Laboratory Protocol

Assessment of Changes in TFA Concentration in Blood

Analytes: **Fatty Acids**

Matrix: **EDTA-Plasma or Serum**

Method: **Analysis of four *trans*-fatty acids in plasma or serum by GC-NCI-MS**

Analytes:

Vaccenic acid	C18:1n-7t
Elaidic acid	C18:1n-9t
Palmitelaidic acid	C16:1n-7t
Linoelaidic acid	C18:2n-6t,9t
Myristic acid	C14:0
Myristoleic acid	C14:1n-5c
Palmitic acid	C16:0
Palmitoleic acid	C16:1n-7c
Stearic acid	C18:0
Oleic acid	C18:1n-9c
cis-Vaccenic acid	C18:1n-7c
Linoleic acid	C18:2n-6c,9c
γ-Linolenic acid	C18:3n-6c, 9c,12c
α-Linolenic acid	C18:3n-3c,6c,9c
Arachidic acid	C20:0
Gondoic acid	C20:1n-9c
Eicosadienoic acid	C20:2n-6c,9c
Dihomo-γ-Linolenic acid	C20:3n-6c,9c,12c
Behenic acid	C22:0
Arachidonic acid	C20:4n-6c,9c,12c,15c
Eicosapentaenoic acid	C20:5n-3c,6c,9c,12c,15c
Lignoceric acid	C24:0
Docosatetraenoic acid	C22:4n-6c,9c,12c,15c
Nervonic acid	C24:1n-9c
Docosapentaenoic acid 6	C22:5n-6c,9c,12c,15c,18c
Docosapentaenoic acid 3	C22:5n-3c,6c,9c,12c,15c
Docosahexaenoic acid	C22:6n-3c,6c,9c,12c,15c,18c

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The protocol was reviewed and updated in 2024.

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1 SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

1.1 Clinical and Public Health Relevance

Trans fats or trans-fatty acids (TFA) are unsaturated fatty acids that contain at least one double bond in the *trans* configuration. The three-dimensional structure of TFA is more similar to saturated fatty acids than to regular unsaturated fatty acids, which have their double bond in the c*is* configuration. The *trans* configuration substantially alters the physical properties of the fatty acids, and thus the properties of the oil containing these TFA for cooking and food manufacturing. Also, it substantially alters the biologic and health effects of the fatty acids when consumed [1].

A positive linear trend between TFA intake and total cholesterol as well as LDL cholesterol concentration was established, which links elevated levels of TFA in blood with increased risk of coronary heart disease. Epidemiological data suggests that there is no threshold under which the association between TFA concentration and lipid profiles become undetectable [2]. National and international efforts to eliminate trans fatty acids from the food supply are ongoing. Elimination of industrially produced TFA from the global food supply by 2023 is a World Health Organization (WHO) flagship priority [3, 4].

To evaluate the effectiveness of these public health activities, changes in TFA intake before and after policy implementation should be measured. Such changes can be assessed using food intake survey data along with TFA data from food samples. It can also be assessed by measuring TFA concentrations in blood. Information obtained by both approaches complement each other. For example, changes in TFA intake between 2003 and 2010 estimated by The U.S. Food and Drug Administration (FDA) were similar to the changes observed in blood in the NHANES survey using only four trans fatty acids [5]. These findings also indicate that the assessment of changes in TFA concentration in blood due to policy implementation does not require blood measurements of all TFA reported in food. Furthermore, measurement of the four trans fats was found to be sufficient to assess the impact of TFAs on cardiovascular disease risk markers [2].

The aim of this laboratory protocol is to measure four selected trans fatty acids to assess the impact of policies on reducing TFA in food supply. The scope of this protocol does not include determining all identified TFA content in blood.

Fatty acid content in blood is reported either as concentration (i.e., in nmol/L) or as percent of total fatty acids. The latter requires measurement of regular fatty acids in blood, which are included in this protocol.

The method described in this procedure was adopted from the method used at the United States Centers for Disease Control and Prevention (US CDC), National Center for Environmental Health (NCEH) [6]

1.2 Test Principle

This measurement procedure determines the total (free and esterified) content of selected TFA and regular fatty acids in plasma and provides results in concentration units. Based on the concentration units, the percent units (TFA as percent of total fatty acids) can be calculated.

The fatty acids in plasma are converted into free fatty acids by subsequent acidic and alkaline hydrolysis. The free fatty acids are extracted from the sample solution using liquid-liquid extraction and derivatized with pentafluorobenzyl-bromide (PFB-Br) in the presence of triethylamine to form pentafluorobenzyl esters [7, 8]. The derivatized fatty acids are separated by capillary gas chromatography and detected by mass spectrometry using negative chemical ionization.

The fatty acids are identified based on their chromatographic retention time and on the specific mass to charge ratio of the carboxylate anion formed in the source. Retention times are compared against those obtained with known standards. Quantitation is performed with standard solution using stable isotope-labeled fatty acids as internal standards.

To calculate TFA as percent of total fatty acids, 27 fatty acids are determined with this measurement procedure (for the names of the specific fatty acids determined in this procedure see Appendix 1). These fatty acids cover over 95% of all fatty acids reported in plasma [9]. This method determines the following four TFA: *trans*-9-hexadecenoic acid (palmitelaidic acid, C16:1n-7t), *trans*-9-octadecenoic acid (elaidic acid, C18:1n-9t), *trans*-11-octadecenoic acid (vaccenic acid, C18:1n-7t-), *trans*-9, *trans*-12-octadecadienoic acid (linolelaidic acid, C18:2n-6t,9t).

The procedure described in this document consists of 6 parts (also see flow chart in Appendix 2):

1. Preparation of the samples for analysis
2. Acidic and alkaline hydrolysis of the samples
3. Isolation of the free fatty acids by liquid-liquid extraction
4. Derivatization of fatty acids
5. Analysis of derivatized fatty acids by GC-MS
6. Data processing and calculations

Acid treatment hydrolyzes most lipids but may lead to partial or complete decomposition of functional groups such as epoxy, hydroperoxy, cyclopropenyl, cyclopropyl and possibly hydroxyl and acetylenic fatty acids. It will also isomerize some *cis/trans* and *cis/cis* conjugated linoleic acid isomers to their *trans/trans* isomers [10]. Thus, this method is not suitable for measuring these particular fatty acids.

The method was developed for measuring TFA in plasma but can also be used with serum and red blood cells. This method was not validated for use with other matrices such as food.

1.3 Scope

The measurement procedure described in this document is intended for quantitatively measuring the fatty acids described in Section 1.2. in human serum or plasma for situations where limited specimen is available such as in human biomonitoring studies. It addresses all aspects related to the measurement process (specimen collection, storage, processing, analysis, and reporting). Specific details related to equipment maintenance and operations are not addressed in this document and need to be created for each laboratory separately. Further, this document is not intended to provide information on data interpretation.

The aim of this laboratory protocol is to measure four selected trans fatty acids to assess the impact of policies on reducing TFA in food supply. The scope of this protocol does not include determining all identified TFA content in blood.

Fatty acid content in blood is reported either as concentration (i.e., in nmol/L) or as percent of total fatty acids. The latter requires measurement of regular fatty acids in blood, which are included in this protocol.

2 SAFETY PRECAUTIONS

2.1 General Safety

All plasma or serum specimens should be considered potentially positive for infectious agents, including HIV and the hepatitis B virus. Hepatitis B vaccination series is recommended for all analysts performing this measurement procedure.

Universal precautions should be observed: protective gloves, laboratory coats, and safety glasses must always be worn during all steps of this method.

Disposable bench covers must be used during sample preparation and sample handling and must be discarded after use. All work surfaces must be appropriately disinfected after work is finished.

2.2 Chemical Hazards

All acids, bases, and all the other reagents and organic solvents used in this measurement procedure must be handled with extreme care; they are caustic, flammable and toxic and they must be handled only in a well-ventilated area or, as required, under a chemical fume hood. Before handling chemicals and reagents described in this procedure, safety information, such as Safety Data Sheets (i.e., at http://www.ilpi.com/msds/index.html), should be obtained and reviewed. Appropriate personal protective equipment (gloves, safety glasses and lab coats) must be worn at all times while handling the following chemicals:

Hydrochloric acid: Handle with extreme care. Concentrated HCl is corrosive. Avoid breathing vapors and avoid contact with skin and eyes. Handle only inside a properly operating chemical fume hood with the sash placed between the operator and the chemicals. Store container in a cabinet designated for acids/corrosives.

Sodium hydroxide: Handle with extreme care. Sodium hydroxide is caustic and toxic. Avoid contact with skin and eyes. Eye contact may result in permanent eye damage, and contact with skin causes skin irritations. Store containers in the designated Base cabinet.

Acetonitrile: May cause eye and skin irritation. May be harmful if swallowed, inhaled or absorbed through the skin. Keep from contact with oxidizing materials. Store in a tightly closed container in a designated flammable cabinet.

Toluene: Irritating to eyes, respiratory system and skin. Flammable and Harmful. Keep away from heat. Store in a flammable cabinet in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid contact with skin and eyes. Keep away from incompatibles, such as oxidizing agents.

Hexane: Irritating to eyes, respiratory system and skin. Flammable and Harmful. Avoid contact with skin or eyes. Store container in a designated flammable cabinet.

Methanol: Danger of permanent damage through inhalation, eye and skin contact and if swallowed. Flammable and Toxic. Avoid contact with skin or eyes. Store container in a designated flammable cabinet.

Pentafluorobenzyl bromide: PFB-Br is a lachrymator and is very damaging to eyes and mucous membranes. Always wear gloves, safety glasses or face shields, lab coat, and work only inside a properly operating chemical fume hood with the sash placed as far down as possible between the operator and the chemicals.

Triethylamine: Avoid contact with skin or eyes. It is corrosive. Always wear gloves, safety glasses or face shields, lab coat, and work only inside a properly operating fume hood with the sash placed as far down as possible between the operator and the chemicals. Store container in a Base cabinet.

CAUTION! Acetonitrile, toluene, methanol, and hexane are volatile organic compounds. Wear gloves, safety glasses, lab coat and/or apron, and work only inside a properly operating chemical fume hood. Keep container tightly closed and sealed in the designated flammable cabinet until ready for use.

CAUTION! Hydrogen gas used for analysis by GC-MS is categorized as a Hazardous Material Class 2, in the Compressed Gases category and is flammable. Laboratory staff should be trained appropriately before handling hydrogen gas.

2.3 **Mechanical Hazards**

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Analysts must read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of analytical equipment and instrumentation unless all power is 'off'. Generally, mechanical and electronic maintenance and repair must only be performed by qualified technicians.

Follow the manufacturer's GC-MS operating instructions and hydrogen safety instructions for your laboratory. Turn off the hydrogen at its source every time the GC or MSD are shut down or while the MSD is vented. Use leak-checking equipment to periodically check for hydrogen leaks.

2.4 Waste Disposal

All solid waste used in sample preparation process, (disposable plastic pipette tips, gloves, bench diapers, caps, etc.) as well as any residual sample material, should be placed into the appropriate biohazard autoclavable bags and proper disposal procedures should be followed.

All glass pipette tips and any sharps, (i.e., broken glass) must be placed into the appropriate sharps containers and autoclaved or follow all appropriate disposal procedures.

All liquid waste disposal must be performed in compliance with local policies and regulations for waste management and chemical tracking.

2.5 Training

Analysts performing this measurement procedure must successfully complete laboratory safety trainings, such as,

- Safety Trainings: general laboratory Safety, Bloodborne Pathogens Safety
- Hazardous Chemical Waste Management Training
- Records Management Training

Further, the analyst must have received training on the specific instrumentation and software used with this measurement procedure from designated staff and from the instrument manufacturer as needed.

Analysts must be familiar with:

- Laboratory safety
- Biological exposure safety
- Chemical hazards including Safety Data Sheets

3 COMPUTERIZATION AND DATA-SYSTEM MANAGEMENT

3.1 Software and Knowledge Requirements

This measurement procedure requires work with different software operated instruments. Specific training to operate these software is required to ensure appropriate and safe instrument function. These training is typically provided by the instrument manufacturer.

Further, calculations of results obtained with the GC-MS software can be performed using calculation templates created with software such as Microsoft Excel. The calculation results are transferred to a database that is created and maintained by the laboratory. Assessment of bench Quality Control results is performed using a specific program and maintained by the laboratory.

