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Short communication

Fractionation of *cis*- and *trans*-oleic, linoleic, and conjugated linoleic fatty acid methyl esters by silver ion high-performance liquid chromatography[1]

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Abstract

Silver ion high-performance liquid chromatography (Ag-HPLC) using two commercially available columns connected in series and a solvent of 0.5% acetonitrile in hexane (1.0 ml/min), was used to fractionate a mixture of conjugated 18:2 isomers (as methyl esters) and resulted in an elution order (10 μg sample size) of: 9-*trans*,11-*trans*-18:2; 9-*cis*,11-*trans*-18:2. A more complex mixture of *cis* and *trans* 9-18:1, 9,11-18:2 and 9,12-18:2 methyl esters could also be fractionated within 25 min. The elution order (10 μg sample size) was found to be: 9-*trans*-18:1; 9-*trans*,11-*trans*-18:2; 9-*trans*,12-*trans*-18:2; 9-*trans*,11-*cis*-18:2; 9-*cis*,11-*trans*-18:2; 9-*cis*-18:1; 9-*trans*,12-*cis*-18:2; 9-*cis*,12-*trans*-18:2; 9-*cis*,12-*cis*-18:2. The Ag-HPLC elution orders differed significantly from those obtained via gas chromatography on a polar stationary phase (30 m SP2380 capillary column; Elution order: 9-*trans*-18:1; 9-*cis*-18:1; 9-*trans*,12-*trans*-18:2; 9-*cis*,12-*trans*-18:2; 9-*trans*,12-*cis*-18:2; 9-*cis*,12-*cis*-18:2; 9-*cis*,11-*trans*-18:2; 9-*trans*,11-*cis*-18:2; 9-*trans*,11-*trans*-18:2). The Ag-HPLC system was also used in the semi-preparative separation of a 10 mg sample of 9-*trans*,11-*trans*-18:2 and 9-*cis*,11-*trans*-18:2 fatty acid methyl esters. Published by Elsevier Science B.V.

Keywords: Fatty acid methyl esters; Oleic acid; Linoleic acid; Conjugated linoleic acid

1. Introduction

Silver ion high-performance liquid chromatography (Ag-HPLC), utilizing columns packed with 5–10 μm Nucleosil SA™ (phenylsulfonic acid groups bonded to a silica substrate) or similar substrate in which the sulfonic acid protons have been exchanged with Ag ions, is a useful technique for the separation and isolation of *cis* and *trans* geometric and positional fatty acid methyl ester (FAME) and triacylglycerol (TAG) isomers. Over the last decade, Ag-HPLC has been applied to the separation/quantitation of *cis* and *trans* FAMEs, FAME positional isomers from partially hydrogenated vegetable oils, conjugated FAMEs, FAMEs labelled with deuterium atoms on the double bond carbons, TAG isomers and to the separation of FAME or TAG mixtures containing FAs of widely

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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differing chain lengths (see [1–3] for excellent reviews of this technology).

While conjugation of the double bonds is considered an intermediate step in 9-*cis*,12-*cis*-18:2 peroxidation [4], conjugated linoleic acid (CLA; 9-*cis*, 11-*trans*-octadecadienoic acid) has been associated with the reduction of chemically induced cancers in mice and rats and the suppression of atherosclerosis in rats [5–7]. The 9-*cis*,11-*trans*-isomer is considered to be the active constituent, but contributions by other isomers (9-*trans*,11-*trans*-, 10-*trans*,12-*cis*-, 10-*cis*,12-*cis*- or 10-*trans*,12-*trans*-18:2) have not been ruled out. Preparation of deuterium-labelled CLA isomers was undertaken [8] for tissue studies and to study their metabolism in humans and animals. In this manuscript we apply Ag-HPLC to the analysis and semi-preparative purification of conjugated 9,11-18:2 FAME isomers synthesized in our laboratory. We also compare the Ag-HPLC elution order obtained for a mixture of *cis*- and *trans*-9-18:1, 9,11-18:2 and 9,12-18:2 FAME isomers with that observed with gas chromatography using a polar stationary phase (SP 2380).

