ELSEVIER

Journal of Chromatography A, 954 (2002) 285–289

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Utilisation of reversed-phase high-performance liquid chromatography as an alternative to silver-ion chromatography for the separation of *cis*- and *trans*-C18:1 fatty acid isomers

Pierre Juanéda*

INRA, Unité de Nutrition Lipidique, 17 Rue Sully, BP 86510, 21065 Dijon Cédex, France

Received 26 November 2001; received in revised form 13 February 2002; accepted 20 February 2002

Abstract

Silver ion-high-performance liquid chromatography (HPLC) has been commonly used for the separation and the analysis of *trans*-18:1 isomers in partially hydrogenated oils and milk fat. This paper describes an easy HPLC method using two reversed-phase columns. The *cis*- and *trans*-18:1 fatty acids isomers as methyl esters were eluted as two separate fractions. The collected fractions were analysed by gas chromatography (GC). The purity of the two fractions were tested by GC–MS and GC-Fourier transform IR. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Silver-ion chromatography; Trans fatty acid methyl esters

1. Introduction

A number of methods were developed to determine the position and the double bond configuration of the 18:1 *trans* isomers in order to study the nutritional impact of these fatty acid isomers [1]. Gas chromatography (GC) cannot resolve the complete mixture of *trans* monoenes considering the overlap with the *cis* isomers [2]. However the separation of *cis* or/and *trans* isomers can be achieved using silver-ion chromatography [3,4] could be followed by GC analysis on capillary columns [5].

The purpose of this work was to use a reversed-phase high-performance liquid chromatography (HPLC) method to obtain pure fractions of *cis* and *trans* isomers of octadecenoic acid which are then analysed by GC on highly polar capillary columns. Furthermore, we have also shown that analysis on a BPX70 column permits separation of the Δ13t and Δ14t isomers, which were not resolved previously on a CP Sil88 column.

2. Experimental

2.1. Chemicals and dairy samples

The chemical reagents were obtained from Sigma (L'Isle d'Abeau, France). All the solvents were distilled before use. Acetonitrile was of UV grade (SDS, Peypin, France).

The dairy products, butter and milk, were purchased in a local supermarket. The butter and milk fats were extracted as previously described [6]. All the samples were converted into fatty acid methyl

*Tel.: +33-3-8063-3110; fax: +33-3-8063-3223.

E-mail address: pierre.juaneda@dijon.inra.fr (P. Juanéda).

0021-9673/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(02)00179-6

esters (FAMEs) using sodium methoxide (0.5 *M*) for 10 min at 50 °C [7].

2.2. High-performance liquid chromatography

The HPLC analyses were performed using a Varian solvent pump (Varian, Les Ulis, France) equipped with a Valco injector fitted with a 100 μl loop. The detector was a differential refractometer, (Waters Model 410, Saint Quentin en Yvelines, France). The FAMEs (up to 2 mg/100 μl in acetone) were separated using two Kromasil-C_{18} columns in series (5 μm, 250 mm×10 mm I.D., ThermoHypersil, Les Ulis, France). Acetonitrile was used as mobile phase at a flow-rate of 4 ml/min. The fractions containing the C18:1 *cis* and *trans* isomers were collected.

2.3. Gas chromatography

The FAMEs were analysed by GC using a HP5890 serie II (Hewlett-Packard, Palo Alto, CA, USA), fitted with a split–splitless injector (250 °C) and a flame ionisation detector (280 °C) coupled with a Diamir integrating system (JMBS, Grenoble, France). CP Sil88, 100 m×0.25 mm I.D., 0.2 μm film thickness (Varian) and BPX70, 120 m×0.25 mm I.D., 0.25 μm film thickness (SGE, Melbourne, Australia) columns were utilised. For both columns, hydrogen was used as carrier gas at a velocity of 34.5 cm/s at 60 °C. The oven was programmed from 60 °C to 160 °C at 20 °C/min and the final temperature was maintained for 50 min.

2.4. Gas chromatography–mass spectrometry

The collected fractions were converted into 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives according to Yurawecz et al. [8]. The analysis of DMOX derivatives was achieved using a HP6890 coupled to a HP5973 mass spectrometer. A BPX70 (50 m×0.33 I.D., 0.25 μm film thickness) capillary column was used with a temperature programming from 50 °C to 190 °C at 15 °C/min. Helium was the carrier gas at 36 cm s^{-1} of velocity. The injector in splitless mode was maintained at 250 °C. The electron impact mass spectra were recorded at 70 eV between 100 and 400 u.

