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Quantitation of trans-fatty acids in human blood via isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry

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Abstract

Trans-fatty acids (TFA) are geometric isomers of naturally occurring cis-fatty acids. High dietary TFA intake has been associated with risk factors for cardiovascular disease. However, little is known about TFA levels in humans. To address this data need, we developed and validated a new isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry (ID-GC-NCI-MS) method for quantitation of 27 fatty acids (FA) including 4 major TFA in human plasma, serum, and red blood cells (RBC) from 66 donors. Quantitation was performed with 18 isotope labeled internal standards and results are presented in μM and % of total FA. This method has high sensitivity and specificity due to use of pentafluorobenzyl-bromide derivatization combined with NCI-MS and a 200 m column to optimize positional and geometric FA isomer separation. The four major TFA, palmitelaidic acid, elaidic acid, trans-vaccenic acid, and linoelaidic acid, were detected in all samples, with median total TFA concentrations of 17.7 μM in plasma, 19.6 μM in serum, and 21.5 μM in RBC. The % of total FA for the TFA was 0.20% in plasma, 0.20% in serum, and 0.30% in RBC. Patterns for % FA are similar to those reported in other studies. We developed a highly specific, ID-GC-NCI-MS method to quantitate TFA and other FA in humans.

Keywords

Trans-fatty acids; Plasma; Serum; Red blood cells; GC–MS; Silver ion chromatography

1. Introduction

Trans-fatty acids (TFA) are unsaturated fatty acids (FA) with at least one double-bond in the trans- configuration. They are not synthesized in the human body. TFA are the geometric

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isomers of naturally occurring cis-FA that are formed industrially during partial hydrogenation of vegetable oils or naturally through biohydrogenation by rumen bacteria in ruminant animals [1,2]. The same trans-isomers can be formed in both processes, but the amount of each positional isomer can vary [3–5]. Industrially produced TFA have been shown to include more than 10 different C18:1 positional trans-isomers from C18:1n-2 t through C18:1n-13 t, with elaidic acid (C18:1n-9 t) as the most abundant trans-isomer in hydrogenated oils, margarines, and shortenings [5,6]. TFA in fat from ruminant animals, such as milk fat, was reported to also contain over 10 different C18:1 TFA isomers with the trans-vaccenic acid (C18:1n-7 t) isomer being the most abundant [7]. Partially hydrogenated vegetable oils have been commonly used in the manufacture of a wide range of food products. People are exposed to TFA through consumption of food containing these oils, as well as food derived from ruminant animals.

High dietary TFA intake is associated with increased low-density lipoprotein cholesterol levels in blood and increased concentrations of other risk factors for cardiovascular disease [8]. Reduction of TFA intake to decrease the risk of cardiovascular diseases in people is a major public health objective, and there are ongoing federal, state, and local activities to achieve this objective [9,10]. TFA exposure assessments to monitor the impact of these activities and their impact on lipid profiles are commonly performed using dietary questionnaires together with food composition databases to estimate intake, or through intervention studies [11]. However, little is known about lipid profiles and the actual TFA concentrations in human blood [12].

Few analytical methods were described for measuring TFA in human blood [13–15]. These methods typically use 100 m gas chromatography (GC) columns with non-specific flame ionization detectors (FID) and present TFA results as a percentage of all detected (total) FA instead of presenting concentration results. Over 65 different FA including TFA have been reported in humans [16–21]. The various analytical methods described in the literature measure different numbers of FA, and thus calculate total FA differently. This limits the comparability of data obtained with these methods. Furthermore, the large number of C18:1 cis- and trans-isomers in particular, pose analytical challenges, because these isomers have the same molecular mass and very similar chemical structures. Therefore, they cannot be distinguished from each other by mass or mass fragmentation using mass spectrometry (MS) and need to be separated chromatographically before they can be detected and quantitated. The separation of all cis- and trans-isomers requires very high chromatographic resolution. In addition, these cis- and trans-isomers occur in blood in very different amounts ranging from micro molar to millimolar concentrations [22,23]. This requires analytical methods that can measure FA over a wide concentration range. Current analytical methods do not address all these challenges, creating the need for new, very sensitive and specific analytical methods with a wide measurement range suitable to measure TFA and regular FA in the general population.

Here we report a sensitive and specific analytical method that enables the quantitative analysis of 4 major TFA and 23 regular FA in plasma, serum, and red blood cells (RBC) via GC-MS.

2. Materials and methods

2.1. Chemicals and reagents

Individual fatty acid methyl esters (FAMEs) (Table 1) and FAME mixtures were purchased from Nu-Chek Prep (Elysian, MN). Stable isotope-labeled FA and FAMEs for use as internal standards (IS) (Table 1) were obtained from Sigma-Aldrich (St. Louis, MO), Iso-Sciences (King of Prussia, PA), Cayman Chemical (Ann Arbor, MI), and Cambridge Isotope Laboratories (Andover, MA). FA and FAMEs for interference testing were purchased from Nu-Chek Prep and Sigma-Aldrich (Supplemental Table S1). All other reagents were analytical grade and were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Calibration and internal standards

Calibration curves were created by combining solutions of individual FAMEs in toluene to make a working solution. This working solution was used as the highest calibrator level and was diluted 2, 4, 10, and 25 times to create four additional calibrator levels. FAMEs were used as standards for calibrator preparation in order to mimic bound fatty acids in vivo. The calibrators, like the samples, must undergo hydrolysis to free the fatty acids for analysis. Solutions of individual stable isotope labeled standards in toluene were combined to create an IS solution (for concentrations of calibrator and IS solutions see Table 1). The five calibrators and a reagent blank were processed in each batch of samples in the same manner as patient samples.

2.3. Quality control samples and blood samples

Quality control (QC) samples at low, medium, and high TFA levels, covering the concentration range typically observed in the general population, were prepared from individual plasma units (Bioreclamation, Inc., Westbury, NY). The QC samples were analyzed in each sample batch in the same manner as patient specimens. Acceptance criteria for values obtained with the QC materials were established by measuring the QC samples in duplicate over 20 days and processing the data as described previously [24]. QC evaluation was performed using SAS (version 9.1, Cary, NC). EDTA-plasma, serum, and RBC samples from 66 adult donors were procured from Bioreclamation, Inc. The company has IRB approval to collect blood and urine and obtains informed consent from donors. CDC's use of the blood and urine is consistent with the IRB approval and donor consent. No personal identifiers were provided to CDC.

2.4. Sample preparation

Plasma, serum, and RBC samples were prepared for analysis as described by Lagerstadt et al. [25]. In brief, 100 μL of sample was combined with 100 μL of IS solution. Consecutive acidic (2 mL of 10% v/v 6 N HCl in acetonitrile) and alkaline (2 mL of 10% v/v 10 N NaOH in methanol) hydrolysis reactions were each carried out at 104 °C for 45 min, followed by neutralization with 6 N HCl. The free FA were extracted with hexane (3 times, 2 mL each), and the solvent was removed under vacuum (Genevac, Stone Ridge, NY). Samples were derivatized at room temperature for 15 min with 100 μL of 7% pentafluorobenzyl-bromide (PFB-Br) in acetonitrile and 10 μL triethylamine. The PFB-esters were extracted with 500

µL hexane and transferred to an autosampler vial for GC–MS analysis. Samples were handled in glass vials to minimize contamination of samples with FA from plastic supplies.