2. Experimental

2.1. Materials and reagents

Hexane (Allied Fisher Scientific, Orangeburg, NY, USA) and acetonitrile (ACN; E. Merck, Darmstadt, Germany) were used as received. Non-conjugated fatty acid methyl ester standards were obtained from Nu-Chek-Prep (Elysian Fields, MN, USA). *Cis*/*trans* isomer mixtures of 9-18:1 and 9,12-18:2 FAMEs were prepared by the *p*-toluenesulfinic acid catalyzed isomeration of the *cis*-precursors [9]. Conjugated (9,11-18:2) FAME samples were obtained by low-temperature crystallization from alkali-isomerized [10] methyl linoleate (9-*cis*,12-*cis*-octadecadienoate) and from the partial reduction of methyl alpha-eleostearate (9-*cis*,11-*trans*,13-*trans*-octadecatrienoate) with hydrazine [11].

2.2. High-performance liquid chromatography

A Spectra-Physics P2000 solvent delivery system (Spectra-Physics Analytical, Fremont, CA, USA), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 20 μl injection loop, and an ISCO V4 absorbance detector (Isco, Lincoln, NE, USA) at wavelengths of 206 or 212 nm were used. The Chromspher Lipids columns (Cat. No. 28313; 4.6 mm I.D.×250 mm stainless steel; 5 μm particle size; silver ion impregnated) were purchased from Chrompack International (Middelburg, The Netherlands), and used as received. Two Lipids columns were connected in series to improve sample capacity and peak-to-peak resolutions. Solvent flow was standardized at 1.0 ml/min; isocratic conditions (0.15–0.5% ACN in hexane; 23°C) were used to minimize variations in FAME retention(s) and resolution(s). The void volume of this system was 4.2 ml.

2.3. Gas chromatography

The fatty acid methyl ester samples were collected in scintillation vials and the solvents were evaporated. The fractions were analyzed on a Varian 3400 gas chromatograph (GC; Varian Instruments, Palo Alto, CA, USA) equipped with a 30 m×0.32 mm SP2380 (Supelco, Bellefonte, PA, USA) capillary column, flame ionization detector (FID) and utilizing He as carrier gas. Operating conditions were: injector, 240°C; split ratio, 100:1; oven temperature programmed from 155°C to 220°C at 3°C/min with an initial hold of 15 min; detector, 280°C.

3. Results

The Ag-HPLC elution pattern for a synthesized sample of 9-*cis*,11-*trans*-18:2 FAME is illustrated in Fig. 1. Fig. 2 depicts the separation by Ag-HPLC of a mixture composed of *cis* and *trans* 18:1, conjugated-18:2 and non-conjugated-18:2 FAMEs. For comparison purposes, the GC elution pattern (SP2380 capillary column) for the mixture used in Fig. 2 is presented in Fig. 3. The 9-*cis*,11-*cis*- and 10-*cis*,12-*cis*-18:2 isomers were identified in Fig. 3 (GC; peak 9) by comparison with synthesized standards and from GC elution patterns obtained by other researchers [11]. The semi-preparative separation of a 10 mg mixture of 9-*trans*,11-*trans* and 9-*cis*,11-*trans*-18:2 FAMEs is illustrated in Fig. 4 (peaks 1 and 2).

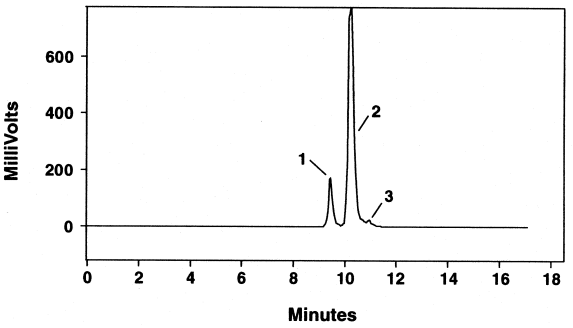

Fig. 1. Fractionation of methyl 9-*cis*,11-*trans*-18:2 by Ag-HPLC. Sample size: 20 μg; flow-rate: 1.0 ml/min 0.5% ACN in hexane. UV detection at wavelength of 206 nm. Peak no. 1=9-*trans*,11-*trans*-18:2; peak no. 2=9-*cis*,11-*trans*-18:2; peak no. 3=9-*cis*,11-*cis*-18:2.