2.5. Gas chromatography–Fourier transform infrared spectrometry (FT-IR)

All the GC–FT-IR analyses were performed with a Bio-Rad Digilab (Cambridge, MA, USA), Model FTS 60A spectrometer connected via a Digital Tracer direct deposition interface to a HP5980 serie II gas chromatograph [9]. The FAMEs were analysed on a CP Sil88 column as previously described. A narrow band (4000–760 cm^{-1}) mercury cadmium telluride detector was used.

3. Results and discussion

Reversed-phase HPLC is a technique commonly used to separate FAMEs as a function of the number of carbons and the number of double bonds [10]. The separation of Δ9*cis* and Δ9*trans* 18:1 isomers was tested using two Kromasil-C_{18} columns in series, and acetonitrile (100%) as solvent. Fig. 1 shows the separation of Δ9*cis*- and Δ9*trans*-18:1 as standard

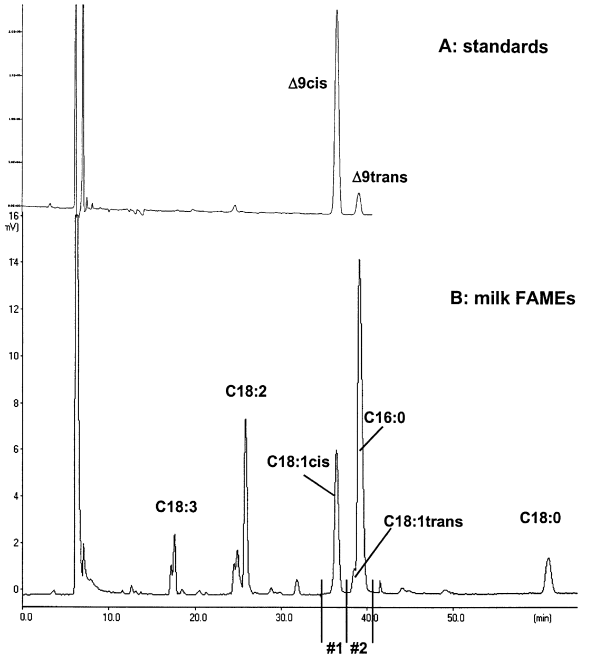

Fig. 1. C_{18}-HPLC separation of Δ9*cis* and Δ9*trans* standards (A) and milk FAMEs (B). Two Kromasil-C_{18} columns, acetonitrile, flow-rate=4 ml/min, RI detector. ''#n'' between two ticks indicates the fraction collected.

