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Preparation, chromatographic separation and relative retention times of *cis*/*trans* heptadecaenoic (17:1) fatty acids

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ABSTRACT

In recent years, several countries have implemented new regulations regarding limitations or labeling of the *trans* fatty acid (*t*FA) content in foods. In order to comply with the new requirements, gas chromatographic methods for fatty acid (FA) analysis have been refined toward the quantitation of a larger number of FAs . Increased attention is also being paid to those present in lower quantities. This article describes a simple procedure for obtaining, pure or in mixtures, geometric and positional isomers of a commercially available monounsaturated FA . *cis* 10–17:1 Fatty acid methyl ester (FAME) was isomerized into its positional/geometrical isomers by repeated hydrobromination/dehydrobromination of its double bond. Reaction products were fractionated into *cis* and *trans* geometric isomers by silver ion HPLC. Pure *cis*-17:1 FAME positional isomers were obtained by reversed-phase HPLC fractionation and identified by gas chromatography – covalent adduct chemical ionization MS/MS using acetonitrile as the reacting gas. The isomerization with *p*-toluenesulfinic acid of the purified FAME yielded the corresponding *trans* isomers; these products were analyzed by GC with flame ionization detection using a Supelco 2560 capillary column in order to determine their elution order and retention times (t_R). A novel procedure was developed to determine t_r for 17:1 FAME positional/geometrical isomers relative to that of the commercially available *cis* 10–17:1 FAME.

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1. Introduction

In recent years, several countries have implemented new regulations regarding limitations or labeling of *trans* fatty acid (*t*FA) content in foods [1–4]. Current analytical methods for quantitation of *t*FA in foods are primarily based on gas chromatography (GC) [5–7]. Methodologies based on infrared spectroscopy (IR), by transmission or total attenuated reflection (ATR), are also available and have been refined in recent years [8–13]. However, the higher limits of quantitation for *t*FA by IR based methods compared with those possible with GC, and the inability of IR methods to provide information regarding the content of other fatty acids (FA), make these methods less desirable for *t*FA quantitation in foods [5,12,13]. *trans* monounsaturated fatty acids (MUFAs), the primary source of *t*FA in food, are of limited availability as reference materials. 18:1 MUFAs occur in large amounts in foods, and the GC separation/quantitation of their geometric/positional isomers has been widely studied [5,6,14]. With the exception of limited data regarding the separation of 16:1 fatty acid methyl esters (FAMEs) positional/geometrical isomers [14,15], identification of the GC elution profile of MUFAs, other than 18:1 FAMEs, is limited to that of the available reference materials.

The current study focuses on the preparation of 17:1 FAMEs, pure or in mixture, that can be used as reference for identification purpose. 17:1 FAs are considered minor components in foods. However, Ollivier et al. reported that 88 of the 564 samples of French virgin olive oil analyzed in their survey had a 17:1 FAs content higher than 0.3% [16]. Alves et al. identified as *cis* 9–17:1 FA the major 17:1 FA present in ruminant fats, observing that *cis* 8- and *cis* 10–17:1 FA are also present as minor positional isomers [17]. Both studies are based on the identification of the 17:1 FAMEs by refined GC–MS methodologies.

The procedure described here for the isomerization of *cis* 10–17:1 FAME can be applied to the isomerization of other MUFAs with the double bond not located in proximity to the carboxylic group, or in the terminal position. The process is based on the hydrobromination of the MUFA double bond yielding monobrominated FAs , followed by dehydrobromination yielding back the double bond in the same or adjacent position. Both reactions have been extensively studied in an earlier period of FA research (1930–1960), and the relevant literature was reviewed by Sonntag in 1961 [18]. Observations and conclusions reported by Sonntag

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should be evaluated in the light of analytical and separation techniques available at that time.

Silver ion HPLC (Ag^+-HPLC) has been widely applied to the separation of FAs based on double bond number, geometric configuration, and position [19–22]. In the case of medium chain length MUFAs, Ag^+-HPLC was observed to provide remarkable separation based on double bond geometric configuration, partial separation based on double bond position, and limited separation based on the FA chain length [22].

In recent years, reversed-phase HPLC has found only limited application in the separation of FAs [23,24]. Destaillats et al. used reversed-phase HPLC to collect the MUFA fraction from food samples as “a single peak” prior to GC analysis [23,24]. The reversed-phase separation applied in the current study is based on the unique selectivity of the Vydac 201TP54 column (Grace Vydac) eluting at sub ambient temperature. The Vydac 201TP54 column has been used for the separation of several compounds based on its selectivity toward analytes’ double bonds, including polycyclic aromatic hydrocarbons, vitamin A, vitamin E and β-carotene [25–28]. In the current study, Ag^+-HPLC was used to separate MUFAs into *cis*/*trans* isomers and reversed-phase HPLC was used to separate positional *cis*-17:1 FAME isomers.

