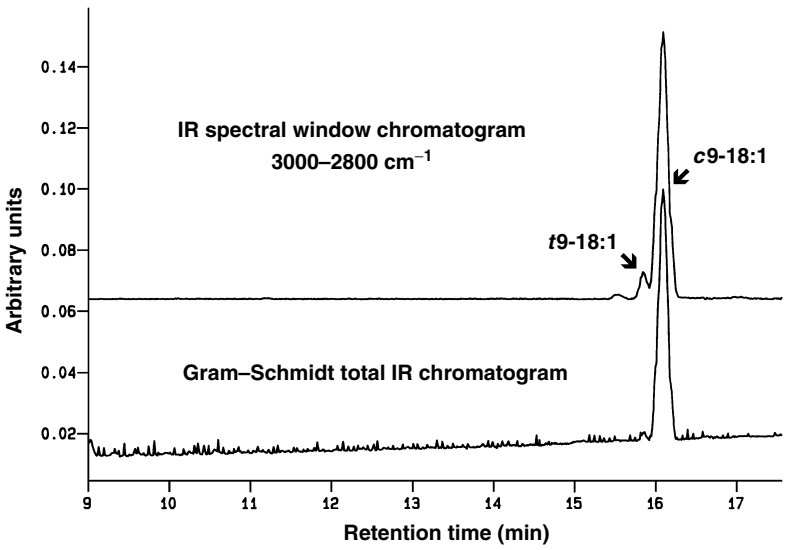

FIG. 1. Gram–Schmidt chromatogram and infrared (IR) spectral window (3000–2800 cm^{-1}) of a solution of methyl elaidate (10 ng) and methyl oleate (100 ng).

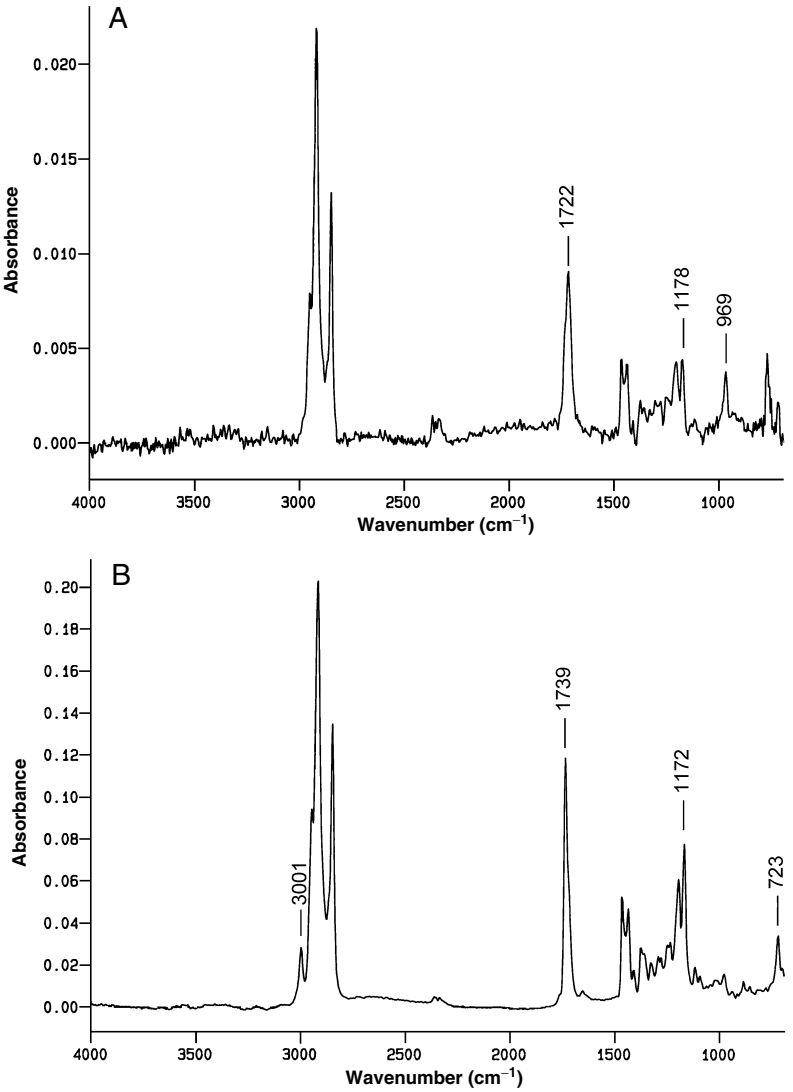

FIG. 2. Gas chromatography/direct-deposition/Fourier transform infrared spectrum of 10 ng of methyl elaidate (A) and of 100 ng of methyl oleate (B).