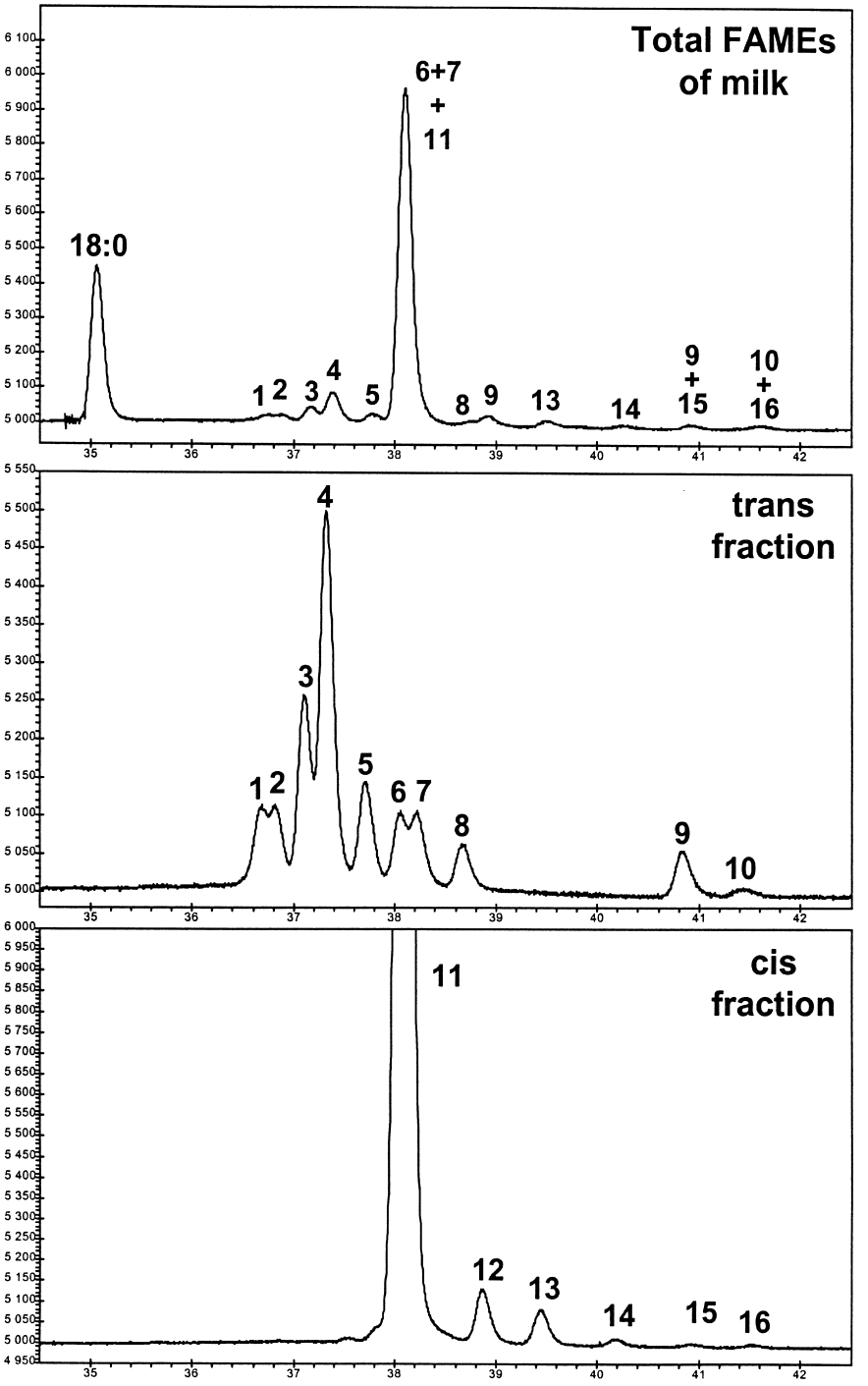

Fig. 2. Analyses of total milk FAMEs and of *cis* and *trans* fraction using a BPX70 column (120 m). Hydrogen was used as carrier gas. The oven was programmed from 60 °C to 160 °C at 20 °C/min and the final temperature was maintained during 50 min. 1: Δ6-7-8*trans*, 2: Δ9*trans*, 3: Δ10*trans*, 4: Δ11*trans*, 5: Δ12*trans*, 6: Δ13*trans*, 7: Δ14*trans*, 8: Δ15*trans*, 9: Δ16*trans*, 10: Δ17*trans*, 11: Δ9*cis*, 12: Δ11*cis*, 13: Δ12*cis*, 14: Δ13*cis*, 15: Δ14*cis*, 16: Δ15*cis*.

(A) and one of the milk FAME sample (B). The resolution between Δ9*cis* and Δ9*trans* 18:1 is 3.32 and the final time of analysis is about 1 h. The volumes of retention (V_r) of the peaks containing 18:1 isomers were V_r(*cis*-18:1)=142.0 ml and V_r(*trans*-18:1)=150.5 ml.

The determination of the double bond position and the purity of the two fractions were tested by GC–MS of the DMOX derivatives and by GC–FT-IR of the FAMEs. The interpretation of the spectra of the DMOX derivatives is relatively simple [11,12]. A close examination of the different DMOX spectra revealed no cross contaminations between the *cis* and *trans* fractions. The FAMEs were analysed by GC–FT-IR. The absence of *trans* band and the presence of a band at 3001.46 cm^{-1} in the *cis*-18:1 fraction confirm that this fraction only contains *cis* double bond. In the *trans*-18:1 fraction, the spectrum shows the presence of band at 966.21 cm^{-1} (*trans* band) and the absence of a *cis* band (3001 cm^{-1}) [9,11]. These data confirmed those obtained by GC–MS, showing no cross contamination between the cis and trans fraction. However, HPLC injections of more than 2 mg of FAMEs result in a pollution of the *trans* fraction by 18:1 Δ9*cis*, under our experimental conditions.