2.5. GC–MS analysis

The analysis of 27 FA and 18 IS was carried out on a 7890/5975C GC–MSD from Agilent Technologies (Santa Clara, CA). The inlet temperature was 240 °C and 1 µL sample injections were performed with a 100:1 split ratio using a Gerstel Multipurpose Sampler MPS (Gerstel, Mülheim an der Ruhr, Germany) equipped with a cool drawer set at 10 °C. The carrier gas was hydrogen at a flow rate of 2 mL/min. Separation was achieved using an Agilent Select FAME (200 m × 250 µm × 0.25 µm) column. The column temperature was ramped from 50 °C to 160 °C at 40 °C/min, held at 160 °C for 10 min, increased 1 °C/min to 175 °C, increased 0.5 °C/min to 210 °C, and increased 35 °C/min to 260 °C then held for 25 min. Selected Ion Monitoring (SIM) analyses were carried out in negative chemical ionization (NCI) mode using methane as the reagent gas. The transfer line, source, and quadrupole temperatures were 260, 230, and 150 °C, respectively. The electron multiplier voltage was adjusted throughout the chromatographic run to increase sensitivity for low abundant FA and prevent detector saturation for high abundant FA.

The impact of NCI as compared to electron ionization (EI) on FA fragmentation and signal intensity was assessed by analyzing calibrator solutions with both ionization techniques. Separation of FAMEs for the EI analyses was carried out as described above for the PFB-esters. SIM analysis was performed using the M^+ ion and a confirmation ion of either $[M-31]^+$, or $[M-32]^+$ to ensure specificity.

2.6. Method quantitation and validation

The FA were identified based on their chromatographic retention time compared to known standards and on the specific mass to charge (*m/z*) ratio of the ion formed in the ion source using SIM (Table 1). Peak area analysis was performed using Mass Hunter B.07.00 (Agilent Technologies). Analyte peak areas were normalized to the IS peak area. Quantitation was performed with calibrator solutions using stable isotope-labeled FA as IS. Calibration curves were constructed using linear regression.

2.6.1. Sensitivity and linearity—The limits of detection (LOD) were determined using Taylor's method [26]. The assay linear ranges were determined by measuring four replicates of each calibrator solution, along with additional calibrators both above and below the regular calibration curve, and assessing them according to CLSI EP6 using EP Evaluator (Data Innovations, Burlington, VT).

2.6.2. Accuracy—Accuracy of the method was assessed using two independent standard materials containing the TFA and the regular FA. Each standard was prepared at three concentrations, 4 mg/mL, 2 mg/mL and 0.2 mg/mL, in order to ensure that all analytes fell within the calibration range. Measurements were made in quintuplicate.

2.6.3. Precision and stability—The intraday and inter-day precision, expressed as percent coefficient of variation (%CV), was assessed using duplicate samples of each QC

pool, prepared and analyzed by five analysts, on six instruments, over 20 days ($N = 40$) and analysis of variance (SAS version 9.4, Research Triangle Institute, Research Triangle Park, NC). The stability of the analytes was assessed for neat and prepared samples at −70 °C over 12 months, and for prepared samples at 10 °C for 5 days.

2.6.4. Specificity—Method specificity was assessed by analyzing 58 commercially available FA (Supplemental Table S1) as potential interferences and comparing their retention time and *m/z* with our analytes. Additionally, silver ion solid phase extraction (SPE) was used to isolate TFA from regular FA [27]. Samples were hydrolyzed and extracted as described in Section 2.4. A mixture of known FA was used to determine the optimal SPE conditions with special focus on separating C16:1 and C18:1 trans-isomers from their corresponding cis-isomers. Free FA in hexane were loaded onto a silver ion cartridge (Discovery Ag-Ion, 750 mg/6 mL, Supelco, Bellefonte, PA) preconditioned with 4 mL each acetone and hexane. Five fractions were obtained by stepwise elution with 6 mL of each hexane:acetone (90:10 v/v, Fraction 1 containing saturated FA [SFA]), hexane:acetone (88:12 v/v, Fraction 2 containing trans-monounsaturated FA [MUFA]), hexane:acetone (84:16 v/v, Fraction 3 containing cis-MUFA and trans, trans-polyunsaturated FA [PUFA]), acetonitrile: acetone (60:40 v/v, Fraction 4 containing cis, trans- and trans, cis- PUFA), and acetonitrile (Fraction 5 containing cis, cis-PUFA), respectively. FA in each fraction were then derivatized and analyzed as described in Sections 2.4 and 2.5.

3. Results

3.1. Chromatographic separation and specificity

A 200 m Select FAME GC column was used to separate 28 FA (Fig. 1). Special attention was given to separate TFA isomers palmitelaidic acid (C16:1n-7 t), elaidic acid, and trans-vaccenic acid from adjacent peaks. Using the conditions described in Section 2.5, at least a 75% chromatographic resolution of all TFA and FA isomers quantitated by this method was achieved. Chromatographic separation consistency was verified on 6 different 200 m Select FAME columns over 20 days as part of the precision assessment. We also assessed other GC columns commonly used for FA analysis [14,15,28], finding the best separation for the TFA and FA isomers in human blood was achieved with the 200 m Select FAME column.

The chromatographic resolution of cis- and trans-isomers was verified by fractionating cis- and trans-isomers using silver ion SPE. GC–MS analysis of fractions obtained with plasma samples, showed that the TFA quantitated with our method are sufficiently resolved from other cis- and TFA, allowing for accurate and reliable quantitation of FA in human samples (Figs. 2–4). Furthermore, it showed that our method is able to separate and detect over 30 other TFA and cis-isomers. However, due to the lack of appropriate standards, these TFA were not quantitated.

As potentially interfering compounds, we tested 15 MUFA, 14 SFA, 8 PUFA, 19 TFA, and 2 hydroxy-FA (Supplemental Table S1). All 58 compounds were found to have either distinctly different retention times and/or *m/z* values from our analytes of interest.

3.2. Method quantitation and validation

3.2.1. Sensitivity and linearity—The PFB-esters are cleaved in the ion source of the mass spectrometer using NCI. This approach produced a single intact carboxylate anion instead of the large number of fragment ions typically observed in EI. This resulted in a twentyfold increase in sensitivity (Fig. 5). The TFA LODs were 0.07 μM for palmitelaidic acid, 0.28 μM for elaidic acid, 0.43 μM for trans-vaccenic acid, and 0.02 μM for linoelaidic acid (C18:2n-6 t,9 t). The LODs of regular FA were of similar magnitude (Table 2). With this sensitivity, we were able to detect all four TFA and all regular FA in every blood sample we analyzed.

The dynamic range of the detection systems spans four order of magnitude (Table 2). The calibration curves are linear for low abundance TFA such as linoelaidic acid (linear range: 0.15–9.20 μmol/L) as well as high abundance regular FA such as linoleic acid (C18:2n-6,9; linear range: 160–8010 μmol/L). We considered several unweighted and weighted calibration curves and the best fit was obtained using unweighted linear curves.

3.2.2. Accuracy—The accuracy for the four TFA was 96% (95% CI: 94%–97%) for palmitelaidic acid, 92% (95% CI: 88%–95%) for elaidic acid, 90% (95% CI: 86%–93%) for trans-vaccenic acid, and 90% (95% CI: 88%–91%) for linoelaidic acid. The accuracy for all regular FA averaged 96% (individual values in Table 2).