4. Discussion

That the 9-*trans*,11-*trans*-18:2 and 9-*cis*,11-*cis*-18:2 isomers can be readily separated from the 9,11-*cis*/*trans* isomer pair by Ag-HPLC is demonstrated in Fig. 1 (peaks 1, 3 and 2, respectively). While Ag-HPLC is capable of separating the non-conjugated *cis*/*trans* isomers of 9,12-18:2 FAME (Fig. 2, peaks 7 and 8), it is less successful in separating the conjugated 9-*trans*,11-*cis*- and 9-*cis*,11-*trans*-18:2 isomers (Fig. 2, peaks 4 and 5). GC, however, gives rise to baseline separation of this isomer pair (Fig. 3, peaks 7 and 8).

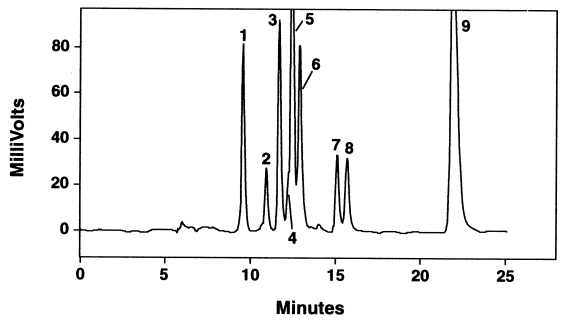

Fig. 2. Fractionation of a mixture composed of *cis* and *trans* 18:1, conjugated-18:2 and non-conjugated-18:2 FAMEs by Ag-HPLC. Sample size: 10 μg; flow-rate: 1.0 ml/min 0.5% ACN in hexane. UV detection at wavelength of 206 nm. Peak no. 1=9-*trans*-18:1; peak no. 2=9-*trans*,11-*trans*-18:2; peak no. 3=9-*trans*,12-*trans*-18:2; peak no. 4=9-*trans*,11-*cis*-18:2; peak no. 5=9-*cis*,11-*trans*-18:2; peak no. 6=9-*cis*-18:1; peak no. 7=9-*trans*,12-*cis*-18:2; peak no. 8=9-*cis*,12-*trans*-18:2; peak no. 9=9-*cis*,12-*cis*-18:2.

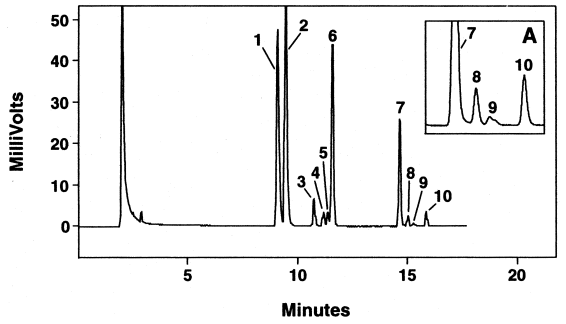

Fig. 3. Elution pattern of a mixture composed of *cis* and *trans* 18:1, conjugated-18:2 and non-conjugated-18:2 FAMEs by gas chromatography (see Section 2 for details). Peak no. 1=9-*trans*-18:1; peak no. 2=9-*cis*-18:1; peak no. 3=9-*trans*,12-*trans*-18:2; peak no. 4=9-*cis*,12-*trans*-18:2; peak no. 5=9-*trans*,12-*cis*-18:2; peak no. 6=9-*cis*,12-*cis*-18:2; peak no. 7=9-*cis*,11-*trans*-18:2; peak no. 8=9-*trans*,11-*cis*-18:2; peak no. 9=9-*cis*,11-*cis*-18:2 and 10-*cis*,12-*cis*-18:2; peak no. 10=9-*trans*,11-*trans*-18:2. Insert A: peak nos. 7–10.

The elution order depicted in Fig. 2 may be compared to the results described by Chobanov et. al. [13] using silver nitrate-impregnated silica thin layer chromatography (Ag-TLC). The order described by Chobanov differs from that observed with our system in that we found 9-*trans*,12-*trans*-18:2 to elute just after, rather than before, 9-*trans*,11-*trans*-18:2. Our system also allowed improved resolution of the *cis*/*trans* 9,12-18:2 isomer pair (Fig. 2, peaks 7 and 8) and a very slight separation of the 9,11-18:2 isomer pair (the *trans*/*cis* isomer elutes first; Fig. 2, peaks 4 and 5).