The trans fractions of FAMEs were analysed on BPX70 and CP-Sil88 capillary columns. For both columns, the Δ6-7-8 *trans* isomers eluted under the same peak as previously described [7,11]. The advantage of using a BPX70 column is mainly the separation of Δ13 and Δ14 *trans*. But, for both capillary columns, some *trans*-18:1 isomers overlap with the *cis* ones, as, for example, Δ9*cis* with Δ13*trans*+Δ14*trans*, Δ14*cis* with Δ16*trans* and Δ15*cis* with Δ17*trans* [2,3,13] as shown in Fig. 2.

This method also permits one to quantify the 18:1 isomer composition in the total FAMEs. As the *trans*-18:1 and the C16:0 are collected together (Fig. 1), it is very easy to determine the percentage of all the 18:1 isomers by using the 16:0 fatty acid as internal standard. The proportions of the *cis*- and *trans*-18:1 isomers in the dairy products, butter and milk, are reported in Table 1. These relative proportions are in good agreement with those described in the literature [14,15].

Silver ion chromatography is usually a tedious method while the utilisation of reversed-phase HPLC for the separation of *cis* and *trans* monoenes could be an attractive alternative technique as demonstrated above. Moreover, this technique has a very good reproducibility and permits to quantify the level of all the *cis*- and *trans*-18:1 isomers in dairy samples. It could be also applied to other lipid samples.

Table 1
cis- and *trans*-octadecenoic acid contents (% of total 18:1 isomers) in butter and milk fat samples

Fatty acid	Butter	Milk
Δ6-7-8*trans*	0.79	1.37
Δ9*trans*	1.04	1.35
Δ10*trans*	1.65	3.27
Δ11*trans*	4.37	7.17
Δ12*trans*	2.02	2.06
Δ13*trans*	1.63	1.33
Δ14*trans*	2.23	1.47
Δ15*trans*	1.45	0.85
Δ16*trans*	1.57	0.90
Δ17*trans*	0.23	0.20
Δ9*cis*	76.30	74.37
Δ11*cis*	2.78	2.74
Δ12*cis*	1.86	1.42
Δ13*cis*	0.43	0.57
Δ14*cis*	0.25	0.29
Δ15*cis*	1.40	0.65
	100.00	100.00

Acknowledgements

The author thanks Etienne Sémon, UMR INRA-ENESAD (Dijon, France) for his help for GC–FT-IR analyses.

References

[1] W.M.N. Ratnayake, in: J.L. Sébédio, W.W. Christie (Eds.), Trans Fatty Acids in Human Nutrition, Oily Press, Dundee, 1998, p. 115, Chapter 4.
[2] R.L. Wolff, C.C. Bayard, J. Am. Oil Chem. Soc. 72 (1995) 1197.
[3] J. Molkentin, D. Precht, Chromatographia 41 (1995) 267.
[4] B. Nikolova-Damyanova, B.G. Hersslof, W.W. Christie, J. Chromatogr. 609 (1992) 133.
[5] R.O. Adlof, L.C. Copes, E.A. Emken, J. Am. Oil Chem. Soc. 72 (1995) 571.

[6] N. Sehat, J.K.G. Kramer, M.M. Mossoba, M.Y. Yurawecz, J.A.G. Roach, K. Eulitz, K.M. Morehouse, Y. Ku, Lipids 33 (1998) 963.
[7] W.W. Christie, J.L. Sébédio, P. Juanéda, Inform 12 (2001) 147.
[8] M.P. Yurawecz, J.K. Hood, J.A.G. Roach, M.M. Mossoba, D.H. Daniels, D.H. Kuand, S.F. Chin, J. Am. Oil Chem. Soc. 71 (1994) 1149.
[9] E. Semon, S. Ferary, J. Augier, J.L. Le Quéré, J. Am. Oil Chem. Soc. 75 (1998) 101.
[10] M.I. Aveldano, M. VanRollins, L.A. Horrocks, J. Lipid Res. 24 (1983) 83.
[11] M.M. Mossoba, R.E. McDonald, J.A.G. Roach, D.D. Fingerhut, M.P. Yurawecz, N. Sehat, J. Am. Oil Chem. Soc. 74 (1997) 125.
[12] W.W. Christie, G.W. Robertson, W.C. McRoberts, J.T.G. Hamilton, Eur. J. Lipid Sci. Technol. 102 (2000) 23.
[13] M. Ledoux, L. Laloux, R.L. Wolff, Analusis 28 (2000) 402.
[14] J. Fritsche, H. Steinhart, Fett/Lipid 100 (1998) 190.
[15] R.L. Wolff, OCL 1 (1994) 209.