3.2.3. Precision and stability—The TFA intraday and inter-day imprecisions range from 2 to 12%CV and 0 to 13%CV, respectively (Table 2). For certain fatty acids there is no measurable imprecision beyond the intraday imprecision resulting in an inter-day imprecision of 0%CV. We did not observe any changes in analyte concentration over the 12 month period for QC samples at −70 °C or the 5 day period for prepared samples at 10 °C.

3.3. Measurement of TFA and FA in plasma, serum, and RBC

We used plasma, serum, and RBC samples from 66 individuals to evaluate our method. Quantitation of the 27 FA was performed using 18 isotope labeled IS. FA concentrations are presented in μM and as % of total FA (Table 3). Palmitelaidic acid, elaidic acid, trans-vaccenic acid, and linoelaidic acid were detectable in all samples, with the median total TFA concentrations of 17.7 μM in plasma, 19.6 μM in serum, and 21.5 μM in RBC. In RBC, the concentrations for lignoceric acid (C24:0), nervonic acid (C24:1-n9), and docosatetraenoic acid (C22:4n-6) were well above the measurement range of our method for all samples, so no RBC data is reported for these analytes. The sum % of total FA for the four TFA was 0.20% in plasma, 0.20% in serum, and 0.30% in RBC.

Overall, the plasma contained 30.7% (2700 μM) SFA, 20.6% (1830 μM) MUFA, and 47.4% (4220 μM) PUFA. The serum consisted of 33.2% (3370 μM) SFA, 20.4% (1970 μM) MUFA, and 44.8% (4410 μM) PUFA. The RBC were comprised of 45.4% (3260 μM) SFA, 14.2% (1030 μM) MUFA, and 40.6% (2910 μM) PUFA.

Plasma FA concentrations were on average 13% lower than the serum concentrations for all FA measured, while the % of total FA values were similar for both matrices. The shorter chain FA (C14:0 through C18:3) were present at higher concentration and % of total FA

levels in the plasma and serum than in the RBC, with the exception of stearic acid (C18:0) which was higher in RBC as were the longer chain FA (C20:0 and above). In plasma and serum, linoleic acid, palmitic acid (C16:0), and oleic acid (C18:1n-9) were the most abundant FA. In RBC, palmitic acid, arachidonic acid (C20:4n-6,9,12,15), and stearic acid were the most abundant. Linoelaidic acid and palmitelaidic acid were the lowest abundance FA in all three matrices. FA patterns within each matrix and across matrices were consistent regardless of whether data was presented in concentration or as % of total FA (Fig. 6).

4. Discussion

The purpose of this study was to develop and validate a method for the accurate quantitation of four major TFA in humans, in addition to regular FA, suitable to obtain information about TFA exposure in humans and to monitor changes of TFA levels in the population.

The method is highly specific and accurate. One of the major challenges in measuring FA in humans is the large number of FA and FA isomers occurring in blood. With this method, 4 TFA and 23 regular FA were quantitated. Thirty-seven other FA, predominately C16:1, C18:1, and C18:2 isomers, can be detected in plasma with this method. However, due to the lack of appropriate standards these additional FA were not quantitated. The specificity of this method was verified by assessing 58 potentially interfering FA. In addition, we separated cis- and trans-isomers in plasma samples and standard solutions using silver ion chromatography to ensure that no co-elution of cis- and trans-isomers measured with this method occurs. Both experiments showed no interferences with the FA and TFA measured with this method. Additionally, assessments using independent standard materials showed a high level of accuracy, which suggests that the method is not affected by interfering compounds. Derivatization of FA and TFA with PFB-Br and detection using NCI-MS resulted in ions with *m/z* ratios specific to the carboxylate anion of the FA. This enabled the separation of FA with different *m/z* ratios. However, mass spectrometry was not able to distinguish between different isomers. Isomer separation was achieved using a 200 m Select FAME column with a temperature program that was optimized to achieve an appropriate separation of FA and TFA, especially for C16:1 and C18:1 isomers. Furthermore, the identity of the FA was ensured using stable isotope labeled IS. This is the first method that allows for the simultaneous separation and reliable quantitation of 23 FA and 4 TFA isomers. Other FA analyses in the literature describe the separation of large numbers of regular FA, were optimized to separate and quantitate specific FA isomers, or use multiple chromatographic runs in order to measure TFA and regular FA [13,18,29].

The method is highly sensitive and has a wide dynamic range. The limited amount of specimen available in epidemiological studies in combination with the very low abundance of TFA in blood requires highly sensitive methods to be able to reliably quantitate TFA. At the same time, the high abundance of regular FA can cause detector saturation and, as a consequence, incorrect quantitation. High sensitivity was achieved for this method by using PFB-Br derivatization in combination with NCI. This approach resulted in an approximately 20-fold sensitivity increase compared to traditional EI. Detector saturation with high abundance FAs was avoided by adjusting the electron multiplier voltage accordingly throughout the chromatographic run. Using stable isotope labeled IS and analyte specific

calibrators ensured accurate quantitation even when different instruments with instrument specific sensitivity settings were used. The linearity of the calibration curves suggests that no detector saturation exists, which otherwise would have resulted in non-linear curves. The achieved sensitivity is sufficient for measuring FA and TFA in plasma, serum, and RBC samples collected in the general population. This is reflected in all FA being detectable in all the samples analyzed in this study. Furthermore, less than 1% of the analytes quantitated in this study had FA or TFA levels that were outside the analytical measurement range of the method for plasma and serum. This indicates that the dynamic range of the method is sufficient for analyzing FAs and TFAs in plasma and serum in the general population. In RBC, 3 out of 27 FA had concentrations well above the measurement range and different calibrator solutions and sample preparation procedures would be needed for their quantitation.

The method is precise and reproducible. The precision of the method is within the range suggested for bioanalytical methods [30] and similar to those reported for other FA methods [16,25]. The precision data was obtained by 5 analysts using 6 different GC-MS systems. The low imprecision demonstrates that the method is very reproducible.

The TFA measured with our method are the major TFA reported in other studies, including linoelaidic acid, with two double bonds in the trans-configuration in order to obtain information about trans-PUFA in blood. The FA concentrations in plasma were lower than in serum, while the % of total FA was consistent between the two matrices.

The levels of regular FA we measured are in good agreement with the levels reported by others (Fig. 6) in plasma [13,15,18,23,31], serum [29,32,33], and RBC [13,14,18,31,34].

The TFA % of total FA in our analyses were comparable to those measured by Enke et al. [13] for elaidic acid, trans-vaccenic acid, and linoelaidic acid in plasma and RBC, and Liu et al. [29] for palmitelaidic acid and elaidic acid in serum. Otherwise, our % of total FA results for the TFA were generally lower than those reported by others [14,15,29,31,34]. The reasons for these differences are not fully understood and could be related to differences in timeframe of sample collection and different isomer resolution. Specimens used in some studies [15,31] were collected between 1989 and 2001; prior to the FDA requirement to include TFA on nutrition labels. We previously found plasma TFA levels to be substantially lower in 2009–2010 than in 1999–2000 in fasted adults from the National Health and Nutrition Examination Survey (NHANES) [22]. Our samples were collected in 2013, which could account for some discrepancy between our TFA measurements and those of other labs. The presence of many challenging to separate positional and geometric isomers in the C16:1, C18:1, and C18:2 regions could also be a contributing factor to differences in TFA measurements. No information was available to compare the level of isomer separation, specifically positional and geometric isomers of C16:1, C18:1, and C18:2 FAs.