C18:2 FAME geometrical isomers that were conjugated in positions Δ9 and Δ11. Figure 3A presents the spectrum of the *trans-trans* isomer. The band that characterizes this configuration is situated at 990 cm^{-1} (CH out-of-plane deformation). This out-of-plane deformation absorption displays a 20 cm^{-1} shift toward the largest wavenumbers and is stronger than the corresponding one in the *t*9–18:1 spectrum. However, its position and relative intensity are comparable to those found in the GC/MI/FTIR spectra of *trans,trans*-18:2 conjugated diene isomers (18). Moreover, a =C-H stretching band is displayed at 3016 cm^{-1} in this *trans-trans* conjugated spectrum. An equivalent band at 3023 cm^{-1} can also be observed in the CH stretch region of the GC/MI/FTIR *trans*,*trans* 18:2 FAME (18). The *cis*,*trans* FAME spectrum (Fig. 3B) exhibits three out-of-plane medium bands at 726, 950 and 986 cm^{-1}. The first one is attributed to the *cis* configuration of one of the two double bonds, whereas the two other bands discriminate the *trans* configuration of the second conjugated unsaturation. Two =C-H stretching bands are observed in the region of 3000–3020 cm^{-1}. These results are consistent with those of Mossoba *et al.* (18), which were acquired with a GC/MI/FTIR system. Further studies are underway to attribute the correct geometry to each of these two double bonds.

The direct-deposition interface in GC–FTIR provides sensitive detection of unsaturated FAME and reveals characteristic spectral information, such as double bond configurations. This intrinsic sensitivity allows the complete study of complex mixtures, especially when a highly unbalanced concentration range is clearly a handicap for the more conventional light-pipe interface.

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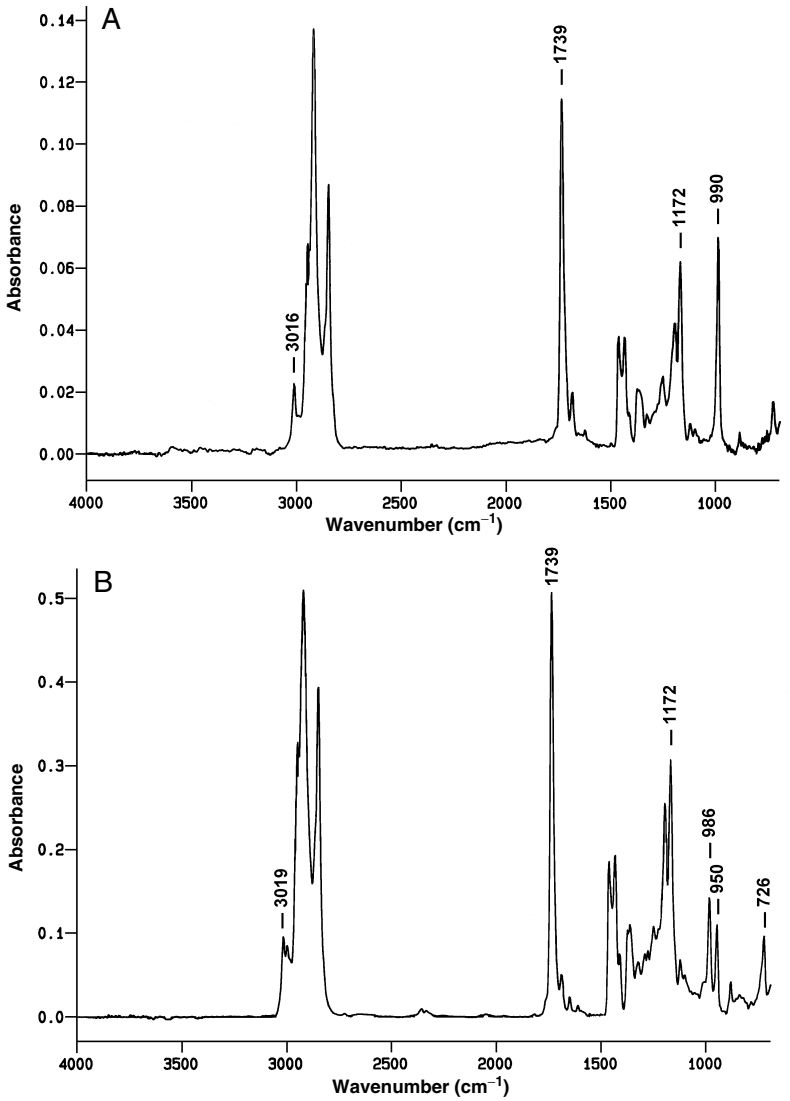

FIG. 3. Gas chromatography/direct-deposition/Fourier transform infrared spectrum of a *trans,trans*-octadecadienoic acid methyl ester (A) and a *cis,trans*-octadecadienoic acid methyl ester (B) from a human milk sample.

