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Short communication

Silver ion high-performance liquid chromatographic separation of fatty acid methyl esters labelled with deuterium atoms on the double bonds

R.O. Adlof*, E.A. Emken

US Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Food Quality and Safety Research, 1815 N. University Street, Peoria, IL 61604, USA

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Abstract

Silver ion HPLC (acetonitrile in hexane as solvent) was used to separate non-deuterated fatty acid methyl esters (*cis*-9-octadecenoate, *cis*-9,*cis*-12-octadecadienoate and *cis*-9,*cis*-12,*cis*-15-octadecatrienoate) from their analogues labelled with deuterium atoms on one or more of the double bonds. Placement of the deuterium atoms on the double bonds increased the retention time of the fatty acid methyl esters and could have useful applications in the analysis or isolation of deuterium-labelled fats and other metabolic products produced during the synthesis and metabolism of deuterium-labelled fatty acids. The % composition data, obtained by silver ion HPLC, can be used to evaluate isotopic purity.

1. Introduction

Silver ion high-performance liquid chromatography (Ag-HPLC) is a useful tool for separating a variety of unsaturated compounds by the number, configuration (*cis* vs. *trans*) or position of (location in the molecule) the double bonds [1–5]. Since separation by silver ion chromatography is primarily due to the interaction of the silver ion(s) with the double bond(s) π electrons of the compound, any neighboring group having an effect on the π electron density of the double bond would affect the retention of unsaturated compounds on a silver ion column. This effect has been documented for olefins and allenes [6] utilizing a silver nitrate–ethylene glycol gas chromatography (GC) column. In addition, steric effects can be inferred during the Ag-HPLC separation of *cis*- and *trans*-pheromone isomers containing branched side-chains adjacent to the double bonds (results not shown). Placement of deuterium atoms on the double bond(s) of fatty acid methyl esters (FAMEs) was therefore expected to effect retention times when compared with non-deuterated FAME analogues.

2. Experimental[1]

2.1. Materials and reagents

Hexane (Allied Fisher Scientific, Orangeburg, NY, USA) and acetonitrile (ACN; E. Merck,

* Corresponding author.

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended over other firms or similar products not mentioned.

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Darmstadt, Germany) were HPLC grade and used as received. Isooctane (E. Merck) was ACS grade. Non-deuterated samples of methyl oleate and methyl linolenate were obtained from NuCheck Prep, Elysian, MN, USA.

2.2. Syntheses

Samples of methyl oleate (18:1; *cis*-9-octadecenoate), methyl linoleate (18:2; *cis*-9,*cis*-12-octadecadienoate) and methyl linolenate (18:3; *cis*-9,*cis*-12,*cis*-15-octadecatrienoate) were prepared with deuterium atoms on one or more of the double bonds. [9,10-$^2H_2$]Methyl oleate (methyl oleate-9,10-d_2) was prepared [7] by reduction (Lindlar catalyst, deuterium gas) of methyl stearolate (9-octadecynoate). Methyl linoleate-12,13-d_2 was synthesized by the reduction (Lindlar catalyst, deuterium gas) of *Crepis alpina* seed oil and subsequent transesterification with HCl–methanol [8]. Since *Crepis alpina* seed oil originally contains about 14% non-deuterated linoleic acid [1.2% 14:0; 4.1% 16:0; 1.0% 18:0; 2.2% 18:1; 2.4% *cis*-9,*trans*-12-18:2; 14.3% 18:2; 74.0% crepenynic (9-*cis*-octadecen-12-ynoic) acid], the methyl linoleate isolated after reduction contained roughly an 80:20 ratio of deuterated to non-deuterated 18:2 and was used as such. Methyl linolenate-9,10,12,13,15,16-d_6 was prepared by the synthesis and subsequent reduction (Lindlar catalyst, deuterium gas) of the triacetylenic precursor [analogous to preparations described in Ref. 9]. Sample FAME mixtures were eluted with petroleum ether through a silica gel Sep-Pak (Waters Assoc., Milford, MA, USA) to remove any oxidation products and dissolved (ca. 10 mg/ml) in isooctane.

2.3. High-performance liquid chromatography

The liquid chromatography system consisted of a ChromSpher Lipids column (catalogue No. 28313; 250 mm × 4.6 mm I.D. stainless steel; 5 μm particle size; silver ion impregnated) purchased from Chrompack (Middelburg, Netherlands), a Spectra-Physics (Freemont, CA, USA) P2000 solvent-delivery system, a Rheodyne (Cotati, CA, USA) 7125 injector with a 20-μl injection loop and an ISCO (Lincoln, NE, USA) V4 UV absorbance detector at 206 nm. A strip-chart recorder (Houston Instruments, Austin, TX, USA) was used during sample collection, etc., while a recording integrator (Model 3390A; Hewlett-Packard, Avondale, PA, USA) was included during method reproducibility studies. Samples were eluted under isocratic solvent conditions (ACN in hexane); solvent flow was standardized at 1.0 ml/min. Solvent compositions and injected sample sizes are presented in Fig. 1.

2.4. Analyses

FAME fractions were collected from the HPLC and analyzed in triplicate by GC. GC–mass spectrometry (MS) was used to determine the % deuterated and non-deuterated FAMEs. Analyses were made on a Hewlett-Packard Model 5890A GC–MS system (quadrupole; positive chemical ionization mode; isobutane as ionizing gas) equipped with a 30 m × 0.25 mm Supelcowax 10 fused-silica capillary column (Supelco, Bellefonte, PA, USA). Data collection and manipulation have been described previously [10].

