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Short communication

A rapid GC–MS method for quantification of positional and geometric isomers of fatty acid methyl esters

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ABSTRACT

So far the most frequently used method for fatty acid (FA) analysis is GC coupled to flame ionization detector (FID). However, GC–FID does not allow profiling of FA synthesis and metabolism using stable isotopes. Here we present a rapid and sensitive GC–MS method for determination of fatty acid methyl esters (FAMEs). Fatty acid methylation was carried out by transesterification with acetyl-chloride and methanol. FAME separation applies a short and polar cyano-column resulting in an analysis time of 17.2 min. Separation was achieved for positional and geometrical (*cis*/*trans*) isomers with chain lengths between C8 and C28. Partial overlap of FAMEs (e.g. for C20:2 (*n*−6) and C21:0) could be resolved using selected ion monitoring (SIM). The precisions for human plasma samples were better than 10% coefficient of variation (CV) except for very low abundant FAs and LODs were in the low femtomol range on column. The developed GC–MS method also allows quantification of conjugated FAs such as conjugated linoleic acid (CLA) isomers because lowering the derivatization temperature from 95 °C to room temperature prevented cis to trans double bond isomerization. Finally, profiling of fatty acid synthesis and metabolism was exemplified with stable isotope labeling of macrophages using fatty acid precursors or deuterated fatty acids. In summary, we present a fast and robust GC–MS method for fatty acid profiling of positional and geometrical isomers including CLAs as well as very long chain fatty acids (VLCFAs). The method is suitable for both clinical studies and basic research including application of stable isotope compounds.

1. Introduction

Several studies have shown that *n*−3 poly-unsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) protect against coronary heart disease [1]. In contrast to *n*−6 fatty acids, *n*−3 fatty acids are precursors of signaling molecules with mainly anti-inflammatory effects [2]. Besides *n*−3 fatty acids, conjugated linoleic acid (CLA) has been reported to have various beneficial effects for human health, such as anti-atherogenic and anti-diabetic effects. CLA refers to a group of positional isomers of octadecadienoic acid with conjugated double bonds [3,4].

Thus, the rapid and precise determination of fatty acid profiles within nutritional, epidemiological and clinical studies is becoming of increasing interest for basic research and human health. Numerous different methods have been employed for total fatty acid analyses in biological and cellular samples. Most methods determining fatty acids in biological samples use gas chromatography coupled to a flame ionization detector (GC–FID). A powerful alternative to GC–FID for fatty acid analysis is gas chromatography coupled to mass spectrometry (GC–MS) combining the separation power of GC with structural information obtained by MS detection [5,6]. Additionally, GC–MS in contrast to GC–FID is suitable for metabolic studies with stable isotopes.

GC–MS methods for the analysis of fatty acids described so far suffer from analysis times longer than 30 min [7,8] or do not show separation of geometric and positional isomers [9,10]. Here we present a novel, fast GC–MS method based on a short, small diameter polar column characterized by a run time of 17.2 min and separation of geometrical and positional isomers of fatty acids.

2. Experimental

2.1. Reagents and standards

Acetyl-chloride, methanol and n-hexane were purchased from Merck. As standard a commercial fatty acid methyl ester (FAME)

Abbreviations: CLA, conjugated linoleic acid; CV, coefficient of variation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl esters; FID, flame ionization detector; PUFA, poly-unsaturated fatty acids; SIM, selected ion monitoring; VLCFA, very long chain fatty acids.

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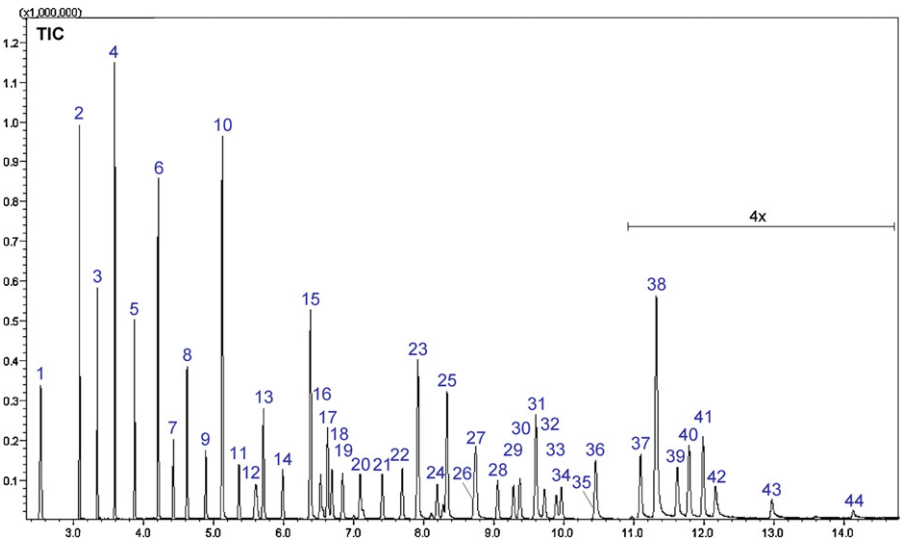