The database activities and QC calculations are performed by dedicated and specially trained staff. Initial calculations are performed by the analysts after receiving specific training from qualified laboratory staff.

3.2 Sample Information

All samples must have unique identifiers. Aliquots of the same sample need to be identified as such. No personal identifiers are used, and all samples are referenced to a blind coded sample identifier. For sample specimen handling procedures, see the flow chart in Appendix 2.

3.3 Data Maintenance

Information about samples and related analytical data should be checked prior to being entered into the database for transcription errors and overall validity. Filing of electronic and physical files and their maintenance is the responsibility of designated staff in the laboratory. The database is maintained by laboratory staff and is routinely backed up by the organization.

3.4 Information Security

Information security should be managed at multiple levels. The information management systems that contain the final reportable results should be restricted through using appropriate security settings as required by the local laboratory. Confidentiality of results should be protected by referencing results to blind coded sample IDs (no names or personal identifiers).

4 PROCEDURE FOR COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR SPECIMEN REJECTION

4.1 General Specimen Requirements

Specimens for TFA analysis may be fresh or frozen plasma, serum, or red blood cells. Gels used in serum separator tubes may contain fatty acid contaminations and should be tested for contamination before use. A minimum of 150 µL sample is needed; a 0.5-mL sample is preferable to allow for repeat analyses. A sample volume of 100 µL is required for analysis. Additional plasma sample volume may be needed if blood clots are present in the vial.

Fasting samples (i.e., samples collected in the morning after overnight fast) are preferred to minimize variability caused by recent food consumption.

The specimen should be transported in appropriate cryogenic vials with external screwcaps. These cryovials should be labeled uniquely as described in section 3.2.

Other specimen handling conditions or procedures used in the local laboratory may apply.

4.2 Specimen Storage

The specimens collected can be shipped frozen on dry ice. Specimens can be kept refrigerated for 3 days. For long-term storage, samples are stored at -70 ºC. Studies have shown that storage of fatty acids in all lipid classes at -60 °C resulted in negligible changes in concentration [11].

Samples are stable for 5 years if stored at -70 °C [12]. While trans fatty acids are stable for longer, oxidation of polyunsaturated fatty acids may occur. Similarly, oxidation may occur with more than five freeze-thaw cycles, or when refrigerated for more than one week. Samples that show extensive hemolysis may give results for certain regular fatty acids that are different to those observed in regular plasma or serum, and are not recommended for analysis.

4.3 **Unacceptable Specimens**

Specimens that are not acceptable for this measurement procedure are those specimens that do not meet the above-mentioned criteria (specimen storage conditions), those transported at room temperature, or those that have evidence of leakage.

5 PREPARATION FOR REAGENTS, CALIBRATION MATERIALS, CONTROL MATERIALS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION.

5.1 **Equipment, Chemicals, and Consumables**

The chemicals, equipment and other materials described below, or others with similar characteristics/capabilities, can be used in this measurement procedure. Verify that alternative chemicals, equipment, and consumables used are evaluated prior to use, to ensure that it is fit for the intended purpose and do not compromise the analytical performance of the measurement procedure. Manual procedures, such as shaking, and pipetting can be used in place of automated equipment.

All materials used can contain contamination with fatty acids. Therefore, batches of materials need to be screened for fatty acid contamination before use. Glassware may be cleaned with appropriate solvent and dried before use. Use of trade names and commercial sources is for identification only and does not constitute endorsement by WHO or by the US CDC.

5.1.1 Equipment, Chemicals, and Consumables Used for Reagent Preparation

1. Various glass beakers (25 mL, 50 mL, 100 mL)
2. 100 mL graduated cylinders
3. Capped 250 mL bottles, class A glassware
4. Sodium Hydroxide, 10N solution, Certified ACS/ASTM
5. Acetonitrile, HPLC Grade Reagent
6. Hydrochloric Acid, 6N Solution, Certified
7. Methanol, 99.8+% A.C.S.

5.1.2 Equipment, Chemicals, and Consumables Used for Sample Processing

1. Vortex: (e.g., T Genie 2)
2. Multi-Tube Vortexer (120 V, speed range 50-2500 rpm)
3. Evaporator: Evaporation System with a steady stream of nitrogen (e.g., GeneVac EZ-2, or TurboVap® LV)

4. Optional: Liquid Handler, with appropriate conductive tips (e.g., Hamilton Microlab STARLet Liquid Handler with Microlab Vector Software version 4.11.5878)
5. Convection oven with temperature range from up to 325°C (such as, Freas Mechanical Convection oven 625, 230 V)
6. Optional: Shaker (i.e. Eberbach Model E6010 Fixed-Speed Reciprocal)
7. Centrifuge with A-4-62 rotor (e.g., Eppendorf Centrifuge 5810 R V4.2)
8. Barcode Scanner
9. Analytical Balance (e.g., AX 205, with printer)
10. Optional: Label Maker (e.g., Brady Label Maker IP300 printer)
11. Rotator (i.e., Boekel Orbitron Platform Rotator I)
12. Displacement pipettes 10-100 µL and 100-1000 µL (i.e., Gilson Positive Displacement Pipettes; Gilson M100 and M1000)
13. Repeater Pipette Adapter
14. 5 mL Repeater Tips (e.g., Combitips)
15. 2 mL cryovials with external thread
16. Disposable glass culture tubes, (threaded, 11.5 mL, 16x100 mm
17. Disposable glass culture tubes (extraction vials, rimless,11.5 mL,16x100 mm)
18. Phenolic screw caps, PTFE-faced rubber liner (e.g., Kimble black caps)
19. Disposable glass Pasteur pipettes, 5 3/4 inch
20. Pasteur pipette bulbs
21. 5 mL disposable glass pipettes
22. Acetonitrile, HPLC Grade Reagent
23. Pentafluorobenzyl Bromide (PFB-Br)
24. Triethylamine (TEA) 99.7%, extra pure
25. Hexane, Reagent Plus $\geq$ 99%
26. Hydrochloric Acid, 6N Solution, Certified

5.1.3 Equipment, Chemicals, and Consumables Used for Sample Measurement

1. GC-MS, with an injection port and mass detector (i.e., Agilent GC-MSD 6890 Gas chromatograph and 5975B Mass selective detector for EI, PCI and NCI)
   Note: the GC-MS may be a gas chromatograph with a split/splitless injection port, capable of operating constant flow temperature programs, combined with a quadrupole mass detector (or equivalent single stage mass detector) capable of negative chemical ionization and meeting the limits of quantification reported in Table 9 (page 27)
2. Data Processing Software (e.g., , Agilent MSD ChemStation version E.02.02 or higher and Mass Hunter version B.07 or higher)
3. Autosampler with cooled drawer (4 °-40 °C) (i.e., Gerstel Multipurpose Sampler MPS 2) with Peltier Cooled drawer; with associated software
4. Hydrogen Generator Outlet, Flowrate, 500mL/min.; Purity, 99.9999%; Pressure, 10-100 psi
5. Chemical Ionization Gas Purifier
6. Non-stick Fluorocarbon Liner Viton O-ring
7. Ultra-Inert, Split, Low Pressure drop, Glasswool Liner
8. Fixed Tapered Needle Syringe 10 µL
9. Big Universal Trap 1/8” Fittings, Hydrogen
10. Chemical Ionization Gas Regulator
11. Advanced Green Non-stick 11mm Septa
12. Capillary Column CP 7421 Select FAME 200 m x 250 µm x 0.25 µm

13. Acetone, GC Resolve
14. N-Hexane, Reagent Plus ≥ 99%
15. GC vials 1.5-mL, Footed, Amber Glass
16. Caps with septa, blue PTFE/Silicone/PTFE
17. Toluene, Certified ACS/ASTM
18. Deionized water with resistance to at least 18 megaOhm/cm
19. Synthetic fatty acid standards (Refer to Appendix 3 for calibrators and internal standards information)

5.2 **Preparation of Reagents Used for Sample Preparation**

5.2.1 Preparation of Acetonitrile:6N hydrochloric acid (90:10, v:v)

This solution is used for the acidic hydrolysis step described in section 7.1.3. Prepare 100 mL of this solution by transferring 10 mL of 6N hydrochloric acid to a 100 mL graduated cylinder and adding acetonitrile up to the 100 mL mark. Transfer to the labeled 250 mL bottle for use in sample preparation. The solution can be prepared weekly and stored at room temperature.

5.2.2 Preparation of Methanol:10N sodium hydroxide (90:10, v:v)

This solution is needed for the alkaline hydrolysis step as described in section 7.1.3. Prepare 100 mL of this solution by transferring 10 mL of 10N sodium hydroxide to a 100 mL graduated cylinder and adding methanol up to the 100 mL mark. Transfer to the labeled 250 mL bottle for use in sample preparation. The solution can be prepared weekly and stored at room temperature.

5.2.3 Preparation of 7.5% Pentafluorobenzyl bromide (PFB-Br) in Acetonitrile

This solution is needed to derivatize the fatty acids for GCMS analysis as described in section 7.1.5. Using a positive displacement pipette add 376 µL PFB-Br to 5 mL of acetonitrile in a threaded culture tube. 5 mL of this solution is sufficient for 49 samples. This solution is prepared on the day of experiment. Store at room temperature in the designated cabinet protected from light by covering the vial with aluminum foil.

5.3 **Calibration Materials**

5.3.1 Preparation of Calibrator Solutions

All fatty acid standards need to be analyzed for purity and integrity and results need to be compared with the certificate of analysis provided with the standard for consistency before use as calibrators.

Laboratories may have specific instruction for preparing calibrator solutions and for documenting the preparation of the solutions. It is recommended to use calibrated and verified pipettes and glassware for these tasks.

Once calibrator solutions are prepared, appropriate procedures need to be applied to verify the accuracy and consistency of the new calibrator solutions before they are used with study samples. For example, the new calibrator solutions can be treated as samples and analyzed with

the old calibrator solutions to verify the concentration of the new calibrator solution, or new calibrator solutions are used to analyze certified reference materials, and results are compared with the assigned target values.

Calibrator concentrations provided herein are appropriate for analysis of fatty acids in the U.S. population. Adjustment to calibration levels may be required for other populations.

The volumes described here are for orientation only and each lab may need to determine different volumes based on their need.

The standards described in Appendix 3 are used to create fatty acid stock solutions in 25 or 50 mL of toluene with the following concentrations:

Table 1: Fatty Acid Stock Solutions and concentrations (for Analyte codes see Appendix 3)

Note: The calibration ranges can be adjusted to encompass the expected concentration ranges of the various fatty acids for different study populations. It is recommended to use preliminary data from the specific population of interest to adjust calibrator concentrations.

Analyte Code	Fatty Acid Concentration (mmol/L)	Analyte Code	Fatty Acid Concentration (mmol/L)	Analyte Code	Fatty Acid Concentration (mmol/L)	Analyte Code	Fatty Acid Concentration (mmol/L)
HDT	1	OD9	6	OD1	6	OTT	0.4
ALN	20	DP6	5	LG1	10	PL1	60
AR1	10	DTA	5	LNA	200	PM1	200
ARA	100	ED1	2.5	ML1	5	ST1	50
DA1	10	EN1	3	MR1	30	VC1	40
DE1	2.5	EPA	40	NR1	10		
DHA	50	GLA	10	OC6	12.5		
DP3	10	HGL	12.5	OL1	150		

1. Calculate the amount of fatty acid needed to create the target concentration stated in the table using the molecular weight of the standard.
2. Weigh the amount of pure fatty acid needed (+/- 15%) in a 25 mL or 50 mL (LNA, OL1, PM1, and ST1) volumetric flask using an analytical balance. Note the mass of fatty acid weighted is used to calculate exact concentration of the fatty acid stock solution.
3. Fill the volumetric flask to just below the line and bring the flask to 20 ^{o}C in a water bath with shaking over 30 minutes. Fill to the mark with toluene.
4. Using the fatty acid stock solutions, 500 mL of a so called “level 40” (TFAC40) calibrator working solution is created using the amounts detailed in Table 3. The target concentration of each analyte in this working solution are as followed:

Table 2: Target Concentration (µmol/L) for the "level 40" Calibrator Working Solutions

Analyte Code	Fatty Acid Concentration (µmol/L)	Analyte Code	Fatty Acid Concentration (µmol/L)	Analyte Code	Fatty Acid Concentration (µmol/L)	Analyte Code	Fatty Acid Concentration (µmol/L)
HDT	25	OD9	125	OD1	125	OTT	7
ALN	400	DP6	100	LG1	200	PL1	1200
AR1	200	DTA	100	LNA	8000	PM1	8000
ARA	2000	ED1	50	ML1	100	ST1	2000
DA1	200	EN1	50	MR1	600	VC1	800
DE1	50	EPA	800	NR1	200		
DHA	1000	GLA	200	OC6	250		
DP3	200	HGL	250	OL1	6000		

5. Pipette from the individual fatty acid stock solutions the volumes listed in the table below in a 500 mL volumetric flask and fill the flask to just below the 500 mL mark with toluene. Bring the flask to 20 ^{o}C in a water bath with shaking over 30 minutes, then fill to the mark with toluene.