The identification of the FA double bond position by GC–EI-MS requires the derivatization of the fatty acids into suitable derivatives such as picolinyl esters or 2-alkenyl-4,4-dimethyl oxazoline derivatives (DMOX)[29,30]. In the current study MUFA FAME chain length and double bond position identifications are achieved without derivatization, through the methodology developed by Brenna and co-workers [31–34]. MUFAs as FAMEs are analyzed with a GC coupled to an ion trap by acetonitrile covalent adduct chemical ionization MS/MS (GC–CACI–MS/MS). Acetonitrile, used as a chemical ionization reagent, produces by self reaction the 1-methyleneimino-1-ethenylium ion (*m*/*z* 54) that reacts with double bond of monounsaturated FAMEs yielding covalent adduct ions of mass $(M+54)^+$. Collisional dissociation of these ions causes cleavage adjacent to either allylic sites or the double bond, permitting its unambiguous localization.

p-Toluenesulfinic acid (PTSA) provides *cis*/*trans* isomerization of double bonds without modifying the double bond position on the aliphatic chain [35]. The conditions for the GC separation of FAME isomers were chosen in accordance with Ce-1h05 AOCS Official Method of Analysis [36].

The separation of FAMEs provided by the cyanopropyl capillary columns used for FAME analysis was observed to be affected by slight changes in column polarity [37] caused by column age or different production lots. As an example, the elution order of 21:0 FAME and conjugated linoleic acid positional isomers was shown to vary when analyzing the same test sample with CP-Sil 88 columns (Varian, Walnut Creek, CA) of different age [37]. *cis* 10–17:1 FAME was chosen to calculate relative retention times (RRT) values based on the fact that slight changes in capillary column polarity provide limited effect on the relative retention of FAME with the same chain length and unsaturation.

2. Materials and methods

FAME reference materials, including *cis* 10-heptadecenoic FAME, were purchased from Nu-Chek-Prep. (Elysian, MN, USA). HBr solution in glacial acetic acid (Purum grade, >33%, Fluka), anhydrous *tert*-butanol (> 99.5%, Sigma), potassium *tert*-butoxide (reagent grade, 95%, Sigma), 1,4-dioxane, and PTSA sodium salt (>95%, Aldrich) were purchased from Sigma Aldrich (St. Louis, MO, USA). UV grade acetonitrile, light petroleum (bp 30–60 °C, PE), isooctane and hexane were purchased from J. T. Baker (Phillipsburg, NJ, USA). Anhydrous diethyl ether (DE), suitable for FA analysis, and verified to be peroxide free, was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Pre-packed tubes containing 3 g of sodium sulphate were purchased from Alltech (currently Grace Davison, Deerfield, IL, USA) or containing 1 g from Varian. BF_3 14% in methanol was purchased from Supelco (Bellefonte, PA, USA).

2.1. Synthetic process

2.1.1. Addition of HBr

100–500 mg of monounsaturated FAME were placed in a 20 ml screw cap test tube and purged with argon. About 10 ml of 33% HBr in glacial acetic acid were placed in a separate screw cap test tube, and both test tubes were placed in a Nestlab 40 RTE chiller (Thermo Electron, Newington, NH, USA) at −15 °C. Two millilitre of chilled HBr in acetic acid were pipetted into the FAME test tube, while minimizing the amount of entering air. The chiller was covered with aluminum foil to prevent light exposure and the test tube was kept 3 h at −15 °C. The chiller temperature was then raised to 5 °C, and maintained constant for 48 h. Ten millilitre of 50:50 (v/v) DE/PE were added to the tube, swirled, and the content was transferred to a 125 ml separatory funnel. The test tube was rinsed two times with 10 ml of 50:50 PE/DE. Fifty millilitre of 50:50 saturated $NaHCO_3$/distilled water were slowly added to the separatory funnel and the pH was verified to be about neutral after the addition was completed. The neutralization reaction produces a large amount of carbon dioxide, and the separatory funnel must be continuously vented during mixing. The organic phase was gently washed with 50 ml of distilled water, filtered through an anhydrous sodium sulfate tube, and the solvent was removed under a gentle stream of argon. The addition of 2–3 drops of 6 M HCl was observed to reduce the formation of emulsions.

2.1.2. Elimination of HBr

1 M potassium *tert*-butoxide solution in anhydrous *tert*-butanol was prepared by dissolving 11.2 g of potassium *tert*-butoxide in 100 ml of anhydrous *tert*-butanol. Five millilitre of potassium *tert*-butoxide solution were added to the 20 ml crew cap test tube containing the reaction products of the HBr addition. The test tube was purged with argon, placed for 24 h in a silicon oil bath maintained at 100 °C, and covered with aluminum foil. After cooling the test tube to room temperature, 10 ml of 50:50 DE/PE were added and the content transferred to a125 ml separatory funnel. The test tube was rinsed two more times with 10 ml of DE/PE. Five millilitre of 6 M HCl were added, followed by 50 ml of 50:50 saturated $NaHCO_3$/distilled water. The aqueous phase pH was verified to be about neutral, and if still basic, corrected by adding 6 M HCl. The $NaHCO_3$ solution must be added slowly and mixing of the separatory funnel must be performed with continuous venting. The organic phase was washed with 50 ml of distilled water (with the addition of two drops of 6 M HCl), and dried through an anhydrous sodium sulfate tube. The solvent was removed under a gentle stream of argon.

2.1.3. Methylation

Final products of reaction were methylated according to previously published principles [38]. About 20–100 mg of the previous reaction products were placed in a 20 ml screw cap test tube, followed by 2–3 ml of 14% BF_3 in methanol. The tube was purged with argon and placed for 15 min in a silicon oil bath at 100 °C. After chilling to room temperature, 2–4 ml of isooctane were added and the tube was swirled. The test tube was then filled with NaCl saturated solution, mixed, and the organic phase was filtered through an anhydrous sodium sulfate tube. The extraction of FAME was repeated with 2–4 more ml of isooctane.