5. Conclusion

We have developed a sensitive and specific analytical method that enables the quantitative analysis of 4 major TFA and 23 regular FA in plasma, serum, and red blood cells. The

chromatography has been optimized for human blood samples, focusing on FA positional and geometric isomer resolution using a 200 m GC column. This method is suitable for the analysis of TFA in humans in large population studies such as NHANES.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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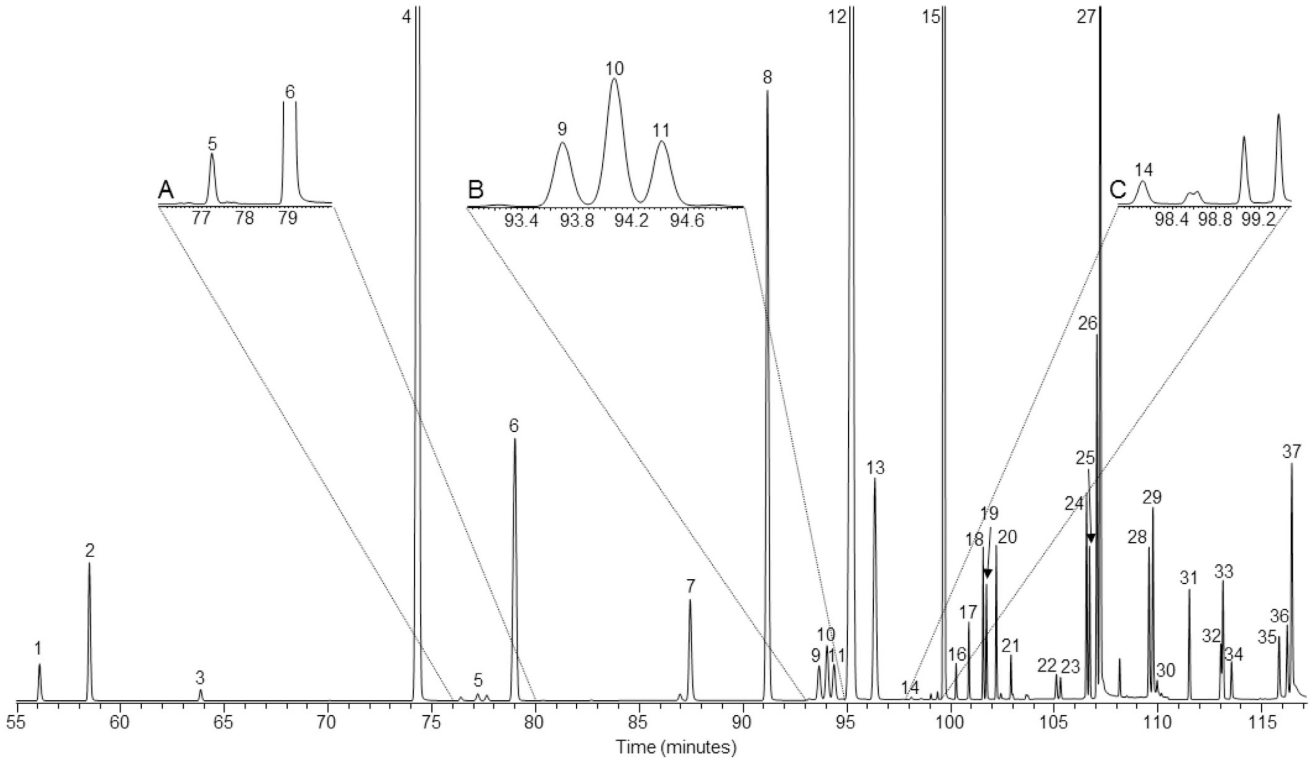

Fig. 1.

Total Ion Chromatogram of a mix of synthetic fatty acid standards analyzed by gas chromatography-negative chemical ionization-mass spectrometry on a CP-7421 Select FAME column. The trans-fatty acids are highlighted in zoomed extracted ion chromatograms (A) *m/z* 253.3 showing palmitelaidic acid (5) and palmitoleic acid (6); (B) *m/z* 281.3 showing elaidic acid (9), petroselinic acid (10), and trans-vaccenic acid (11); and (C) *m/z* 279.3 showing linoelaidic acid (14). All other peak identities are listed in Table 1.

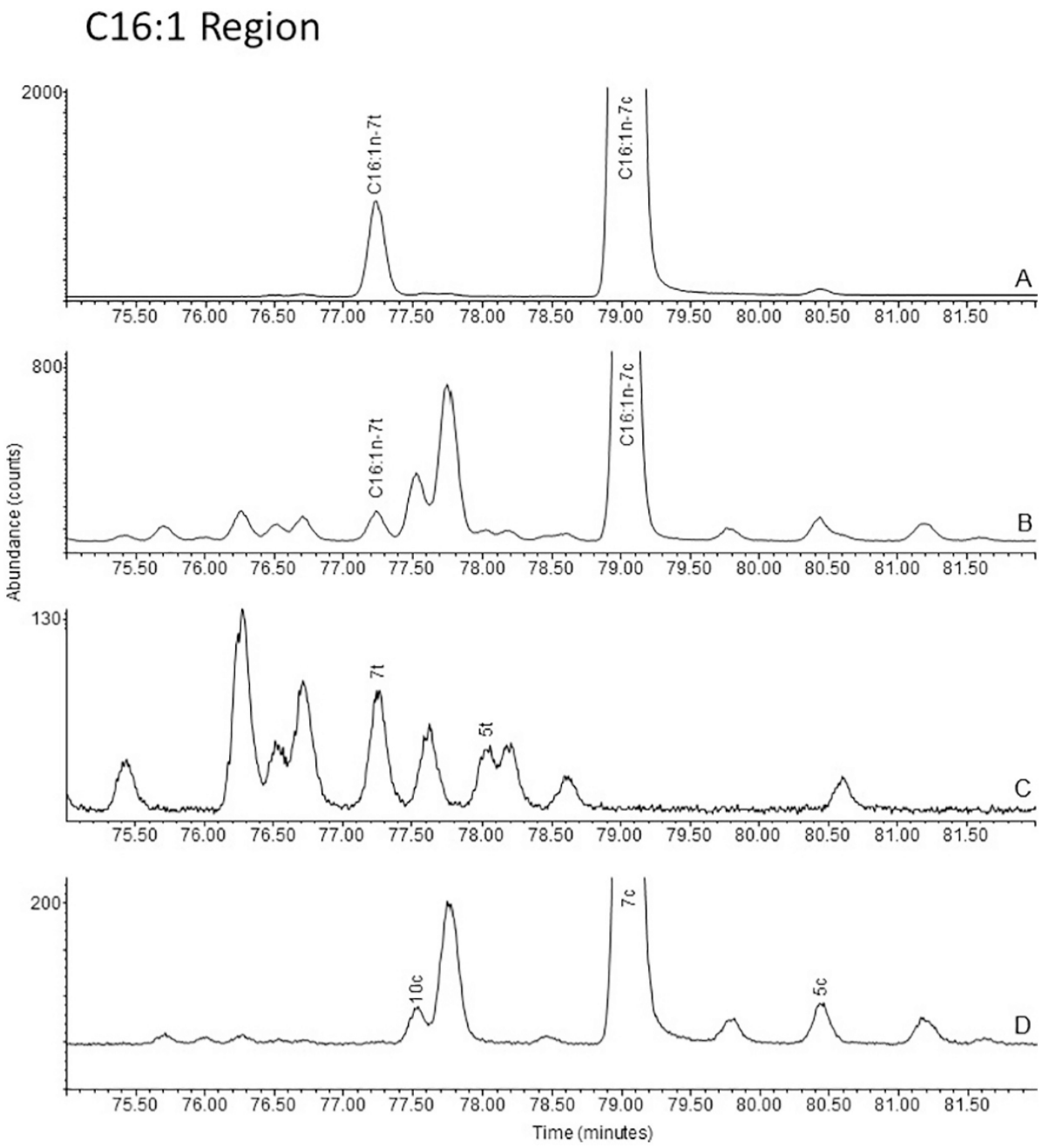

Fig. 2.