Table 3: Fatty Acid Stock Solution Needed for the "level 40" Calibrator Working Solutions

Analyte Code	Volume of Fatty Acid Stock Solution (mL)	Analyte Code	Volume of Fatty Acid Stock Solution (mL)	Analyte Code	Volume of Fatty Acid Stock Solution (mL)	Analyte Code	Volume of Fatty Acid Stock Solution (mL)
HDT	10	OD9	10	OD1	10	OTT	10
ALN	10	DP6	10	LG1	10	PL1	10
AR1	10	DTA	10	LNA	20	PM1	20
ARA	10	ED1	10	ML1	10	ST1	20
DA1	10	EN1	10	MR1	10	VC1	10
DE1	10	EPA	10	NR1	10		
DHA	10	GLA	10	OC6	10		
DP3	10	HGL	10	OL1	20		

6. Prepare four levels called "35", "30", "20", "10" calibrator working solutions in toluene using the dilution scheme described in the following table (use volumetric flasks and volumetric pipettes to prepare these solutions):

Table 4: Dilution Table for the Preparation of Level "35", "30", "20", "10" Calibrator Working Solutions

Calibrator working solution	Volume of Level 40 to use (mL)	Dilute to volume (mL)	Name of solution created
Level 40	100	200	Level 35 (TFAC35)
Level 40	50	200	Level 30 (TFAC30)
Level 40	20	200	Level 20 (TFAC20)
Level 40	10	250	Level 10 (TFAC10)

The target concentrations of the calibrators are listed in **Appendix 3.**
Calibrator working solutions are stable for at least two years when stored at -70^{o}C

5.3.2 Preparation of Internal Standard Solutions

This method is highly sensitive and initial investigations detected odd-numbered fatty acids in blood. Therefore, it is not recommended to use such fatty acids as internal standards with this method. The internal standards listed in this section may not always be available and fewer or different internal standards can be used as long as the analytical performance requirements are fulfilled.

All fatty acid standards need to be analyzed for purity and integrity and results need to be compared with the certificate of analysis provided with the standard for consistency before use as internal standards.

Laboratories may have specific instruction for preparing internal standard solutions and for documenting the preparation of the solutions. If the calibrator concentrations have been changed, then the internal standard concentrations should be adjusted accordingly.

Once internal standard solutions are prepared, appropriate procedures need to be applied to verify the consistency of the new internal standard solutions before they are used with study samples.

The internal standards described in Appendix 3 are used to create internal standard fatty acid stock solutions in toluene with the concentrations listed in Table 5.

Table 5: Desired Internal Standard Fatty Acid Stock Solution Concentration
Note: The volumes described here are for orientation only and each lab may need to determine different volumes based on their need.

Analyte Code	Desired Internal Standard Fatty Acid Stock Solution Concentration (mg/mL)	Volume of Stock Solution used to Prepare Working Solution (mL)	Desired Internal Standard Fatty Acid Concentration of the Working Solution (µmol/L)
HDT_IS	0.26	20	10
OD9_IS	0.86	20	30
OD1_IS	0.86	20	30

OTT_IS	0.57	2	2
ALN_IS	5	20	200
AR1_IS	1.76	20	50
ARA_IS	10	50	800
DA1_IS	2.08	20	50
DHA_IS	10	20	300
EPA_IS	9.22	20	300
LG1_IS	1.92	20	50
LNA_IS	89.48	20	3,000
MR1_IS	5.11	20	200
OL1_IS	45.05	20	1,500
PL1_IS	10	20	500
PM1_IS	57.52	20	2,000
ST1_IS	15.99	20	500
VC1_IS	5.75	20	200

1. Calculate the amount of fatty acid needed to create the target concentration of the internal standard stock solution stated in the table using the molecular weight of the standard.
2. Weigh the amount of fatty acid needed (+/- 15%) in a 25 mL volumetric flask (5 mL flask for OTT_IS) using an analytical balance. Use a separate flask for each fatty acid. Note the mass of fatty acid and use it to calculate exact concentration of the fatty acid stock solution.
3. Fill the flask with toluene to the 25 mL (or 5 mL for OTT_IS) mark.
4. For each fatty acid solution, transfer the volume needed to prepare the working solution as stated in the table in a 2000 mL volumetric flask.
5. Fill the volumetric flask to just below the 2000 mL line and bring the flask to 20 ^{o}C in a water bath with shaking over 30 minutes. Fill to the 2000 mL mark with toluene.
6. Mix well.
7. Aliquot solution in a threaded glass tube with screw cap and store at -70 ^{o}C.

6 CALIBRATION AND CALIBRATION VERIFICATION

6.1 Calibration

6.1.1 Calibration of Instruments and Equipment

All volumetric pipettes are calibrated annually following procedures recommended by the manufacturers. Mass spectrometry instruments are calibrated for mass accuracy regularly as recommended by the manufacturer and following the manufacturer's procedures. Accuracy of other equipment such as oven temperatures are verified regularly according to the manufacturer's recommendation or using established references (i.e., commercial buffer solutions, external thermometers).

All calibration and calibration verification activities are documented appropriately.

6.1.2 Calibration of measurement

Calibrators used in this measurement procedure are traceable to commercial, pure compound standards (for details on pure compound specifications see Appendix 3). Calibration solutions are prepared starting with gravimetric measurements. For Metrological traceability according to ISO 17511 [13], see Appendix 4. Calibrators are analyzed together with each set of samples.

6.2 **Calibration Verification**

Calibration verification of equipment is performed 6 months after calibration was performed or earlier when recommended by the manufacturer.

With each set of samples, five levels of calibration material and duplicate low, mid, and high level quality control materials are analyzed. Possible shifts in calibration are assessed by comparing bench Quality Control material data against predefined acceptance limits using appropriate software programs (see also Section 8).

Calibration is further verified by analyzing commercial standards every 6 months and comparing the results obtained against predefined acceptance limits (+/- 15% from target value).

7 PROCEDURE OPERATION INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

All instruments are checked for correct function using the manufacturer's acceptance criteria. All automated steps in the sample preparation process may also be performed manually.

7.1.1 Specimen Storage and Handling during Testing

All vials are labeled as outlined in this document and according to the laboratory's specific policies and procedures and are scanned/documented during the process of sample preparation, sample transfer and analysis, in order to ensure that individual samples can be tracked throughout the process.

Specimens are allowed to reach room temperature for sample preparation. The unused portion of the patient specimen is returned to the freezer and stored at -70^{o}C. Samples ready for analysis by GC-MS are either stored at -70 ^{o}C in the freezer or at 10±2°C in the GC-MS instrument sample tray.

7.1.2 Preparation of Samples for Analysis

All samples are processed together with 1 reagent blank (toluene), 1 set of calibrators (5 levels: TFAC10, TFAC20, TFAC30, TFAC35, TFAC40), and 6 bench QC samples (2 low, 2 medium and 2 high). Typically, 36 patient samples are processed in one batch (total number of samples per batch: 49, including the Retention Time Standard (RTSTD)).

Note: An automated liquid handler, such as the Hamilton can be used to scan sample barcoded sample IDs in step 3.

1. Assess all samples for acceptability using the criteria described in section 4.2 and 4.3.
2. Thaw all specimens to room temperature before preparation. Frozen plasma samples,

QC samples, Internal Standard (IS) solutions and calibrator solutions are allowed to reach ambient temperature and are homogenized by placing them on the rotator for approximately 30 minutes.

3. Verify and record the IDs of all samples, calibrators, and QCs. Scan the barcodes of all coded vials and reagents if a barcode scanner is available.
4. Working in the hood, transfer 100 µL of samples, QC samples, calibrators and the blank (toluene) to the labeled 16 x 100 mm glass threaded culture tubes using a 100 µL positive displacement pipette.
5. After the transfer of all samples, the analyst must visually inspect all vials for potential blood clots. If blood clots are noted, the vial is discarded, and the sample is manually pipetted using a positive displacement pipette into a new glass threaded culture tube.
6. Transfer 100 µL of Internal Standard solution to all samples, QCs, and calibrators using a 100 µL positive displacement pipette and visually verify successful transfer by checking the solution levels in all vials.
7. Recap sample vials and store remaining sample at dedicated place in -70 ^{o}C freezer.

7.1.3 Hydrolysis of Samples

1. Add 2 mL of 6N HCL (90:10, v:v) in Acetonitrile solution to each vial using a graduated glass pipette.
2. Cap all vials and vortex them for 30 seconds using the Multi-Tube Vortexer at 2500 rpm.
3. Place samples in the Mechanical Convection oven set at 104 (+/-4) ^{o}C for 45 minutes.
4. Remove vials from the oven and place them in the chemical fume hood for 30 minutes to allow them to cool to room temperature.
5. Assess the volume in all vials by comparing it to a vial containing 2.2 mL of water. Adjust any volume lost during the hydrolysis step with acetonitrile using a positive displacement pipette and document action.
6. Add 2 mL of 10N NaOH (90:10, v:v) in methanol to all vials using a graduated glass pipette.
7. Cap all vials and vortex them for 30 seconds using the Multi-Tube Vortexer at 2500 rpm.
8. Place the samples in the Mechanical Convection oven set up at 104 (+/-4) ^{o}C for 45 minutes.
9. Remove vials from the oven and let them cool for 30 minutes to room temperature in the chemical fume hood.
10. Assess the volume in all vials by comparing it to a vial containing 4.2 mL of water, and checking that it is not below the level marked in step 7. Adjust any volume lost during the hydrolysis step with methanol using a positive displacement pipette and document action.
11. Add 500 µL of 6N HCl solution to each vial using a repeater pipette.
12. Cap all vials and vortex them for 5 seconds at highest setting.

Note: Alternatively, evaporation loss after hydrolysis steps can be assessed by marking the solvent levels before placing the samples in the oven and observing if the level drops below the starting volume.

If complete evaporation occurs, sample preparation will need to be repeated for affected samples. If partial evaporation occurs, solvent is added to maintain the appropriate volume ratio for the upcoming extraction steps. The concentration estimates for these samples will be compensated by the internal standards, which are added to the samples before the first hydrolysis step.

7.1.4 Extraction of Free Fatty Acids

Note: An automated liquid handler, such as the Hamilton STARLet, if available, can be used to scan barcoded sample IDs in step 4.

1. Working in the hood, add 2 mL of hexane to each threaded culture tube.
2. Shake all vials in a Fixed Speed Reciprocal Shaker for 15 minutes (no heat) at high setting until the two solvent layers disappear and the sample solution becomes opaque.
3. Transfer the threaded culture tubes to the centrifuge and centrifuge samples for 5 minutes at 21 (+/-1)^{o}C and 3000 rpm to separate the organic layer from the aqueous layer. Before starting the centrifuge, ensure that the load is balanced.
4. Verify that the extraction vials are labelled correctly and that the sample ID's match the corresponding sample vials.
5. Carefully transfer the upper hexane layer from each sample into the extraction vials, using clean glass Pasteur pipets.
6. Repeat steps 2-5 two times (total of 3 extraction steps). The hexane layers from each vial are combined in the same vial used in step 5.
7. Verify that the IDs on the sample vials and the extraction vials match and are in the correct order before transferring each hexane layer.
8. Transfer the extraction vials containing the hexane extracts to the evaporator and dry down with a steady stream of nitrogen.
9. Proceed to derivatization immediately after drying to avoid sample oxidation.

7.1.5 Derivatization of Fatty Acids

1. Remove all the extraction vials from the evaporator and place in the hood. Ensure all vials are dry. Continue drying process if samples contain liquids.
2. Add 100 µL of the PFB-Br solution and 10 µL of the Triethylamine (TEA) to each vial.
3. Vortex all vials for 5 seconds to mix the solutions.
4. Keep vials in the hood at room temperature for 15 minutes for the derivatization reaction to occur.
5. Add 0.5 mL of hexane to each vial.
6. Mix all vials by vortexing for about 5 seconds.