REFERENCES

1. Jensen, N.J., and M.L. Gross, Mass Spectrometry Methods for Structural Determination and Analysis of Fatty Acids, *Mass Spectrom. Rev. 6*:497–536 (1987).
2. Griffiths, P.R., and J.A. de Haseth, *Fourier Transform Infrared Spectrometry,* Wiley-Interscience, New York, 1986, p. 656.
3. Herres, W., *HRGC–FTIR: Chromatography–Fourier Transform Infrared Spectroscopy. Theory and Applications,* Hüthig, Heidelberg, 1987, p. 212.
4. Le Quéré, J.L., Le Couplage CPG/IRTF dans l'Analyse des Arômes, *Analusis 20*:M46–M49 (1992).
5. Le Quéré, J.L., Gas Chromatography–Fourier Transform Infrared Spectrometry in the Analysis of Fatty Acids, in *New Trends in Lipid and Lipoprotein Analyses*, edited by J.L. Sébédio and E.G. Perkins, AOCS Press, Champaign, 1995, Ch. 17.
6. Sébédio, J.L., J.L. Le Quéré, E. Sémon, O. Morin, J. Prévost, and A. Grandgirard, Heat Treatment of Vegetable Oils. II. GC–MS and GC–FTIR Spectra of Some Isolated Cyclic Fatty Acid Monomers, *J. Am. Oil Chem. Soc. 64*:1324–1333 (1987).
7. Grandgirard, A., A. Piconneaux, J.L. Sébédio, S.F. O'Keefe, E. Sémon, and J.L. Le Quéré, Occurrence of Geometrical Isomers of Eicosapentaenoic and Docosahexaenoic Acids in Liver Lipids of Rats Fed Heated Linseed Oil, *Lipids 24*:799–804 (1989).
8. Christie, W.W., E.Y. Brechany, J.L. Sébédio, and J.L. Le Quéré, Silver Ion Chromatography and Gas Chromatography–Mass Spectrometry in the Structural Analysis of Cyclic Monoenoic Acids Formed in Frying Oils, *Chem. Phys. Lipids 66*:143–153 (1993).
9. Dobson, G., W.W. Christie, E.Y. Brechany, J.L. Sébédio, and J.L. Le Quéré, Silver Ion Chromatography and Gas Chromatography–Mass Spectrometry in the Structural Analysis of Cyclic Dienoic Acids Formed in Frying Oils, *Ibid. 75*:171–182 (1995).
10. Reedy, G.T., D.G. Ettinger, J.F. Schneider, and S. Bourne, High-Resolution Gas Chromatography/Matrix Isolation Infrared Spectrometry, *Anal. Chem. 57*:1602–1609 (1985).
11. Mossoba, M.M., R.E. McDonald, J.Y.T. Chen, D.J. Armstrong, and S.W. Page, Identification and Quantitation of *trans*-9,*trans*-12-Octadecadienoic Acid Methyl Ester and Related Compounds

in Hydrogenated Soybean Oil and Margarines by Capillary Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectroscopy, *J. Agric. Food Chem. 38*:86–92 (1990).
12. Bourne, S., A.M. Haefner, K.L. Norton, and P.R. Griffiths, Performance Characteristics of a Real-Time Direct Deposition Gas Chromatography/Fourier Transform Infrared Spectrometry, *Anal. Chem. 62*:2448–2452 (1990).
13. Visser, T., and M.J. Vredenbregt, Cryotrapping: Gas Chromatography–Fourier Transform Infrared Spectrometry in Environmental Analysis: A Pilot Study, *Vib. Spectrosc. 1*:205–210 (1990).
14. Ferary, S., J. Auger, and A. Touché, Trace Identification of Plant Substances by Combining Gas Chromatography–Mass Spectrometry and Direct-Deposition Gas Chromatography–Fourier Transform Infrared Spectrometry, *Talanta 43*:349–357 (1996).
15. Gilbert, A.S., C.J. Moss, P.L. Francis, M.J. Ashton, and D.S. Ashton, Combined Gas Chromatography–Infra-Red Spectroscopy for the Determination of Propanediol in Acyclovir Cream, *Chromatographia 42*:305–308 (1996).
16. Chardigny, J.M., R.L. Wolff, E. Mager, J.L. Sébédio, L. Martine, and P. Juanéda, *Trans* Mono- and Polyunsaturated Fatty Acids in Human Milk, *Eur. J. Clin. Nutr. 49*:523–531 (1995).
17. Carreau, J.P., and J.P. Dubacq, Adaptation of a Macro-Scale Method to the Micro-Scale for Fatty Acid Methyl Transesterification of Biological Lipid Extracts, *J. Chromatogr. 151*:384–390 (1978).
18. Mossoba, M.M., R.E. McDonald, D.J. Armstrong, and S.W. Page, Identification of Minor C_{18} Triene and Conjugated Diene Isomers in Hydrogenated Soybean Oil and Margarine by GC–MI–FT–IR Spectroscopy, *J. Chromatogr. Sci. 29*:324–330 (1991).

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