Selected Ion Monitoring chromatogram of (A) a mix of synthetic fatty acid standards, (B) a human plasma sample, and Silver SPE fractions of a human plasma sample, (C) Fraction 2 containing C16:1 trans-isomers and (D) Fraction 3 containing C16:1 cis-isomers, analyzed by gas chromatography-negative chemical ionization-mass spectrometry on a CP-7421 Select FAME column.

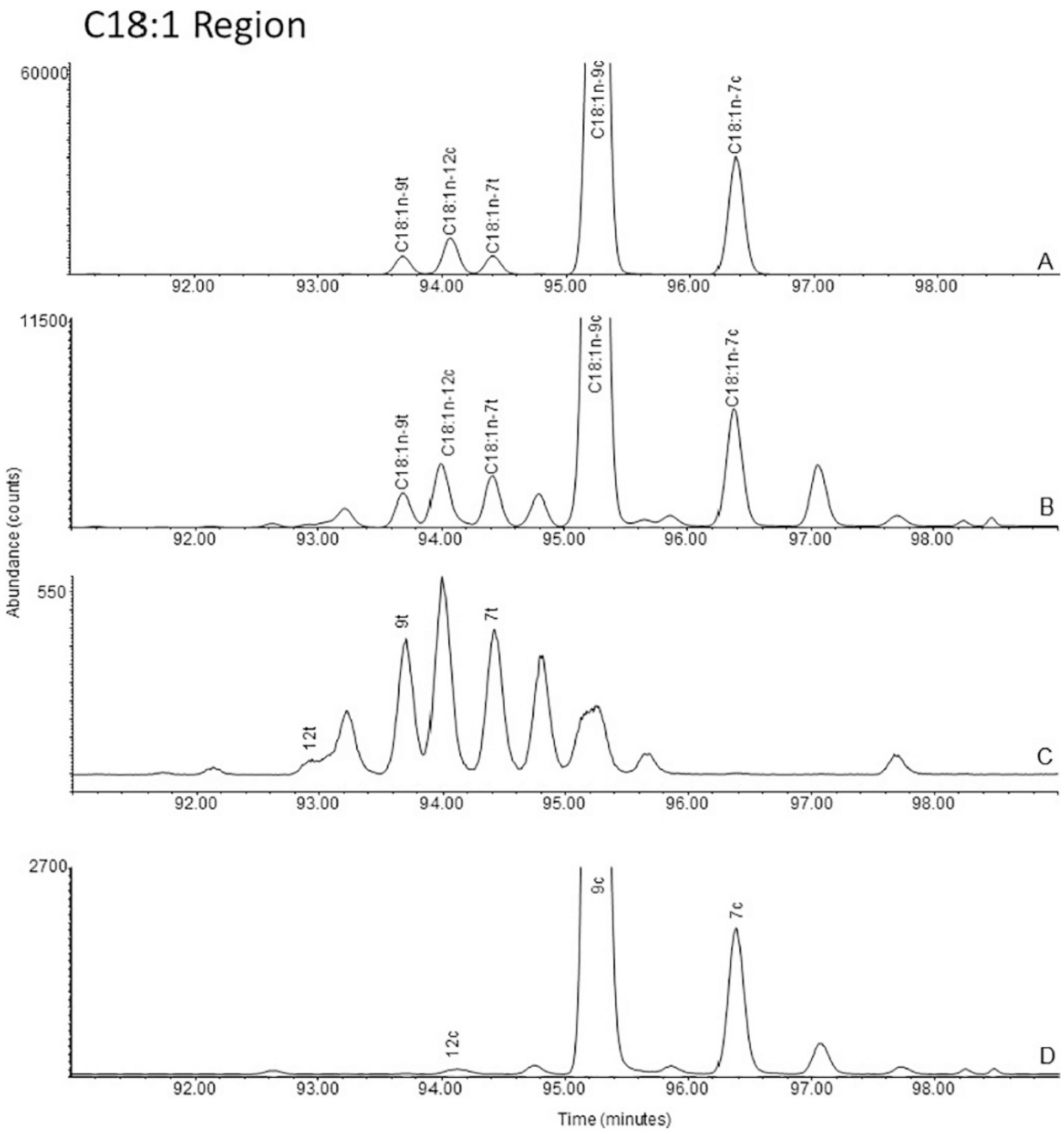

Fig. 3.

Selected Ion Monitoring chromatogram of (A) a mix of synthetic fatty acid standards, (B) a human plasma sample, and Silver SPE fractions of a human plasma sample, (C) Fraction 2 containing C18:1 trans-isomers and (D) Fraction 3 containing C18:1 cis-isomers, analyzed by gas chromatography-negative chemical ionization-mass spectrometry on a CP-7421 Select FAME column.