7. Verify that the GC vials are correctly labelled and are in the correct order before transferring the measurement solution.
8. Transfer the measurement solution, making sure to transfer only the top (hexane) layer to the GC vials.
9. Cap the GC vials and visually inspect successful transfer of the measurement sample solution. Ensure that the sample in the GC vial does not show two layers.
10. Cap and transfer vials to the GC autosampler for GC-MS analysis or store as described in section 7.1.1 until GC-MS analysis.

7.1.6 Analysis of Derivatized Fatty Acids by GC-MS

All samples prepared in one batch are analyzed in one batch on the same instrument. A retention time standard sample containing all analytes is added to each batch.

1. Create an appropriate analytical run sequence file, according to your laboratory protocol. (See Appendix 5 for an example of an analytical sequence).
2. It is recommended that the sequence of analyzing samples is created in a manner that at least one quality control material and one calibrator are analyzed within a 24-hour period. The first sample in a sequence is always an instrument control standard, such as the RTSTD. (See Appendix 5 for an example analytical sequence).
3. The samples are loaded on the GC-MS instrument as stated in the sequence file and the position of samples in the autosampler are verified against the information in the sequence file. Basic instrument function and settings are checked according to the GC-MS manufacturer's instructions. It is assured that the correct instrument method is loaded, and that all method parameters are stable.
4. The instrument run sequence is started.
5. Using the Retention Time Standard sample, the performance of the GC-MS system is assessed by inspecting retention times, peak intensities, and general chromatographic parameters. When instrument malfunction is indicated, the sequence is stopped, samples are stored in the freezer and the problem is addressed.
6. Upon completion of the GC-MS analysis, the GC vials are recapped and stored in the designated space in the freezer at -70 ^{o}C.

The following GC-MS parameters are used (for further specific details see Appendix 6). Typical chromatograms of the retention time standard and low QC are shown in Appendix 7:

Chromatographic conditions

The chromatographic conditions below were found to be suitable to achieve resolution of the four trans-fatty acid isomers from neighboring peaks and to balance the response of high and low abundance peaks to ensure sufficient abundance on the low end while avoiding saturating the column or detector on the high end.

Prior to analyzing samples, it is necessary to ensure appropriate chromatographic conditions to meet the following minimum criteria: 1. greater than 70% resolution for each trans-fatty acid from neighboring peaks; peak shapes should be symmetrical with tailing factors between 0.8 and 1.2 for PM1, OL1, and LNA in the retention time standard or Level 40 calibrator; and S/N ratio better than 10 for OTT and OTT_IS in the lowest calibrator.

Injection:
Injector: Gerstel MPS2
Injection volume: 1 µL
Injection mode: Split (Split ratio: 100:1)
Injector temperature: 240 °C
Gas type: Hydrogen
Gas flow: 2 mL/min
Column: Agilent Select-FAME 200 m x 250 µm x 0.25 µm (length, inner diameter, film thickness)
Oven:
Initial temperature: 50 °C

Table 6: Temperature Program

Step	Start Temperature [°C]	Heating Rate [°C/min]	End Temperature [°C]	Temperature Hold Time (min)
1	50	40	160	10
2	160	1	175	0
3	175	0.5	210	0
4	210	35	260	25

Mass spectrometric conditions
Acquisition mode: Selected Ion Monitoring (SIM)
Solvent Delay: 20 min
MV Mode: Gain Factor
MS source Temperature: 230°C
MS Quadrupole Temperature: 150°C
CI Gas: Methane
CI Flow Rate: 40
CI A/B Gas: 1

Table 7: Analyte and IS selected ion monitoring mass and time segments.
Note: Analytes and Internal standards are listed in order of retention times and are grouped based on the assigned SIM groups in the acquisition method.

	No.	Fatty Acid	Analyte code	SIM mass	Shorthand	Internal Standard
Group 1	1		MR1_IS	254.4	D_{27}-C14:0	
	2	Myristic acid	MR1	227.2	C14:0	MR1_IS
	3	Myristoleic acid	ML1	225.2	C14:1n-5c	MR1_IS
Group 2	4		PM1_IS	271.3	$^{13}C_{16}$-C16:0	
	5	Palmitic acid	PM1	255.3	C16:0	PM1_IS
Group 3	6		HDT_IS	258.4	$^{13}C_{5}$-C16:1n-7t	
	7	Palmitelaidic acid	HDT	253.2	C16:1n-7t	HDT_IS
	8		PL1_IS	269.3	$^{13}C_{16}$-C16:1n-7c	
	9	Palmitoleic acid	PL1	253.2	C16:1n-7c	PL1_IS

Group 4	10		ST1_IS	318.5	D_{35}-C18:0	
	11	Stearic acid	ST1	283.3	C18:0	ST1_IS
Group 5	12		OD9_IS	286.4	$^{13}C_5$-C18:1n-9t	
	13	Elaidic acid	OD9	281.3	C18:1n-9t	OD9_IS
	14	Petroselinic acid	OC6	281.3	C18:1n-12c	OD1_IS
	15		OD1_IS	286.4	$^{13}C_5$-C18:1n-7t	
	16	*trans*-Vaccenic acid	OD1	281.3	C18:1n-7t	OD1_IS
	17		OL1_IS	299.3	$^{13}C_{18}$-C18:1n-9c	
	18	Oleic acid	OL1	281.3	C18:1n-9c	OL1_IS
	19		VC1_IS	286.4	$^{13}C_5$-C18:1n-7c	
	20	*cis*-Vaccenic acid	VC1	281.3	C18:1n-7c	VC1_IS
Group 6	21		OTT_IS	284.4	$^{13}C_5$-C18:2n-6t,9t	
	22	Linoelaidic acid	OTT	279.3	C18:2n-6t,9t	OTT_IS
Group 7	23		LNA_IS	297.3	$^{13}C_{18}$-C18:2n-6c,9c	
	24	Linoleic acid	LNA	279.3	C18:2n-6c,9c	LNA_IS
Group 8	25		AR1_IS	350.7	D_{39}-C20:0	
	26	Arachidic acid	AR1	311.3	C20:0	AR1_IS
	27	γ-Linolenic acid	GLA	277.1	C18:3n-6c, 9c,12c	ALN_IS
	28		ALN_IS	291.5	D_{14}-C18:3n-3c,6c,9c	
	29	α-Linolenic acid	ALN	277.1	C18:3n-3c,6c,9c	ALN_IS
	30	Gondoic acid	EN1	309.3	C20:1n-9c	AR1_IS
Group 9	31	Eicosadienoic acid	ED1	307.3	C20:2n-6c,9c	AR1_IS
	32	Dihomo-γ-Linolenic acid	HGL	305.3	C20:3n-6c,9c,12c	ARA_IS
	33		DA1_IS	382.9	D_{43}-C22:0	
	34	Behenic acid	DA1	339.4	C22:0	DA1_IS
	35		ARA_IS	311.3	D_8-C20:4n-6c,9c,12c,15c	
	36	Arachidonic acid	ARA	303.3	C20:4n-6c,9c,12c,15c	ARA_IS
Group 10	37		EPA_IS	306.3	D_5-C20:5n-3c,6c,9c,12c,15c	
	38	Eicosapentaenoic acid	EPA	301.1	C20:5n-3c,6c,9c,12c,15c	EPA_IS
	39		LG1_IS	414.9	D_{47}-C24:0	
	40	Lignoceric acid	LG1	367.4	C24:0	LG1_IS
Group 11	41	Docosatetraenoic acid	DTA	331.3	C22:4n-6c,9c,12c,15c	DHA_IS
	42	Nervonic acid	NR1	365.4	C24:1n-9c	LG1_IS
	43	Docosapentaenoic acid 6	DP6	329.3	C22:5n-6c,9c,12c,15c,18c	DHA_IS
	44	Docosapentaenoic acid 3	DP3	329.3	C22:5n-3c,6c,9c,12c,15c	DHA_IS
	45		DHA_IS	332.3	D_5-C22:6n-3c,6c,9c,12c,15c,18c	
	46	Docosahexaenoic acid	DHA	327.3	C22:6n-3c,6c,9c,12c,15c,18c	DHA_IS

7.1.7 Data Processing

1. Data files generated by the GC-MS system are transferred to an appropriate location according to the laboratory's specific policies and procedures.
2. Using the appropriate data processing method, relevant chromatographic peaks are identified based on their retention time and m/z. The area under the curve is integrated.
3. Integrated peaks are documented as electronic files (in PDF format) and integration results are saved as Excel files.
4. The integration results are imported into an Excel template where final results are calculated.
5. Integrations and integration results are reviewed by a specially trained and dedicated individual who is not an analyst. Errors detected will be returned to the analyst for correction. Only data that passed this review process will be considered for further processing. See appendix 9 for additional integration guidelines.
6. Integration results and calculation results are combined with relevant operator, instrument and QC sample information and transferred to an appropriate location according to the laboratory's specific policies and procedures.

7.1.8 Data Calculations

1. Area ratios (A/IS = Analyte response/Internal Standard response) are calculated from the analyte and internal standard area counts.
2. Calibration curves are generated with the area ratios from the calibrators and their assigned values using ordinary linear regression.

   A/IS = slope*target concentration + y intercept

   The calibration curve is assessed for outliers and other problems resulting in non-linear behavior of data points. Analytes with invalid calibration curves are not processed further.
3. The analyte concentration in micromole per liter (μmol/L) is calculated using the area ratio calculated for a particular fatty acid and the regression parameters of the corresponding calibration curve.
   Area ratios for analytes outside the established linear range will not be used to calculate analyte concentration. These samples will be reanalyzed after appropriate dilution or concentration.
4. The sum of all fatty acids in one sample in micromole per liter is calculated. The portion of a particular TFA on the total fatty acids is calculated by dividing the concentration of the TFA with the sum of all fatty acid and multiplying this number by 100.

8 QUALITY ASSESSMENT AND PROFICIENCY TESTING

Quality assessment activities should be performed according to the laboratory's specific policies and procedures.

8.1 Quality Control Procedures

8.1.1 Quality Control Materials

Bench QC materials are used in this measurement procedure which consists of three plasma materials with levels of concentration spanning the "low-normal" to "high-normal" ranges for the analytes of interest.

The bench QC specimens are inserted in each sample batch and processed the same as the patient specimens.

8.1.2 Establishing QC Limits and Quality Control Evaluation

Acceptance criteria for values obtained with the bench QC materials ("QC limits") are established according to the procedure described by Caudill et al. [14]. Other procedures typically used to evaluate bench QCs are also acceptable.

8.1.3 Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

When results of control or calibration materials fail to meet the laboratory's established criteria for acceptability, all patient test results obtained in the unacceptable test run and since the last acceptable test run need to be repeated and no results should be reported. Specimen processing and analysis is stopped and should only resume when corrective action(s) has been performed to ensure the reporting of accurate and reliable patient test results.

8.2 Proficiency Testing

No commercial proficiency testing/external quality assessment program exists for the analytes reported with this measurement procedure. Because of this situation, the Audit-Sample Procedure as alternative proficiency testing program as described in the guideline of the Clinical and Laboratory Standards Institute (CLSI) GP29-P [15] was selected.

In this procedure 5 proficiency testing pools with levels spanning the full range of analyte values likely to be encountered in human specimens are prepared, characterized by measuring 30 separate vials from each pool in at least 10 different runs and performance limits are calculated. Individual vials from these pools are blinded by a different CDC laboratory. The 5 blinded vials are analyzed twice a year and results are evaluated by the division statistician. For the Proficiency Testing challenge to pass, at least 4 of the 5 results for each analyte need to be within the established performance limits (80% is considered passing).

If fewer than 4 of the 5 proficiency testing samples are within the limits for a given analyte, the challenge is considered as failed, no patient samples are to be analyzed and appropriate actions to correct this problem need to be initiated. Analysis of patient samples can resume after the problem was corrected and another Proficiency Testing challenge passed successfully.

9 METHOD PERFORMANCE CHARACTERISTICS

Alternative methods can be used if they achieve the analytical performance characteristics listed in this section and equivalence between the method described here and the alternate method is demonstrated. Determination of equivalence can be assessed using the protocols, such as the one described in CLSI guideline EP09 [16].