3. Results

Fig. 1 illustrates the Ag-HPLC elution patterns observed with deuterated and non-deuterated methyl oleate, linoleate and linolenate. In all examples, the non-deuterated isomer eluted first. Retention times of the deuterated FAME (compared to the non-deuterated analogue) increased as the number of deuterium-containing double bonds increased. The separation of the methyl oleate-d_0/-9,10-d_2 and methyl linoleate-d_0/-12,13-d_2 pairs was incomplete (Fig. 1A and B), but baseline separation of methyl linolenate-d_0 and -9,10,12,13,15,16-d_6 (Fig. 1C) was achieved.

The methyl linoleate-d_0 and -d_2 mixture used to generate Fig. 1B was analyzed in triplicate and the results were compared with composition data obtained by GC–MS. The percentages obtained by Ag-HPLC for -d_0 (20.8 ± 1.9) and -d_2 (79.1 ± 1.8) are similar (less than 2% of -d_2

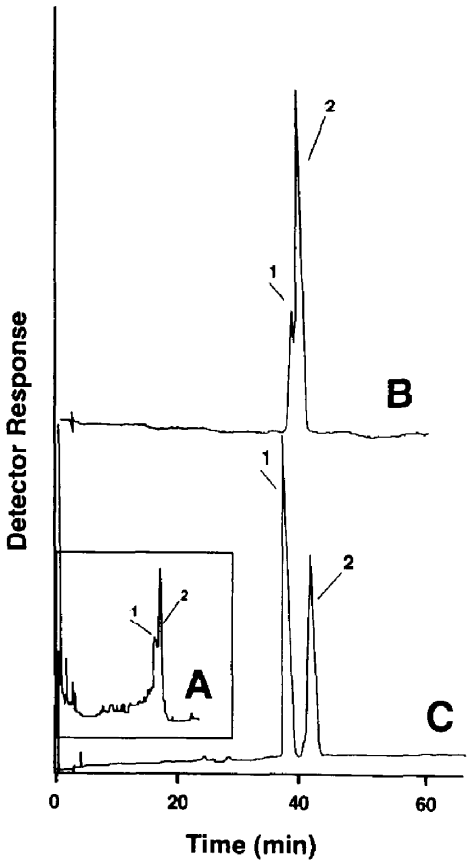

Fig. 1. Separation of deuterium-labelled and unlabelled FAMEs. (A) Methyl oleate-d_0 and -9,10-d_2; sample injected: 5 μl (1:3, w/w; 10 mg/ml isooctane); flow-rate: 1.0 ml/min of 0.07% ACN in hexane; UV detector at 206 nm; chart speed: 20 cm/h; peaks: 1 = oleate-d_0; 2 = oleate-9,10-d_2. (B) Methyl linoleate-d_0 and -12,13-d_2; sample injected: 0.5 μl (1:4, w/w; 10 mg/ml isooctane); flow-rate: 1.0 ml/min of 0.1% ACN in hexane; UV detector at 206 nm; chart speed: 10 cm/h; peaks: 1 = linoleate-d_0; 2 = linoleate-12,13-d_2. (C) Methyl linolenate-d_0 and -9,10,12,13,15,16-d_6; sample injected: 1.0 μl (2:1, w/w; 7 mg/ml isooctane); flow-rate: 1.0 ml/min of 0.2% ACN in hexane; UV detector at 206 nm; chart speed: 10 cm/h; peaks: 1 = linolenate-d_0; 2 = linolenate-9,10,12,13,15,16-d_6.

peak area) to the results obtained by GC–MS (19.3 and 80.7, respectively).

4. Discussion

No simple theory exists to predict the influence of substituents on the stability of the complex formed between silver ions and olefinic double bonds. Both steric effects and effects due to substituents that increase or decrease the electron density of the double bond have been suggested as explanation for observed differences in the stability of Ag–olefin complexes [6]. The fractionation of unsaturated FAMEs with deuterium atoms on the double bond(s) from their non-deuterated analogues by Ag-HPLC may be explained in part by changes in the stability of the complex formed between the Ag ions and the π-electrons of the double bond (see Refs. [11] and [12] for reviews of this topic). The increased electron release to the double bond by the stronger C–^{2}H bonds (compared with C–H bonds) would result in increased Ag-complex stability. Thus substrates with deuterium atoms on the double bonds should be retained longer by Ag-HPLC.

The elution order of deuterium-labelled and non-labelled FAMEs obtained by Ag-HPLC differs from the elution patterns obtained by capillary GC [13]. On both polar and non-polar capillary GC columns, the deuterium-labelled FAME elute first. Similar fractionations and elution orders have been documented in the separation of deuterated and non-deuterated carotenoids by reversed-phase HPLC [14]. The fractionation described in these examples occurs even when the deuterium atoms are not located on double bonds. With Ag-HPLC, however, the elution order is reversed, with non-deuterated FAMEs eluting before their deuterated analogues.

This technology may have future applications in the analysis and isolation of deuterium-labelled fats and other metabolic products produced during the metabolism of deuterium-labelled precursors. Work is currently underway to develop semi-preparative applications.

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