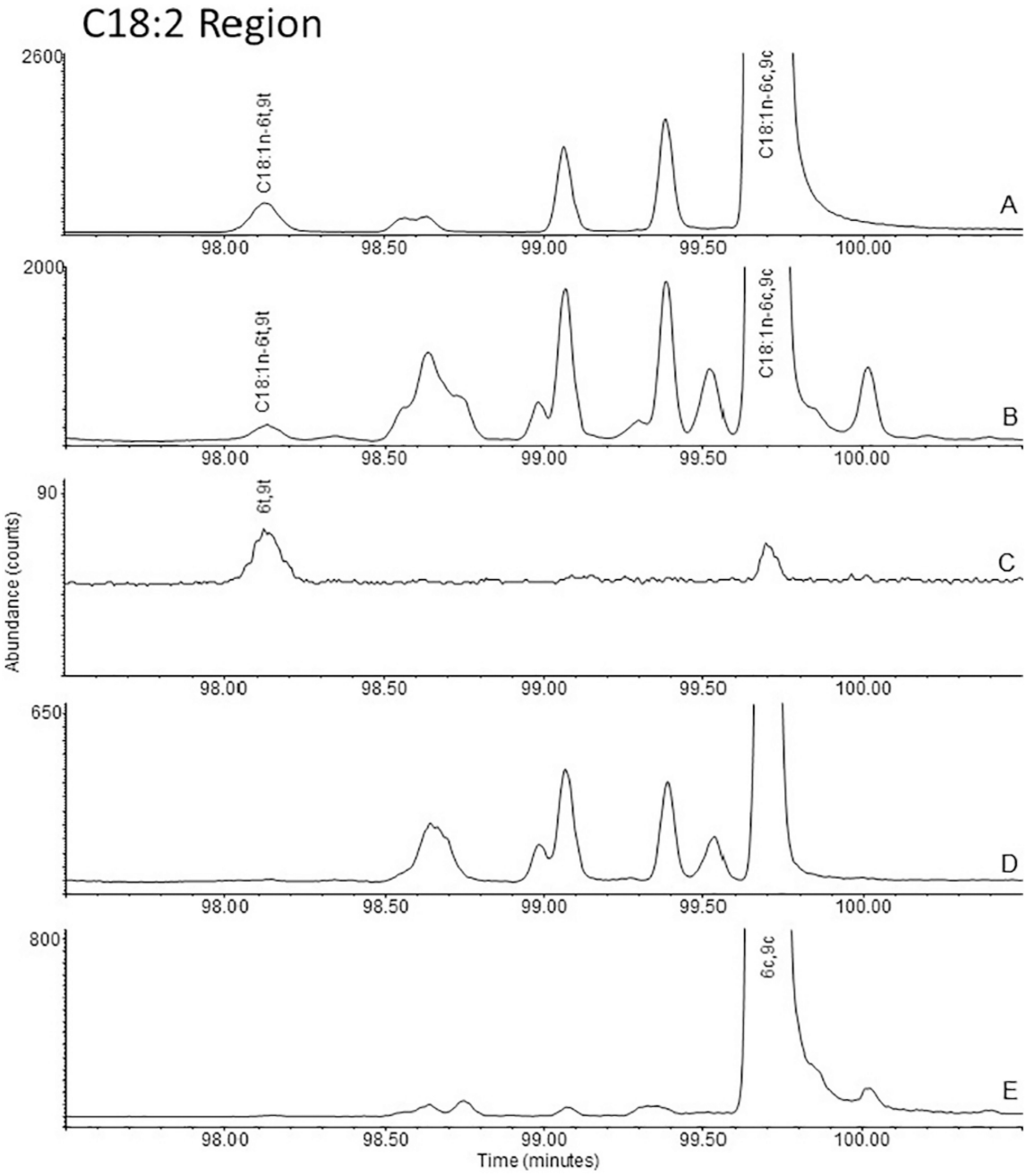

Fig. 4.

Selected Ion Monitoring chromatogram of (A) a mix of synthetic fatty acid standards, (B) a human plasma sample, and Silver SPE fractions of a human plasma sample, (C) Fraction 3 containing C18:2 trans, trans-isomers, (D) Fraction 4 containing C18:2 cis, trans- and trans, cis-isomers, (E) Fraction 5 containing C18:2 cis, cis-isomers, analyzed by gas chromatography-negative chemical ionization-mass spectrometry on a CP-7421 Select FAME column.

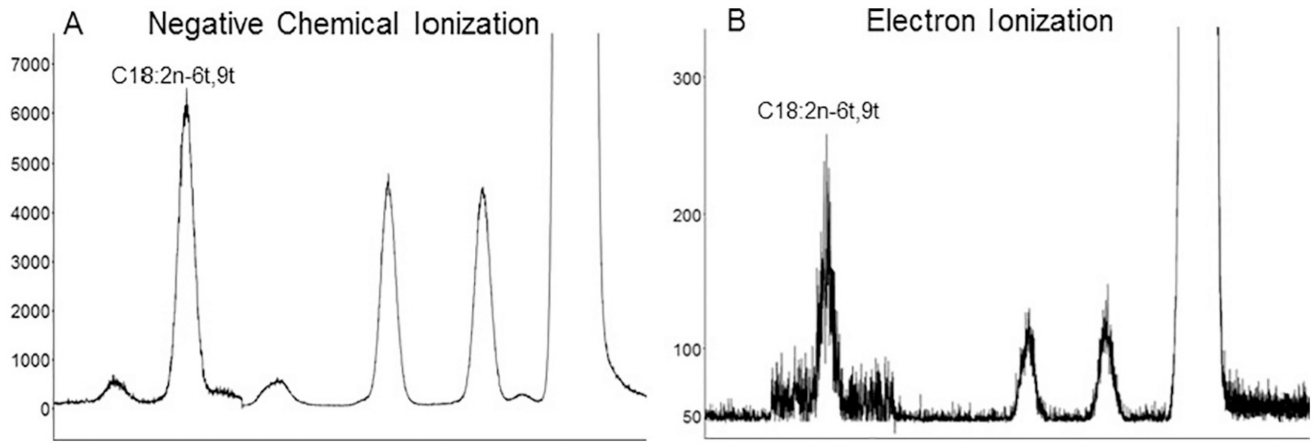

Fig. 5.

Zoomed section of chromatogram showing linoelaidic acid (C18:2n-6 t,9 t) as a PFB-ester ionized using (A) Negative Chemical Ionization at *m/z* 279.3 or as a FAME ionized using (B) Electron Ionization at *m/z* 294.3 and 263.2.

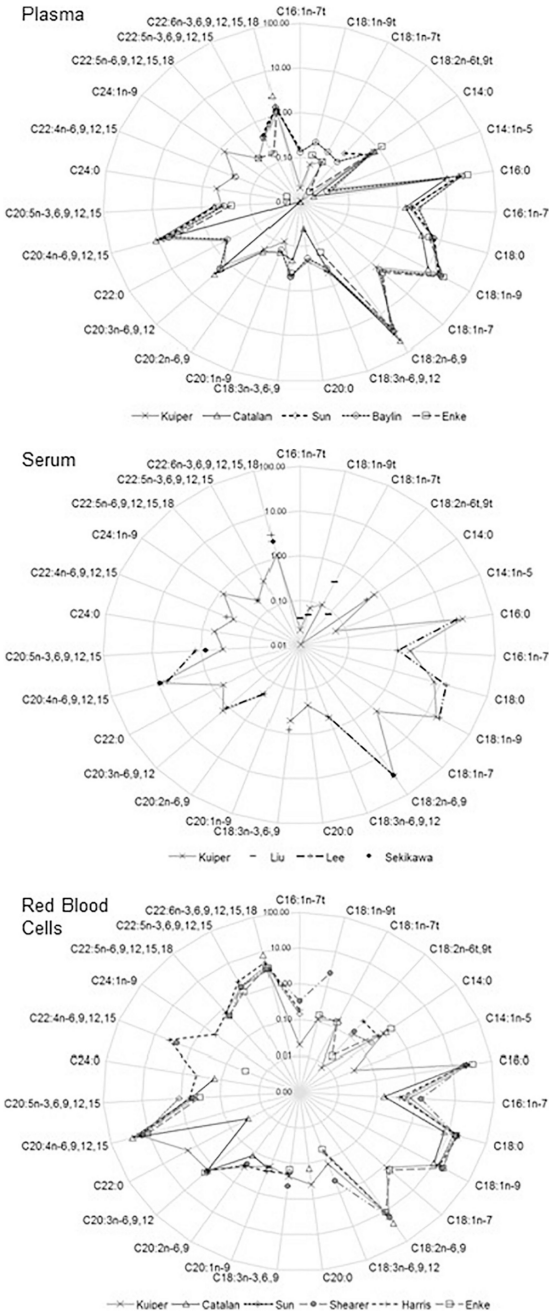

Fig. 6.