9.1 Reportable Range of Results and Linearity Limits

The reportable range of results is the range within linearity of the verified assay. The linearity for the analytes measured in this measurement procedure was determined following CLSI guideline EP6 [17]. The reportable ranges of results are indicated in the table below:

Table 8: Reportable ranges of analytes

Analyte	Analyte code	Linear Range µmol/L
trans 9-hexadecenoic acid	HDT	0.48 - 24.0
trans-9-octadecenoic acid	OD9	2.60 - 130
trans-11-octadecenoic acid	OD1	2.60 - 130
trans-9, trans-12-octadecadienoic acid	OTT	0.14 - 7.00
tetradecanoic acid	MR1	12.1 - 604
cis-9-tetradecenoic acid	ML1	2.12 - 106
hexadecanoic acid	PM1	161 - 8060
cis-9-hexadecenoic acid	PL1	24.6 - 1230
octadecanoic acid	ST1	40.5 - 2023
cis-9-octadecenoic acid	OL1	120 - 5990

cis-11-octadecenoic acid	VC1	14.7 - 737
cis-6-octadecenoic acid	OC6	4.86 - 243
cis-9, cis-12-octadecadienoic acid	LNA	159 - 7980
cis-9, cis-12, cis-15-octadecatrienoic acid	ALN	8.14 - 407
cis-6, cis-9, cis-12-octadecatrienoic acid	GLA	4.12 - 206
eicosanoic acid	AR1	4.06 - 203
cis-8, cis-11, cis-14-eicosatrienoic acid	HGL	5.14 - 257
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA	36.1 - 1810
cis-11-eicosenoic acid	EN1	0.96 - 48.0
cis-11, cis-14-eicosadienoic acid	ED1	1.00 - 50.0
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	EPA	14.5 - 724
docosanoic acid	DA1	3.98 - 199
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	DHA	20.6 - 1030
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	DTA	1.92 - 96.0
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	DP3	4.06 - 203
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	DP6	1.96 - 98.0
tetracosanoic acid	LG1	4.04 - 202
cis-15-tetracosenoic acid	NR1	3.92 - 196

9.2 **Limit of Detection (LOD)**

The limits of detection were determined using Taylor's method [18].

Table 9: Limits of detection (LOD)

Analyte	Analyte code	LOD µmol/L
trans 9-hexadecenoic acid	HDT	0.07
trans-9-octadecenoic acid	OD9	0.28
trans-11-octadecenoic acid	OD1	0.43
trans-9, trans-12-octadecadienoic acid	OTT	0.02
tetradecanoic acid	MR1	0.33
cis-9-tetradecenoic acid	ML1	0.29
hexadecanoic acid	PM1	17
cis-9-hexadecenoic acid	PL1	0.76
octadecanoic acid	ST1	4.02
cis-9-octadecenoic acid	OL1	14.3
cis-11-octadecenoic acid	VC1	1.06
cis-6-octadecenoic acid	OC6	0.4
cis-9, cis-12-octadecadienoic acid	LNA	4.9
cis-9, cis-12, cis-15-octadecatrienoic acid	ALN	0.82
cis-6, cis-9, cis-12-octadecatrienoic acid	GLA	0.43
eicosanoic acid	AR1	0.47
cis-8, cis-11, cis-14-eicosatrienoic acid	HGL	1
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA	0.36
cis-11-eicosenoic acid	EN1	0.84
cis-11, cis-14-eicosadienoic acid	ED1	0.16
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	EPA	1.29
docosanoic acid	DA1	1.77
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	DHA	1.96
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	DTA	0.34
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	DP3	0.51
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	DP6	0.33
tetracosanoic acid	LG1	1.59
cis-15-tetracosenoic acid	NR1	1.38

9.3 **Analytical Specificity**

Analytical specificity is achieved through:

- A sample preparation that isolates the analytes of interest from other components in the sample matrix
- A sample derivatization procedure that only reacts with the analytes and compounds with similar chemical characteristics
- High resolution chromatography that separates the analytes of interest and allows for compound identification based on chromatographic retention time using reference compounds and stable isotope labeled internal standards
- Mass spectrometric ionization mode that only allows for detection of the derivatives created during sample preparation
- Mass selective detection mode that only allows for detection of the mass-to-charge ratios specific to the fatty acids

Analytical specificity was tested

1. By assessing possible chromatographic coelution and MS detection using 63 different fatty acids (for the list of compounds used in this assessment see Appendix 8). None of the tested compounds showed coelution with the analytes reported in this method.
2. High, medium, and low QC pools were analyzed without addition of the internal standard to assess whether compounds in the QC samples coelute with the internal standards. No coelution was detected in this experiment.

9.4 **Accuracy and Precision**

Within-day imprecision was determined from 10 replicates of high, medium, and low QCs. The among day variability is assessed by measuring high, medium, and low QC pools in duplicate each over 20 days and calculating the means and standard deviations.

Table 10: Within-Day Precision

Analyte	Within-Day Precision (%CV) Low	Within-Day Precision (%CV) Medium	Within-Day Precision (%CV) High
trans 9-hexadecenoic acid	2	1	2
trans-9-octadecenoic acid	3	1	2
trans-11-octadecenoic acid	2	1	1
trans-9, trans-12-octadecadienoic acid	10	9	4
tetradecanoic acid	1	1	2
cis-9-tetradecenoic acid	2	2	2
hexadecanoic acid	1	1	1
cis-9-hexadecenoic acid	1	1	1
octadecanoic acid	1	1	1
cis-9-octadecenoic acid	1	1	1
cis-11-octadecenoic acid	1	1	1
cis-6-octadecenoic acid	1	1	2
cis-9, cis-12-octadecadienoic acid	1	1	1
cis-9, cis-12, cis-15-octadecatrienoic acid	1	2	1
cis-6, cis-9, cis-12-octadecatrienoic acid	1	2	2
eicosanoic acid	1	1	1
cis-8, cis-11, cis-14-eicosatrienoic acid	1	3	2
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	1	2	1
cis-11-eicosenoic acid	2	3	2
cis-11, cis-14-eicosadienoic acid	2	3	2
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	1	1	1
docosanoic acid	2	5	4
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	3	3	1
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	4	5	3
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	4	4	3
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	3	6	3
tetracosanoic acid	5	7	3
cis-15-tetracosenoic acid	5	7	3
Sum of fatty acids	1	1	1

Table 11: Among-Day Precision

Analyte	Among-Day Precision (%CV) Low	Among-Day Precision (%CV) Medium	Among-Day Precision (%CV) High
trans 9-hexadecenoic acid	9	4	4
trans-9-octadecenoic acid	10	4	5
trans-11-octadecenoic acid	10	4	5
trans-9, trans-12-octadecadienoic acid	17	12	9
tetradecanoic acid	6	4	4
cis-9-tetradecenoic acid	20	9	5
hexadecanoic acid	5	4	6
cis-9-hexadecenoic acid	6	4	4
octadecanoic acid	4	4	5
cis-9-octadecenoic acid	4	3	5
cis-11-octadecenoic acid	4	3	4
cis-6-octadecenoic acid	12	4	5
cis-9, cis-12-octadecadienoic acid	4	4	6
cis-9, cis-12, cis-15-octadecatrienoic acid	5	4	4
cis-6, cis-9, cis-12-octadecatrienoic acid	6	4	4
eicosanoic acid	8	6	5
cis-8, cis-11, cis-14-eicosatrienoic acid	5	4	5
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	4	3	4
cis-11-eicosenoic acid	11	11	8
cis-11, cis-14-eicosadienoic acid	5	5	4
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	25	13	6
docosanoic acid	7	7	7
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	13	15	6
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	5	5	4
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	9	6	5
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	6	5	5
tetracosanoic acid	8	8	8
cis-15-tetracosenoic acid	7	11	6
Sum of fatty acids	4	3	4

The accuracy was verified by analyzing commercial standards materials (GLC standard GLC-603 and GLC-674, NuCheckPrep, Elysian, MN) and comparing the assigned value to the measured values.

Table 12: Accuracy

Analyte	Analyte code	Average Accuracy (%)	95% CI (%)
trans 9-hexadecenoic acid	HDT	95.6	94.5-96.7
trans-9-octadecenoic acid	OD9	91.7	87.8-95.5
trans-11-octadecenoic acid	OD1	89.5	86.0-93.0
trans-9, trans-12-octadecadienoic acid	OTT	89.8	88.3-91.2
tetradecanoic acid	MR1	98	96.5-99.4
cis-9-tetradecenoic acid	ML1	128.4	127.1-129.7
hexadecanoic acid	PM1	103.3	100.5-106.0
cis-9-hexadecenoic acid	PL1	99.4	97.2-101.6
octadecanoic acid	ST1	97	93.6-100.4
cis-9-octadecenoic acid	OL1	98.4	96.7-100.1
cis-11-octadecenoic acid	VC1	99	97.8-100.1
cis-6-octadecenoic acid	OC6	99.7	97.2-102.12
cis-9, cis-12-octadecadienoic acid	LNA	90.3	89.7-91.0
cis-9, cis-12, cis-15-octadecatrienoic acid	ALN	90.4	88.1-92.8
cis-6, cis-9, cis-12-octadecatrienoic acid	GLA	93	90.7-95.4
eicosanoic acid	AR1	95.6	92.4-98.7
cis-8, cis-11, cis-14-eicosatrienoic acid	HGL	92.9	89.7-96.2
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA	95.4	91.5-99.3
cis-11-eicosenoic acid	EN1	82	80.0-83.9
cis-11, cis-14-eicosadienoic acid	ED1	95	90.0-100.1
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	EPA	90.2	88.7-91.7
docosanoic acid	DA1	98.4	96.6-100.2
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	DHA	92	89.9-94.0
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	DTA	91.9	91.3-92.6
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	DP3	89.5	84.4-94.6
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	DP6	87.5	86.0-89.0
tetracosanoic acid	LG1	97.4	92.2-102.6
cis-15-tetracosenoic acid	NR1	100.6	95.2-106.1

9.5 Limitations of Method, Interfering Substances and Conditions

Interfering conditions

Analysts preparing samples and handling supplies and equipment must always wear gloves to minimize contamination of samples with fatty acids from the skin or skin cream products. Additionally, the chromatogram in the blank sample should be evaluated: if the area count in the blank sample is higher than that of the lowest calibrator, then the calibration curve is rejected.

Limitations of the method

This method was tested for fatty acid analysis in human plasma and serum and may not be suitable for other specimens. The analytical performance parameters need to be reassessed and verified when other specimen matrices are used.

This method does not allow for analysis of fatty acids containing functional groups such as epoxy, hydroperoxy, cyclopropenyl, cyclopropyl and possibly hydroxyl and acetylenic groups. Further, it is not suitable for analysis of cis/trans and cis/cis conjugated linoleic acid isomers as they may be converted to their trans/trans isomers.

Interfering Substances

The method was tested for 63 different substances (for specific details see Appendix 8). None of these substances interfere with the analytes reported with this measurement procedure.

10 REFERENCE RANGES (NORMAL VALUES)

Population-based reference ranges have not been established yet for these trans fatty acids.