The Ag-HPLC elution pattern in Fig. 2 may be

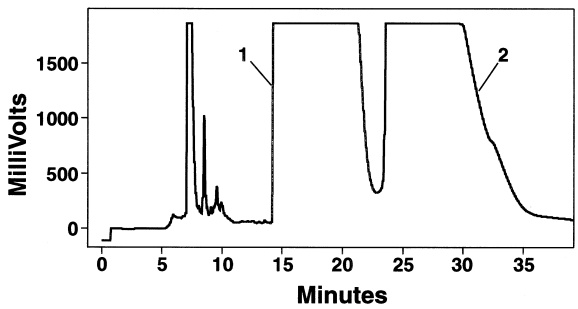

Fig. 4. Semi-preparative fractionation of a mixture of methyl 9,11-18:2 isomers by Ag-HPLC. Sample size: 10 mg; flow-rate: 1.0 ml/min 0.15% ACN in hexane. UV detection at wavelength of 212 nm. Peak no. 1=9-*trans*,11-*trans*-18:2; peak no. 2=9-*cis*,11-*trans*-18:2.

compared to the pattern observed with gas chromatography using the more polar cyanopropyl silicon (SP2380) capillary column (Fig. 3). The GC elution pattern of the FAME isomer mixture is 9-18:1 (*trans,* then *cis*), followed by 9,12-18:2 (*trans*/*trans, cis*/*trans, trans*/*cis,* then *cis*/*cis*), and then by 9,11-18:2 (*cis*/*trans, trans*/*cis*, *cis*/*cis*, then *trans*/*trans*). The GC elution pattern observed for the 9,11-18:2 isomers differs significantly from the 9,12-18:2 pattern, in that the *cis*/*cis* and the *trans*/*trans* isomers (Fig. 3, peaks 9 and 10) elute after the *cis*/*trans, trans*/*cis* isomer pair (Fig. 3, peaks 7 and 8). A similar elution pattern has been observed using a 60 m Supelcowax-10 (Supelco, Bellefonte, PA) capillary column [12]. The two small peaks defined as Peak No. 9 in Fig. 3 (insert A) eluted as one peak in [12], but were labelled as 9-*cis*,11-*cis*-18:2 and 10-*cis*,12-*cis*-18:2. The Ag-HPLC elution order for the 18:2 conjugated and the 18:2 non-conjugated FAME (Fig. 2) is similar to the 9,12-18:2 GC pattern (Fig. 3, peaks 3–6) except that the *cis*/*trans*-18:2 elution orders are reversed (*trans*/*cis* elutes before *cis*/*trans*). The Ag-HPLC pattern (Fig. 2) also demonstrates more overlap between the 9-18:1, the 9,11-18:2 and the 9,12-18:2 FAMEs. In contrast to the elution order observed for GC (Fig. 3), the majority of the 9,11-18:2 isomers elute before the 9,12-18:2 isomers with the Ag-HPLC system.

By manipulation of the percentage of ACN in the hexane, our Ag-HPLC system was also used to purify larger (8–10 mg) samples of CLA isomers. The fractionation of a 10 mg mixture of methyl 9-*trans*,11-*trans*-18:2 and methyl 9-*cis*,11-*trans*-18:2 synthesized in our laboratory is depicted in Fig. 4. A 1.0–2.5 mg sample of the same mixture was fractionated within 35 min using a solvent composition of ca. 0.3% ACN in hexane, while 5–7.5 mg samples were fractionated within 35 min at a solvent composition of 0.15% ACN in hexane. By adjustment of the percentage of ACN in the hexane, the separation of 20 μg–10 mg samples of conjugated FAME isomers was achieved. Purities of the isolated FAMEs were found to be 97–99% by GC. Ag-HPLC was unable to resolve all of the 9,11 CLA isomers studied, but the syntheses could be manipulated to yield only one of the *cis*/*trans*, *trans*/*cis* isomer pair. Ag-HPLC could then be applied for the semi-preparative isolation of these isomers.

Acknowledgements

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