Radar plots comparing our % of total FA results to those found in the literature for plasma, serum, and red blood cells. Plasma data is compared to the work of Catalan (2013) [18], Sun (2007) [31], Baylin (2005) [15], and Enke (2011) [13]. Serum results are compared to Liu (2013) [29], Lee (2008) [32], and Sekikawa (2008) [33]. Red blood cell values are compared to Catalan (2013) [18], Sun (2007) [31], Shearer (2009) [34], Harris (2012) [14], and Enke (2011) [13]. Data is plotted on a log10 scale for ease of visibility of low abundance fatty acids.

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Table 1

GC–MS and method parameters for the trans-fatty acids, regular fatty acids, and internal standards (IS).

Fatty acid	Common name	Peak Number[a]	m/z Ratio	Retention time (min)	Working solution or IS solution concentration (μM)
C16:1n-7 t	Palmitelaidic acid	5	253.2	77.2	25.3
$^{13}C_5$-C16:1n-7 t		5	258.4	77.2	11
C18:1n-9 t	Elaidic acid	9	281.3	93.7	130
$^{13}C_5$-C18:1n-9 t		9	286.4	93.7	30
C18:1n-7 t	Trans-vaccenic acid	11	281.3	94.4	130
$^{13}C_5$-C18:1n-7 t		11	286.4	94.4	30
C18:2n-6 t,9 t	Linoelaidic acid	14	279.3	98.1	9.2
$^{13}C_5$-C18:2n-6 t,9 t		14	284.4	98.1	2
C14:0	Myristic acid	2	227.2	58.5	608
D_{27}-C14:0		1	254.4	56.1	200
C14:1n-5	Myristoleic acid	3	225.2	63.9	106
C16:0	Palmitic acid	4	255.3	74.3	8060
$^{13}C_{16}$-C16:0		4	271.3	74.3	2000
C16:1n-7	Palmitoleic acid	6	253.2	79.0	1230
$^{13}C_{16}$-C16:1n-7		6	269.3	78.9	500
C18:0	Stearic acid	8	283.3	91.2	2020
D_{35}-C18:0		7	318.5	87.5	500
C18:1n-9	Oleic acid	12	281.3	95.3	5990
$^{13}C_{18}$-C18:1n-9		12	299.3	95.2	1500
C18:1n-7	Cis-vaccenic acid	13	281.3	96.4	790
$^{13}C_5$-C18:1n-7		13	286.4	96.3	213
C18:2n-6,9	Linoleic acid	15	279.3	99.7	8010
$^{13}C_{18}$-C18:2n-6,9		15	297.3	99.7	3000
C18:3n-6,9,12	γ-Linolenic acid	17	277.1	100.9	206
C20:0	Arachidic acid	18	311.3	101.6	203
D_{39}-C20:0		16	350.7	100.3	50
C18:3n-3,6,9	α-Linolenic acid	20	277.1	102.2	407

Fatty acid	Common name	Peak Number[a]	m/z Ratio	Retention time (min)	Working solution or IS solution concentration (μM)
D_{14}-C18:3n-3,6,9		19	291.5	101.7	200
C20:1n-9	Gondoic acid	21	309.3	102.9	60.4
C20:2n-6,9		22	307.3	105.1	51.8
C20:3n-6,9,12	Dihomo-γ-linolenic acid	24	305.3	106.6	257
C22:0	Behenic acid	25	339.4	106.7	200
D_{43}-C22:0		23	382.9	105.3	
C20:4n-6,9,12,15	Arachidonic acid	27	303.3	107.2	1980
D_8-C20:4n-6,9,12,15		26	311.3	107.1	800
C20:5n-3,6,9,12,15	EPA	29	301.1	109.8	786
D_5-C20:5n-3,6,9,12,15		28	306.3	109.6	300
C24:0	Lignoceric acid	31	367.4	111.5	202
D_{47}-C24:0		30	414.9	109.9	50
C22:4n-6,9,12,15	Adrenic acid	32	331.3	113.1	104
C24:1n-9	Nervonic acid	33	365.4	113.2	201
C22:5n-6,9,12,15,18		34	329.3	113.6	106
C22:5n-3,6,9,12,15	DPA	35	329.3	115.9	204
C22:6n-3,6,9,12,15,18	DHA	37	327.3	116.5	1030
D_5-C22:6n-3,6,9,12,15,18		36	332.3	116.3	300

[a]Peak number 10 in Fig. 1 is C18:1n-12 (petroselinic acid). Due to coelution with a trans-isomer (Fig. 3), this peak is not quantitated.

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Table 2

Method validation parameters for the trans-fatty acids and regular fatty acids.

Fatty acid	LOD (μM)	% accuracy (95% CI)	Intraday precision (%CV, n =40)			Inter-day precision (%CV, n =40)			Linear range (μM)
			Low	Medium	High	Low	Medium	High	
C16:1n-7 t	0.07	96 (94–97)	3.5	2.0	3.1	7.9	3.5	2.5	0.48–25.3
C18:1n-9 t	0.28	92 (88–95)	4.7	1.9	3.8	9.3	3.3	2.7	2.54–130
C18:1n-7 t	0.43	90 (86–93)	3.0	2.3	2.8	9.7	2.9	3.5	2.54–130
C18:2n-6 t,9 t	0.02	90 (88–91)	11.5	7.1	8.6	12.9	10.1	0.0[a]	0.15–9.20
C14:0	0.33	98 (97–99)	1.9	1.5	2.3	5.6	3.6	2.9	12.1–608
C14:1n-5	0.29	128 (127–130)	4.8	3.1	2.7	19.2	8.4	4.2	2.06–106
C16:0	17.0	103 (101–106)	1.8	1.5	3.8	4.3	3.8	4.2	158–8060
C16:1n-7	0.76	99 (97–102)	1.8	1.3	2.6	5.3	3.9	3.6	24.0–1230
C18:0	4.02	97 (94–100)	1.3	1.4	3.2	3.5	3.1	3.4	40.0–2020
C18:1n-9	14.3	98 (97–100)	1.4	1.3	3.3	3.4	3.1	3.8	120–5990
C18:1n-7	1.06	99 (98–100)	1.3	1.1	2.7	3.4	3.0	3.3	14.8–790
C18:2n-6,9	4.90	90 (90–91)	1.8	1.5	3.0	3.8	3.3	5.0	160–8010
C18:3n-6,9,12	0.43	93 (91–95)	2.6	2.2	2.3	5.4	3.4	3.4	4.09–206
C20:0	0.47	96 (92–99)	1.9	2.2	2.4	7.6	5.9	4.1	4.02–203
C18:3n-3,6,9	0.82	90 (88–93)	2.2	2.4	2.5	4.4	3.1	3.1	7.95–407
C20:1n-9	0.84	82 (80–84)	4.7	4.3	5.0	10.3	10.6	6.2	0.96–60.4
C20:2n-6,9	0.16	95 (90–100)	3.3	3.2	3.3	3.4	3.9	1.7	1.00–51.8
C20:3n-6,9,12	1.00	93 (90–96)	2.8	3.0	3.9	4.0	3.3	2.9	4.98–257
C22:0	1.77	98 (97–100)	4.1	4.3	4.2	6.1	5.0	4.0	3.98–200
C20:4n-6,9,12,15	0.36	95 (92–99)	1.2	1.1	2.6	3.3	3.2	3.5	36.2–1980
C20:5n-3,6,9,12,15	1.29	90 (89–92)	2.7	2.2	2.2	24.2	12.3	5.3	14.4–786
C24:0	1.59	97 (92–103)	4.8	4.8	6.3	6.0	6.2	4.7	4.02–202
C22:4n-6,9,12,15	0.34	92 (91–93)	2.9	2.2	2.7	4.1	3.5	2.9	1.92–104
C24:1n-9	1.38	101 (95–106)	4.4	9.2	5.1	4.6	0.0[a]	2.6	3.92–201
C22:5n-6,9,12,15,18	0.33	88 (86–89)	2.9	2.2	3.2	5.0	4.8	3.4	1.96–106
C22:5n-3,6,9,12,15	0.51	90 (84–95)	2.6	2.1	2.6	7.8	5.2	3.0	4.06–204

Fatty acid	LOD (μM)	% accuracy (95% CI)	Intraday precision (%CV, n =40)			Inter-day precision (%CV, n =40)			Linear range (μM)
			Low	Medium	High	Low	Medium	High	
C22:6n-3,6,9,12,15,18	1.96	92 (90–94)	2.5	1.6	2.2	13.3	16.0	5.5	20.0–1030

[a] A zero result for inter-day precision indicates that there is no measurable variation beyond the intraday variation.