An in-house assessment using plasma samples from 66 individuals, collected in 2013 was performed to obtain information on concentrations that can be expected in the U.S. general population. Reference ranges may be different in other countries. In this study, the following values were determined:

Table 13: Reference ranges of various fatty acids in plasma in US general population

Analyte	Values in µmol/L		Values as percent of total fatty acids	
	Mean (Range)	Median (5^{th}-95^{th} Percentile)	Mean (Range)	Median (5^{th}-95^{th} Percentile)
trans 9-hexadecenoic acid	2.53 (0.671-8.64)	2.13 (1.05-4.83)	0.021 (0.009-0.038)	0.02 (0.012-0.032)
trans-9-octadecenoic acid	12.3 (2.6-120)	9 (4.06-25)	0.08 (0.04-0.21)	0.07 (0.05-0.17)
trans-11-octadecenoic acid	14.7 (3.1-181.2)	10.3 (5.07-28.7)	0.11 (0.04-0.32)	0.1 (0.05-0.22)
trans-9, trans-12-octadecadienoic acid	1.66 (0.36-8)	0.9 (0.455-4.44)	0.011 (0.007-0.022)	0.01 (0.008-0.017)
tetradecanoic acid	96.3 (23.1-325.2)	72.8 (36.6-253.3)	0.86 (0.38-1.92)	0.74 (0.51-1.74)
cis-9-tetradecenoic acid	6.42 (0.69-31.38)	4.67 (1.87-16.73)	0.06 (0.01-0.19)	0.05 (0.02-0.13)
hexadecanoic acid	2272 (1086-5574)	2106 (1335-3663)	22.17 (19.08-27.45)	21.83 (19.66-25.39)
cis-9-hexadecenoic acid	205 (61-897)	162 (76.4-477)	1.92 (0.86-5.03)	1.68 (1.03-4.09)
octadecanoic acid	678 (334-1708)	648 (415-1027)	6.68 (5.4-8.71)	6.69 (5.66-7.74)
cis-9-octadecenoic acid	1785 (628-4610)	1604 (958-3268)	17.14 (12.94-25.76)	16.44 (13.63-21.91)
cis-11-octadecenoic acid	143 (53-353)	134 (79.1-249)	1.37 (0.95-1.83)	1.36 (1.06-1.71)
cis-6-octadecenoic acid	22.1 (5.5-217.5)	16.3 (9.24-41)	0.18 (0.07-0.5)	0.16 (0.1-0.34)
cis-9, cis-12-octadecadienoic acid	3230 (1447-6030)	3142 (2053-4483)	33.89 (22.19-42.47)	34.12 (26.25-40.04)
cis-9, cis-12, cis-15-octadecatrienoic acid	54.6 (19.1-199.6)	45.7 (23.5-117)	0.52 (0.24-0.93)	0.48 (0.33-0.89)
cis-6, cis-9, cis-12-octadecatrienoic acid	52.5 (16.3-127.6)	46.6 (24.9-102)	0.53 (0.18-1.25)	0.49 (0.3-0.91)
eicosanoic acid	21.5 (7.54-39.4)	20.7 (13.3-31.7)	0.22 (0.13-0.3)	0.22 (0.15-0.29)
cis-8, cis-11, cis-14-eicosatrienoic acid	127 (53-290)	118 (66.4-201)	1.26 (0.78-2.21)	1.2 (0.89-1.84)
cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	799 (316-1382)	795 (444-1196)	8.36 (5.15-13.61)	8.45 (5.85-11.13)
cis-11-eicosenoic acid	9.79 (1.55-32.3)	8.6 (4.12-17.9)	0.09 (0.02-0.16)	0.09 (0.06-0.14)
cis-11, cis-14-eicosadienoic acid	19.6 (7.78-43.1)	19 (10.8-32.6)	0.19 (0.08-0.27)	0.19 (0.15-0.24)
cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	42.9 (18.3-160)	37.4 (22.6-68.6)	0.46 (0.13-1.67)	0.41 (0.28-0.77)
docosanoic acid	53.7 (2.8-102)	51.9 (26.6-90.6)	0.53 (0.03-0.86)	0.55 (0.31-0.73)
cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	110 (41-320)	103 (62.1-168)	1.19 (0.58-3.35)	1.14 (0.69-1.7)

Table 14: Reference Range (continued)

Analyte	Values in µmol/L		Values as percent of total fatty acids	
	Mean (Range)	Median (5^{th}-95^{th} Percentile)	Mean (Range)	Median (5^{th}-95^{th} Percentile)
cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	27.9 (7.25-59.1)	27 (14.9-46.5)	0.27 (0.07-0.41)	0.28 (0.19-0.36)
cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	40.6 (15.6-105)	36.7 (22.6-64.9)	0.41 (0.23-1.1)	0.39 (0.3-0.53)
cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	18.9 (5.69-39.5)	18.2 (8.53-28.1)	0.19 (0.08-0.34)	0.19 (0.13-0.29)
tetracosanoic acid	52.7 (3.32-106)	47.3 (29.6-92.7)	0.51 (0.03-0.81)	0.51 (0.33-0.67)
cis-15-tetracosenoic acid	71.8 (4.24-126)	70.2 (40-107)	0.75 (0.04-1.14)	0.77 (0.46-1)
Sum of fatty acids	9972 (4458-20865)	9618 (6071-14689)	NA	NA

11 TEST RESULT REPORTING SYSTEM

Results are reported to 3 significant digits based on assay sensitivity calculations. Measurements are reported in µmol/L. If reporting trans-fats as percent of total fats, the calculations should be based on the µM concentrations and the calculations should be included in the report, including what other trans-fats are measured.

12 PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Following successful completion of analysis, remaining samples are retained until all results have been reported and sufficient time has passed for review of the results. After this time, samples are either returned to the contact person who requested the analysis or are treated according to laboratory policy.

Standard record keeping (e.g., database, notebooks, data files) is used to track specimens. Records (including related QA/QC data) are maintained for 3 years, and duplicate records are kept off-site in electronic format. Study subject confidentiality is protected by providing personal identifiers only to the medical officer if needed or remain with the contact person who requested the analyses.

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14 APPENDICES

Appendix 1. List of Fatty Acids Measured with this Measurement Procedure

Appendix 2. Flow Chart Describing Sample Processing Performed for Fatty Acids Analysis

Appendix 3. Description of Standards Used

Appendix 4. Metrological Traceability of Trans Fatty Acids Measurements

Appendix 5. Example of Analytical Sequence

Appendix 6. GC-MS Settings (Instrument Control Parameters)

Appendix 7. Retention Time Standard And Low QC Sample Chromatogram

Appendix 8. List of Compounds Tested for Interference

Appendix 9. Integration guidelines

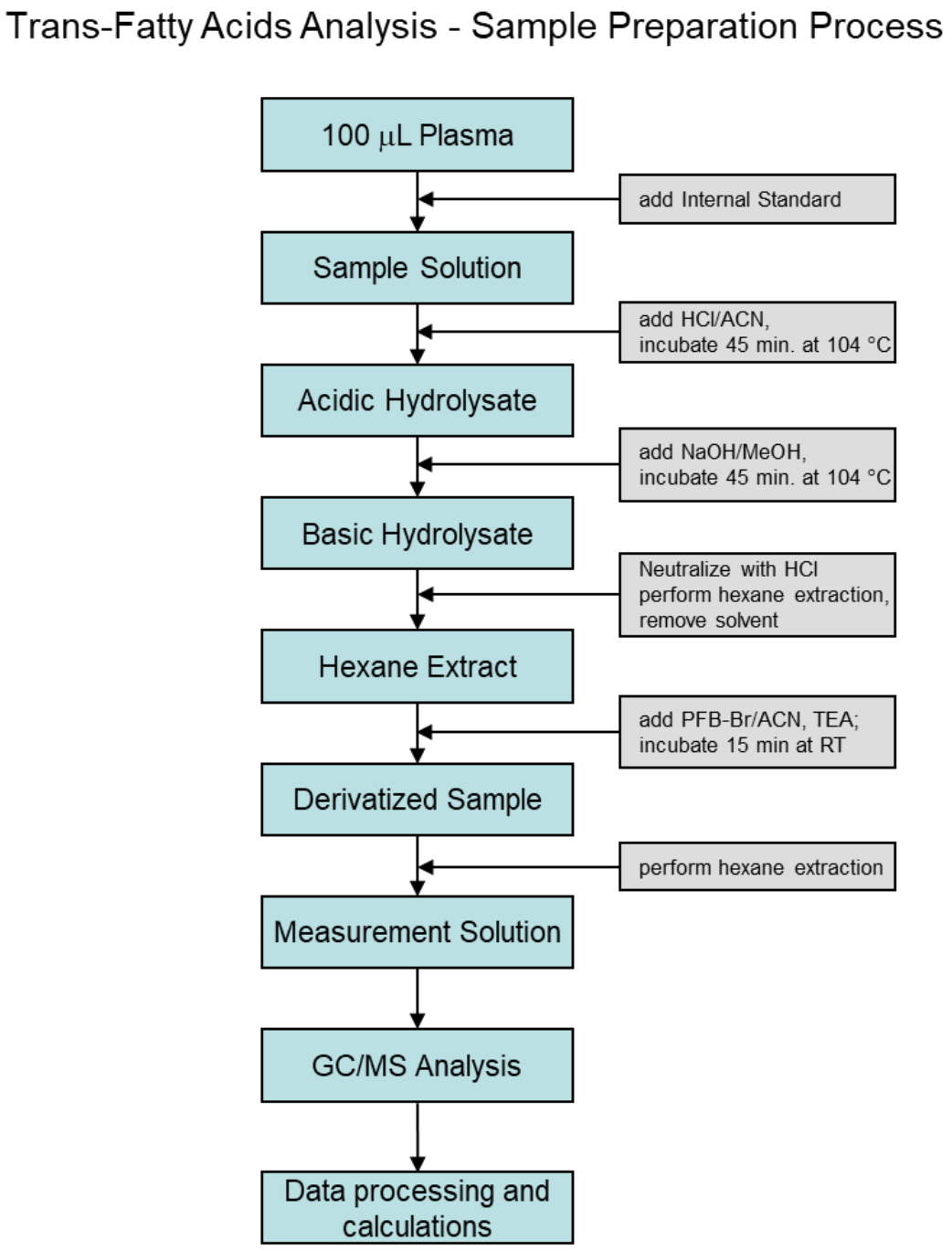
Appendix 10. Symbols, Abbreviations, Terminology

Appendix 1. List of Fatty Acids Measured with this Measurement Procedure

Nr.	IUPAC name	Common Name	Shorthand	Analyte Code
1	trans 9-hexadecenoic acid	Palmitelaidic acid	C16:1n-7t	HDT*
2	trans-9-octadecenoic acid	Elaidic acid	C18:1n-9t	OD9*
3	trans-11-octadecenoic acid	Vaccenic acid	C18:1n -7t	OD1*
4	trans-9, trans-12-octadecadienoic acid	Linolelaidate acid	C18:2n- 6t, 9t	OTT*
5	tetradecanoic acid	Myristic acid	C14:0	MR1*
6	cis-9-tetradecenoic acid	Myristoleic acid	C14:1n-5c	ML1
7	hexadecanoic acid	Palmitic acid	C16:0	PM1*
8	cis-9-hexadecenoic acid	Palmitoleic acid	C16:1n-7c	PL1*
9	octadecanoic acid	Stearic acid	C18:0	ST1*
10	cis-9-octadecenoic acid	Oleic acid	C18:1n-9c	OL1*
11	cis-11-octadecenoic acid	*cis*-Vaccenic acid	C18:1n-7c	VC1*
12	cis-9, cis-12-octadecadienoic acid	Linoleic acid	C18:2n-6c,9c	LNA*
13	cis-9, cis-12, cis-15-octadecatrienoic acid	alpha-Linolenic acid	C18:3n-3c,6c,9c	ALN*
14	cis-6, cis-9, cis-12-octadecatrienoic acid	gamma-Linolenic acid	C18:3n-6c,9c,12c	GLA
15	eicosanoic acid	Arachidic acid	C20:0	AR1*
16	cis-8, cis-11, cis-14-eicosatrienoic acid	Dihomo-gamma-Linolenic acid	C20:3n-6c,9c,12c	HGL
17	cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	Arachidonic acid	C20:4n-6c,9c,12c,15c	ARA*
18	cis-11-eicosenoic acid	Gondoic acid	C20:1n-9c	EN1
19	cis-11, cis-14-eicosadienoic acid	Eicosadienoic acid	C20:2n-6c,9c	ED1
20	cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	Eicosapentaenoic acid	C20:5n-3c,6c,9c,12c,15c	EPA*
21	docosanoic acid	Behenic acid	C22:0	DA1*
22	cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	Docosahexaenoic acid	C22:6n-3c,6c,9c,12c,15c,18c	DHA*
23	cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	Docosatetraenoic acid	C22:4n-6c,9c,12c,15c	DTA
24	cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	Docosapentaenoic acid 3	C22:5n-3c,6c,9c,12c,15c	DP3
25	cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	Docosapentaenoic acid 6	C22:5n-6c,9c,12c,15c,18c	DP6
26	tetracosanoic acid	Lignoceric acid	C24:0	LG1*
27	cis-15-tetracosenoic acid	Nervonic acid	C24:1n-9c	NR1

*For these compounds stable isotope labeled standards are available.