Fig. 1. Chromatogram of a FAME standard mixture. The peaks are assigned in Table 1.

standard mixture (FAME Mix 37, Supelco) spiked with FAMEs of phytanic acid, C18:1 (*cis*-11; *n*−7), C20:4 (*cis*-8,*cis*-11,*cis*-14,*cis*-17; *n*−3), C22:4 (*cis*-7,*cis*-10,*cis*-13,*cis*-16; *n*−6), C25:0, C26:0 and C28:0 (Larodan, Sweden) was used. The internal standards C13:0 and 19-methyleicosanoate (C21:0 *iso*) and the conjugated linoleic acid isomers C18:2 (*trans*-9,*trans*-11), C18:2 (*cis*-9,*trans*-11) and C18:2 (*trans*-10,*cis*-12) were obtained from Larodan (purity of CLAs 89–94%). Stock standard solutions were prepared in n-hexane and stored at −20 °C until usage. Internal standards were dissolved in methanol. The stable isotope labeled 2-^{13}C acetate was obtained from Cambridge Isotope Laboratories, D_3-C16:0 was purchased from Sigma.

Human EDTA-containing plasma was prepared from blood samples freshly drawn from healthy human volunteers; approval was obtained from the University of Regensburg ethics committee. All samples were stored at −80 °C prior to analysis.

2.2. Sample preparation

FAMEs were prepared as described previously [11]. Briefly, 10 μl plasma or cell homogenate corresponding to 50 μg protein were methylated in PTFE screw capped Pyrex tubes. 1 μg each of C13:0 and C21:0 *iso* were added as internal standards in 50 μl methanol. Derivatization was performed with 2 ml of methanolic acetyl-chloride (10%) and 500 μl n-hexane at 95 °C for 1 h under vigorous shaking in a water bath. For CLA analysis derivatization was carried out at RT over night. After cooling down to RT, 5 ml 6% potassium carbonate solution was added. 100 μl of the n-hexane top layer was transferred into a 500 μl auto-sampler vial and crimped.

2.3. Gas chromatography coupled to mass spectrometry

FAMEs were separated by a highly polar BPX70 column (10 m length, 0.10 mm diameter, 0.20 μm film thickness, SGE) coated with 70% cyanopropyl polysilphenyl-siloxane using a GC-2010 coupled to a GCMS-QP2010 detector from Shimadzu. The injection volume was 1 μl and a programmed temperature vaporizer (PTV) was used in the spilt mode 1:20 for 3 s, switched for 1.3 min to the splitless mode and a split ratio of 1:100 until end of the run. Injection temperature was 72 °C; after 3 s it was increased with 240 °C/min to 250 °C and hold for 15 min. The liner was packed with CarboFrit™ (Restek).

The temperature program was as follows: the initial oven temperature 50 °C was hold for 0.75 min, then programmed to increase with 40 °C/min to 155 °C, with 6 °C/min to 210 °C, to reach finally with 15 °C/min 250 °C and hold for 2 min. Helium was used as carrier gas with a constant linear velocity of 50 cm/s. The detector temperature was kept at 250 °C.

Characterization and identification of FAMEs was performed in the scan mode. Quantification was done by selected ion monitoring (SIM) mode of the most intense fragments (saturated: *m*/*z* 74, mono-unsaturated: *m*/*z* 55, di-unsaturated: *m*/*z* 67, poly-unsaturated: *m*/*z* 79). Data acquisition and processing were performed with the GC–MS Solution Software (Shimadzu). Quantification was based on an external calibration with C21:0 *iso* as internal standard. C13:0 added in a constant ratio to C21:0 *iso* was used as a quality control.

2.4. Stable isotope labeling of cells

To study fatty acid synthesis, human primary macrophages were incubated with 10 mM 2-^{13}C acetate for 24 h. For palmitate desaturation and elongation studies cells were incubated with 50 μM D_3-palmitate for 24 h. Cells were harvested in PBS and homogenized by sonication. Enrichment of 2-^{13}C-acetate in palmitate was analyzed by mass isotopomer distribution analysis using SIM of molecular ions (*m*/*z* 270–278) [12,13]. Desaturation and elongation of D_3-C16:0 was determined by SIM of specific molecular or fragment ions of the metabolites (D_3-C16:0: *m*/*z* 273, D_3-C16:1: *m*/*z* 242, D_3-C18:0: *m*/*z* 301).

3. Results and discussion

3.1. Gas chromatographic separation

Aim of this study was to develop an easy and fast method to quantify fatty acids in biological materials. Fatty acid methyl esters (FAMEs) were prepared by transesterification in a one-step reaction with acetyl chloride and methanol based on the protocol by Lepage and Roy [11]. To analyze biological samples with low concentration we decided to use a programmed temperature vaporizer (PTV) as sample inlet. Beside a gain of sensitivity, the use of a PTV showed superior peak shapes compared to conventional split/split-less operation (data not shown). In biological samples it is mandatory to separate positional and geometric isomers of fatty