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Table 3

Fatty acid median (10th–90th percentile) concentrations in plasma, serum, and red blood cells in 66 adults, expressed as μM and % of total fatty acids.

Fatty acid	Plasma		Serum		Red blood cells	
	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentile) % of total FA	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentiles) % of total FA	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentiles) % of total FA
C16:1n-7 t	1.78 (1.08–2.62)	0.02 (0.01–0.03)	2.12 (1.34–3.41)	0.02 (0.01–0.03)	1.39 (0.999–1.71)	0.02 (0.01–0.02)
C18:1n-9 t	6.20 (4.11–13.7)	0.07 (0.05–0.14)	6.98 (4.40–15.6)	0.07 (0.05–0.14)	8.13 (5.93–11.2)	0.11 (0.08–0.16)
C18:1n-7 t	8.76 (5.25–16.8)	0.10 (0.06–0.18)	9.52 (6.50–20.9)	0.10 (0.06–0.18)	11.4 (8.68–16.4)	0.16 (0.12–0.23)
C18:2n-6 t,9 t	0.94 (0.62–1.52)	0.01 (0.01–0.01)	1.01 (0.656–1.94)	0.01 (0.01–0.02)	0.568 (0.441–0.687)	0.01 (0.01–0.01)
C14:0	64.0 (41.7–137)	0.75 (0.54–1.42)	74.8 (44.0–188)	0.77 (0.56–1.56)	34.6 (26.6–54.2)	0.47 (0.37–0.69)
C14:1n-5	4.40 (2.20–13.2)	0.05 (0.03–0.12)	5.52 (2.33–19.5)	0.06 (0.03–0.18)	2.50 (2.31–3.38)	0.03 (0.03–0.04)
C16:0	1930 (1370–2720)	21.98 (20.30–25.30)	2420 (1650–3500)	23.86 (21.82–27.89)	1890 (1720–2120)	26.33 (25.34–27.56)
C16:1n-7	141 (83.5–343)	1.71 (1.07–3.22)	162 (94.4–401)	1.79 (1.13–3.63)	49.0 (35.2–105)	0.70 (0.50–1.26)
C18:0	597 (428–790)	6.65 (5.85–7.56)	739 (536–948)	7.23 (6.40–8.36)	1180 (1040–1350)	16.34 (15.13–17.46)
C18:1n-9	1490 (1020–2400)	16.60 (14.05–21.81)	1590 (1110–2500)	16.28 (13.86–21.02)	879 (749–1070)	12.13 (10.82–14.04)
C18:1n-7	117 (80.3–174)	1.37 (1.11–1.66)	136 (93.6–209)	1.42 (1.16–1.70)	84.2 (71.3–104)	1.19 (1.04–1.35)
C18:2n-6,9	3030 (2150–3750)	34.20 (27.40–39.73)	3030 (2230–4030)	30.99 (24.11–35.64)	1070 (858–1300)	15.07 (12.41–16.88)
C18:3n-6,9,12	45.0 (25.2–76.4)	0.50 (0.33–0.75)	55.4 (26.4–96.6)	0.52 (0.34–0.82)	9.79 (6.38–16.3)	0.14 (0.09–0.20)
C20:0	19.1 (15.2–24.4)	0.22 (0.16–0.28)	21.7 (17.8–28.9)	0.22 (0.17–0.28)	29.5 (24.1–35.1)	0.40 (0.34–0.48)
C18:3n-3,6,9	42.6 (24.1–81.1)	0.49 (0.37–0.81)	50.5 (27.3–102)	0.50 (0.34–0.85)	16.8 (12.5–22.8)	0.24 (0.18–0.31)
C20:1n-9	7.66 (4.43–13.8)	0.09 (0.07–0.13)	_[a]	_[a]	11.5 (9.36–14.0)	0.16 (0.14–0.19)
C20:2n-6,9	17.6 (11.1–24.2)	0.19 (0.16–0.23)	19.3 (13.1–28.9)	0.20 (0.16–0.24)	23.4 (19.7–27.7)	0.32 (0.27–0.37)
C20:3n-6,9,12	109 (74.3–153)	1.21 (0.96–1.72)	131 (87.3–199)	1.35 (1.02–1.86)	112 (83.6–146)	1.51 (1.20–1.95)
C22:0	46.6 (32.8–62.3)	0.55 (0.35–0.67)	59.0 (44.2–76.7)	0.60 (0.40–0.77)	128 (101–160)	1.82 (1.52–2.13)
C20:4n-6,9,12,15	772 (520–940)	8.40 (6.03–10.63)	888 (622–1140)	8.83 (6.50–11.34)	1260 (1080–1470)	17.49 (15.49–19.72)
C20:5n-3,6,9,12,15	32.9 (20.3–51.6)	0.38 (0.28–0.67)	35.9 (19.8–72.4)	0.35 (0.25–0.65)	31.0 (23.7–41.3)	0.43 (0.34–0.56)
C24:0	44.6 (33.5–56.8)	0.51 (0.37–0.65)	53.1 (42.8–68.9)	0.55 (0.41–0.70)	_[a]	_[a]
C22:4n-6,9,12,15	24.1 (16.7–35.6)	0.27 (0.20–0.36)	27.3 (19.8–42.3)	0.28 (0.21–0.36)	_[a]	_[a]
C24:1n-9	68.9 (42.6–89.7)	0.76 (0.54–0.96)	81.4 (64.2–107)	0.83 (0.65–1.08)	_[a]	_[a]
C22:5n-6,9,12,15,18	17.5 (11.2–22.3)	0.19 (0.14–0.25)	19.7 (13.6–29.2)	0.20 (0.14–0.27)	53.4 (41.9–64.5)	0.74 (0.56–0.95)

Fatty acid	Plasma		Serum		Red blood cells	
	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentile) % of total FA	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentiles) % of total FA	Median (10th–90th percentiles) Conc (μM)	Median (10th–90th percentiles) % of total FA
C22:5n-3,6,9,12,15	34.2 (25.4–51.2)	0.39 (0.31–0.49)	39.3 (27.5–61.6)	0.39 (0.32–0.50)	127 (110–155)	1.79 (1.56–2.05)
C22:6n-3,6,9,12,15,18	94.5 (72.1–141)	1.13 (0.84–1.57)	114 (85.2–171)	1.19 (0.87–1.63)	206 (157–295)	2.90 (2.25–4.02)

[a]Values outside the reportable range.