Appendix 2. Flow Chart Describing Sample Processing Performed for Fatty Acids Analysis



Appendix 3. Description of Standards Used

Standards used for creating calibrators

(The chemicals described below can also be purchased from other vendors)

Nr.	Name	Analyte Code	Manufacturer	Purity	MW (g/mol)
1	trans 9-hexadecenoic acid	HDT	Nu-Chek-Prep, Elysian, MN	>99%	254.4
2	trans-9-octadecenoic acid	OD9	Nu-Chek-Prep, Elysian, MN	>99%	282.46
3	trans-11-octadecenoic acid	OD1	Nu-Chek-Prep, Elysian, MN	>99%	282.46
4	trans-9, trans-12-octadienoic acid	OTT	Nu-Chek-Prep, Elysian, MN	>99%	294.51
5	tetradecanoic acid	MR1	Nu-Chek-Prep, Elysian, MN	>99%	228.38
6	cis-9-tetradecenoic acid	ML1	Nu-Chek-Prep, Elysian, MN	>99%	226.38
7	hexadecanoic acid	PM1	Nu-Chek-Prep, Elysian, MN	>99%	256.43
8	cis-9-hexadecenoic acid	PL1	Nu-Chek-Prep, Elysian, MN	>99%	254.43
9	octadecanoic acid	ST1	Nu-Chek-Prep, Elysian, MN	>99%	284.48
10	cis-9-octadecenoic acid	OL1	Nu-Chek-Prep, Elysian, MN	>99%	282.48
11	cis-11-octadecenoic acid	VC1	Nu-Chek-Prep, Elysian, MN	>99%	282.48
12	cis-6-octadecenoic acid	OC6	Sigma-Aldrich, St. Louis, MO	99+%	296.51
13	cis-9, cis-12-octadecadienoic acid	LNA	Nu-Chek-Prep, Elysian, MN	>99%	280.48
14	cis-9, cis-12, cis-15-octadecatrienoic acid	ALN	Nu-Chek-Prep, Elysian, MN	>99%	278.48
15	cis-6, cis-9, cis-12-octadecatrienoic acid	GLA	Nu-Chek-Prep, Elysian, MN	>99%	278.48
16	eicosanoic acid	AR1	Nu-Chek-Prep, Elysian, MN	>99%	312.54
17	cis-8, cis-11, cis-14-eicosatrienoic acid	HGL	Nu-Chek-Prep, Elysian, MN	>99%	306.53
18	cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA	Nu-Chek-Prep, Elysian, MN	>99%	304.52
19	cis-11-eicosenoic acid	EN1	Nu-Chek-Prep, Elysian, MN	>99%	310.54
20	cis-11, cis-14-eicosadienoic acid	ED1	Nu-Chek-Prep, Elysian, MN	>99%	308.53
21	cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	EPA	Nu-Chek-Prep, Elysian, MN	>99%	302.52
22	docosanoic acid	DA1	Nu-Chek-Prep, Elysian, MN	>99%	340.59
23	cis-13-docosenoic acid	DE1	Nu-Chek-Prep, Elysian, MN	>99%	338.59
24	cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	DHA	Nu-Chek-Prep, Elysian, MN	>99%	328.57
25	cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	DTA	Nu-Chek-Prep, Elysian, MN	>99%	332.57
26	cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	DP3	Nu-Chek-Prep, Elysian, MN	>99%	330.57
27	cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	DP6	Nu-Chek-Prep, Elysian, MN	>99%	330.57
28	tetracosanoic acid	LG1	Nu-Chek-Prep, Elysian, MN	>99%	368.64
29	cis-15-tetracosenoic acid	NR1	Nu-Chek-Prep, Elysian, MN	>99%	366.63

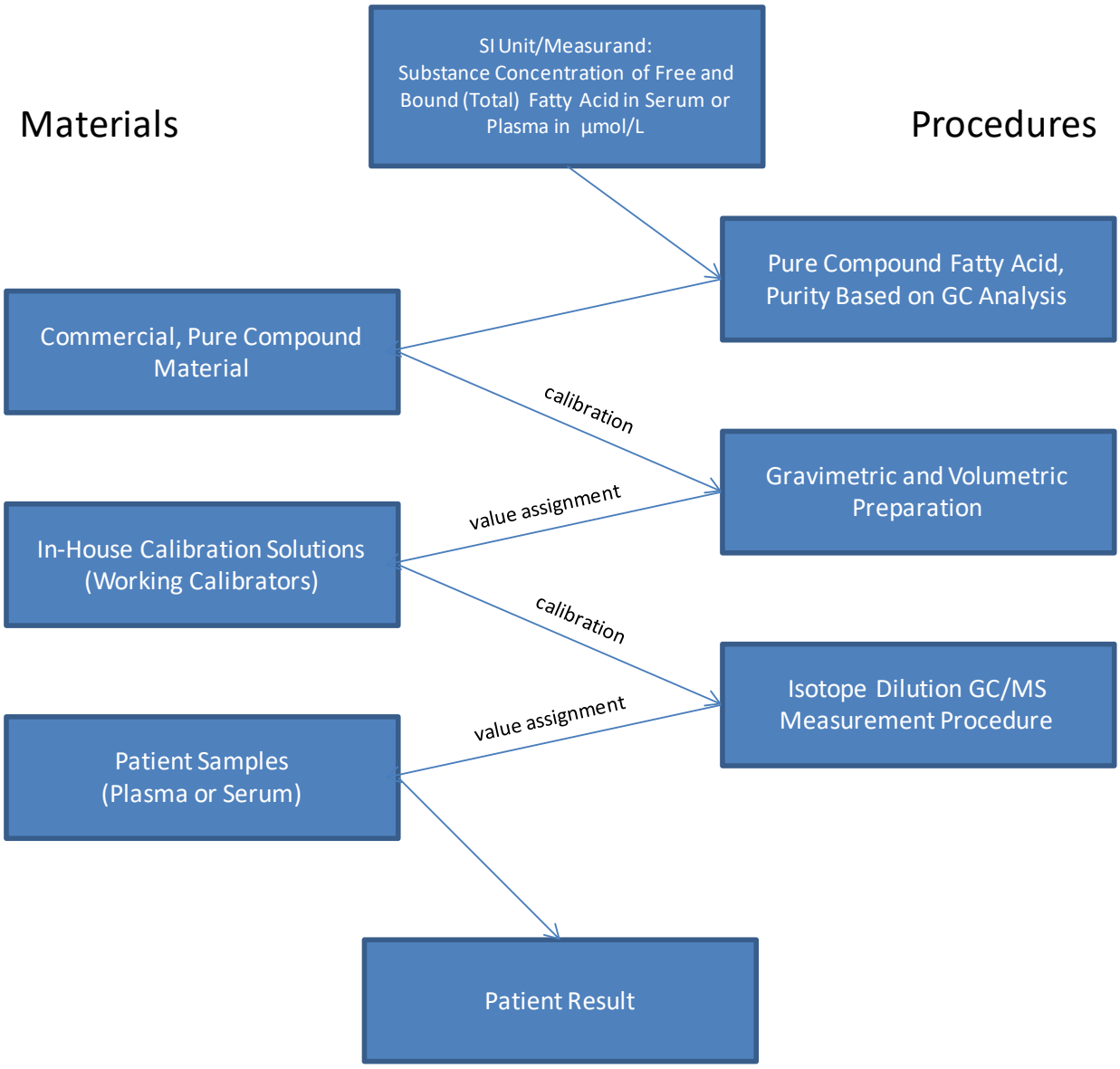
Appendix 3 (continued): Stable isotope-labeled standards used for internal standards

Name	Analyte Code	Manufacturer	Purity	MW (g/mol)
$^{13}C_5$-trans-9-hexadecenoic acid	HDT_IS	Sigma-Aldrich, St. Louis, MO	≥99%	259.37
$^{13}C_5$-trans-9-octadecenoic acid	OD9_IS	Sigma-Aldrich, St. Louis, MO	>99%	287.42
$^{13}C_5$-trans-11-octadecenoic acid	OD1_IS	Sigma-Aldrich, St. Louis, MO	≥99%	287.42
$^{13}C_5$-trans-9, trans-12-octadecadienoic acid	OTT_IS	Sigma-Aldrich, St. Louis, MO	≥98%	285.41
D_{27}-tetradecanoic acid	MR1_IS	Cambridge Isotopes Laboratories, Cambridge, MA	≥98%	255.54
$^{13}C_{16}$-hexadecanoic acid	PM1_IS	Sigma-Aldrich, St. Louis, MO	≥99%	272.31
$^{13}C_{16}$-cis-9-hexadecenoic acid	PL1_IS	Sigma-Aldrich, St. Louis, MO,	>99%	270.29
D_{35}-octadecanoic acid	ST1_IS	Cambridge Isotopes Laboratories, Cambridge, MA	≥98%	319.69
$^{13}C_{18}$-cis 9-octadecenoic acid	OL1_IS	IsoSciences, King of Prussia, PA	≥99%	300.27
$^{13}C_5$-cis-11-octadecenoic acid	VC1_IS	Sigma-Aldrich, St. Louis, MO	≥99%	287.42
$^{13}C_{18}$- cis-9, cis-12-octadecadienoic acid methyl ester	LNA_IS	IsoSciences, King of Prussia, PA	≥99%	298.31
D_{39}-eicosanoic acid methyl ester	AR1_IS	IsoSciences, King of Prussia, PA	≥98%	365.77
D_{14}-cis-9, cis-12, cis-15-octadecatrienoic acid	ALN_IS	Cayman Chemical, Ann Arbor, MI	>99%	292.5
D_{43}-docosanoic acid	DA1_IS	IsoSciences, King of Prussia, PA	≥99%	383.85
D_8- cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA_IS	Cayman Chemical, Ann Arbor, MI	≥96%	312.5
D_5- cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid methyl ester	EPA_IS	IsoSciences, King of Prussia, PA	≥99%	321.52
D_{47}-tetracosanoic acid	LG1_IS	IsoSciences, King of Prussia, PA	≥99%	415.95
D_5- cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid methyl ester	DHA_IS	IsoSciences, King of Prussia, PA	≥97%	347.56

Appendix 3 (continued): Target concentrations of the calibrator solutions

Nr.	Name	Analyte Code	Level 40 TFAC40 (µmol/L)	Level 35 TFAC35 (□mol/L)	Level 30 TFAC30 (µmol/L)	Level 20 TFAC20 (µmol/L)	Level 10 TFAC10 (µmol/L)
1	trans 9-hexadecenoic acid	HDT	25	12.5	6.25	2.5	1
2	trans-9-octadecenoic acid	OD9	125	62.5	31.3	12.5	5
3	trans-11-octadecenoic acid	OD1	125	62.5	31.3	12.5	5
4	trans-9, trans-12-octadienoic acid	OTT	8	4	2	0.8	0.32
5	tetradecanoic acid	MR1	600	300	150	60	24
6	cis-9-tetradecenoic acid	ML1	100	50	25	10	4
7	hexadecanoic acid	PM1	8,000	4,000	2,000	800	320
8	cis-9-hexadecenoic acid	PL1	1,200	600	300	120	48
9	octadecanoic acid	ST1	2,000	1,000	500	200	80
10	cis-9-octadecenoic acid	OL1	6,000	3,000	1,500	600	240
11	cis-11-octadecenoic acid	VC1	800	400	200	80	32
12	cis-6-octadecenoic acid	OC6	250	125	62.5	25	10
13	cis-9, cis-12-octadecadienoic acid	LNA	8,000	4,000	2,000	800	320
14	cis-9, cis-12, cis-15-octadecatrienoic acid	ALN	400	200	100	40	16
15	cis-6, cis-9, cis-12-octadecatrienoic acid	GLA	200	100	50	20	8
16	eicosanoic acid	AR1	200	100	50	20	8
17	cis-8, cis-11, cis-14-eicosatrienoic acid	HGL	250	125	62.5	25	10
18	cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid	ARA	2,000	1,000	500	200	80
19	cis-11-eicosenoic acid	EN1	50	25	12.5	5	2
20	cis-11, cis-14-eicosadienoic acid	ED1	50	25	12.5	5	2
21	cis-5, cis-8, cis-11, cis-14, cis-17-eicosapentaenoic acid	EPA	800	400	200	80	32
22	docosanoic acid	DA1	200	100	50	20	8
23	cis-13-docosenoic acid	DE1	50	25	12.5	5	2
24	cis-4, cis-7, cis-10, cis-13, cis-16, cis-19-docosahexaenoic acid	DHA	1,000	500	250	100	40
25	cis-7, cis-10, cis-13, cis-16-docosatetraenoic acid	DTA	100	50	25	10	4
26	cis-7, cis-10, cis-13, cis-16, cis-19-docosapentaenoic acid	DP3	200	100	50	20	8
27	cis-4, cis-7, cis-10, cis-13, cis-16-docosapentaenoic acid	DP6	100	50	25	10	4
28	tetracosanoic acid	LG1	200	100	50	20	8
29	cis-15-tetracosenoic acid	NR1	200	100	50	20	8

Appendix 4. Metrological Traceability of Trans Fatty Acids Measurements



Appendix 5. Example of Analytical Sequence

Injection order	Sample Type	Vial #	Sample ID
1	Instrument Check Std	49	RT STD
2	Calibrator	4	TFAC20L06
3	Calibrator	5	TFAC10L06
4	Quality Control	8	+021208PA
5	Sample	13	QCS9019PL
6	Sample	14	QCS9029PL
7	Sample	15	QCS9030PL
8	Sample	16	QCS9031PL
9	Sample	17	QCS9033PL
10	Sample	18	QCS9034PL
11	Sample	19	QCS9035PL
12	Sample	20	QCS9036PL
13	Sample	21	QCS9063PL
14	Calibrator	1	TFAC40L06
15	Calibrator	6	TFAC00L06
16	Quality Control	7	+031209PA
17	Sample	22	QCS9064PL
18	Sample	23	QCS9065PL
19	Sample	24	QCS9068PL
20	Sample	25	QCS9069PL
21	Sample	26	QCS9073PL
22	Sample	27	QCS9074PL
23	Sample	28	QCS9075PL
24	Sample	29	QCS9079PL
25	Sample	30	QCS9080PL
26	Sample	31	QCS9081PL
27	Calibrator	3	TFAC30L06
28	Quality Control	9	+011207PA
29	Quality Control	10	+031209PA
30	Sample	32	QCS9082PL
31	Sample	33	QCS9084PL
32	Sample	34	QCS9085PL
33	Sample	35	QCS9086PL
34	Sample	36	QCS9087PL
35	Sample	37	QCS9088PL
36	Sample	38	QCS9089PL
37	Sample	39	QCS9090PL
38	Sample	40	QCS9091PL
39	Sample	41	QCS9092PL
40	Calibrator	2	TFAC35L06
41	Quality Control	11	+021208PA
42	Quality Control	12	+011207PA
43	Sample	42	QCS9094PL
44	Sample	43	QCS9095PL
45	Sample	44	QCS9096PL
46	Sample	45	QCS9097PL
47	Sample	46	QCS9098PL
48	Sample	47	QCS9100PL
49	Sample	48	QCS9101PL

Appendix 6. GC-MS Settings (Instrument Control Parameters)

Parameters listed as documented by Mass Hunter software:

Sample Inlet:	GC	Temperature	
Mass Spectrometer:	Enabled	Setpoint	On
		(Initial)	260 °C
Injection Location:	Front	Post Run	0 °C
		Column	
GC Oven Temperature		Column #1	
Setpoint	On	Flow	
(Initial)	50 °C	Setpoint	Off
Hold Time	0 min	(Initial)	2 mL/min
Post Run	130 °C	Post Run	0.57353 mL/min
Program			
#1 Rate	40 °C/min	Agilent Varian CP7421	
#1 Value	160 °C	FAME SELECT 200 m x 250 µm x 0.25 µm	
#1 Hold Time	10 min	In	
#2 Rate	1 °C/min	Front SS Inlet H2	
#2 Value	175 °C	Out	MSD
#2 Hold Time	0 min	(Initial)	50 °C
#3 Rate	0.5 °C/min	Pressure	40.264 psi
#3 Value	210 °C	Flow	2 mL/min
#3 Hold Time	0 min	Average Velocity	29.639 cm/sec
#4 Rate	35 °C/min	Holdup Time	11.247 min
#4 Value	260 °C		
#4 Hold Time	20 min	Column Outlet Pressure	0 psi
Equilibration Time	0.25 min	GERSTEL MAESTRO SYSTEM SETTINGS	
Max Temperature	290 °C	Maestro Runtime	119.18 min
Maximum Temperature Override	Disabled	GC Cool Down Time	10.00 min
Slow Fan	Disabled		
Cryo	Off	GERSTEL MPS Liquid Injection	
		Syringe	10ul
Front SS Inlet H2			
Mode	Split	SAMPLE PARAMETERS	
Heater	On 240 °C	Sandwich	not used
Pressure	On 40.264 psi		
Total Flow	On 245 mL/min	Inj. Volume	1.0 µL
Septum Purge Flow	On 3 mL/min	Air Volume below	0.0 µL
Gas Saver	On 20 mL/min		
	after 2 min	Inj. Speed	50.00 µL/s
Split Ratio	100 :1	Fill Volume	1.0 µL
Split Flow	240 mL/min	Fill Strokes	3
		Fill Speed	1.00 µL/s
		Viscositiy Delay	0 s
		Eject Speed	50.00 µL/s
		Pre Inj. Delay	0 s
		Post Inj. Delay	0 s
Thermal Aux 2 (MSD Transfer Line)		Inj. Penetration	40.00 mm

Sample Tray Type	VT54
Vial Penetration	35.00 mm

CLEANING PARAMETERS

Preclean Sample : 1

Wash Station 1	Wash1
Preclean Solv.1	0
Postclean Solv.1	8
Fill Speed Solv.1	5.00 µL/s
Viscosity Delay Solv.1	0 s
Eject Speed Solv.1	50.00 µL/s
Information Solv.1	acetone
Wash Station 2	Wash2
Preclean Solv.2	2
Postclean Solv.2	8
Fill Speed Solv.2	5.00 µL/s
Viscosity Delay Solv.2	0 s
Eject Speed Solv.2	50.00 µL/s
Information Solv.2	hexane

MS Information

Acquisition Mode	SIM
Solvent Delay (minutes)	20
EM Setting mode Delta	200
Number of SIM Groups	12

[SIM Parameters]

Group 1	Group ID	1
Resolution		1
Group Start Time		20
Number of Ions		2
Ions		
Dwell In Group (Mass, Dwell):		(171.30, 200)
		(199.30, 200)
Group 2	Group ID	2
Resolution		1
Group Start Time		51
Number of Ions		3
Ions		
Dwell In Group (Mass, Dwell):		(225.20, 125)
		(227.20, 125)
		(254.40, 125)
Group 3	Group ID	3
Resolution		1
Group Start Time		70
Number of Ions		2
Ions		
Dwell In Group (Mass, Dwell):		(255.30, 200)
		(271.30, 200)
Group 4	Group ID	4
Resolution		1
Group Start Time		77.5
Number of Ions		3
Ions		
Dwell In Group (Mass, Dwell):		(253.20, 125)
		(258.40, 125)
		(269.30, 125)
Group 5	Group ID	5
Resolution		1
Group Start Time		84
Number of Ions		2
Ions		
Dwell In Group (Mass, Dwell):		(283.30, 200)
		(318.50, 200)
Group 6	Group ID	6
Resolution		1
Group Start Time		94.5
Number of Ions		3
Ions		
Dwell In Group (Mass, Dwell):		(281.30, 125)
		(286.40, 125)
		(299.30, 125)
Group 7	Group ID	7
Resolution		1
Group Start Time		98.7
Number of Ions		2
Ions		
Dwell In Group (Mass, Dwell):		(279.30, 200)
		(284.40, 200)
Group 8	Group ID	8
Resolution		1
Group Start Time		99.5
Number of Ions		2
Ions		
Dwell In Group (Mass, Dwell):		(279.30, 200)
		(297.30, 200)
Group 9	Group ID	9
Resolution		1

Group Start Time 100.8
Number of Ions 5
Ions
Dwell In Group (Mass, Dwell): (277.10, 80)
(291.50, 80)
(309.30, 80)
(311.30, 80)
(350.70, 80)

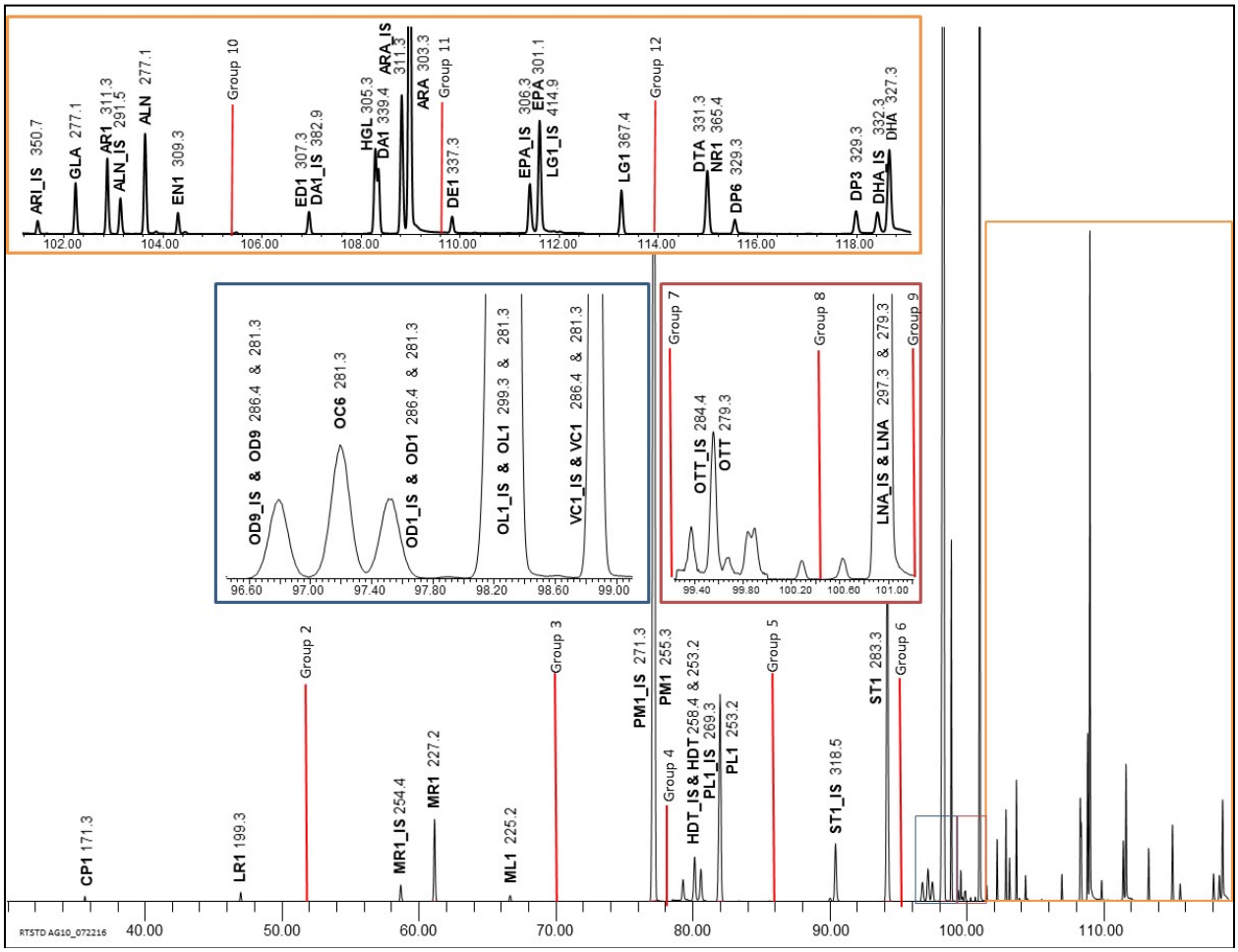
Group 10 Group ID 10
Resolution 1
Group Start Time 104.5
Number of Ions 6
Ions
Dwell In Group (Mass, Dwell): (303.30, 65)
(305.30, 65)
(307.30, 65)
(311.30, 65)
(339.40, 65)
(382.85, 65)

Group 11 Group ID 11
Resolution 1
Group Start Time 109.1
Number of Ions 5
Ions
Dwell In Group (Mass, Dwell): (301.10, 80)
(306.30, 80)
(337.30, 80)
(367.40, 80)
(414.95, 80)
Group 12 Group ID 12
Resolution 1
Group Start Time 113.5
Number of Ions 5
Ions
Dwell In Group (Mass, Dwell): (327.30, 80)
(329.30, 80)
(331.30, 80)
(332.30, 80)
(365.40, 80)

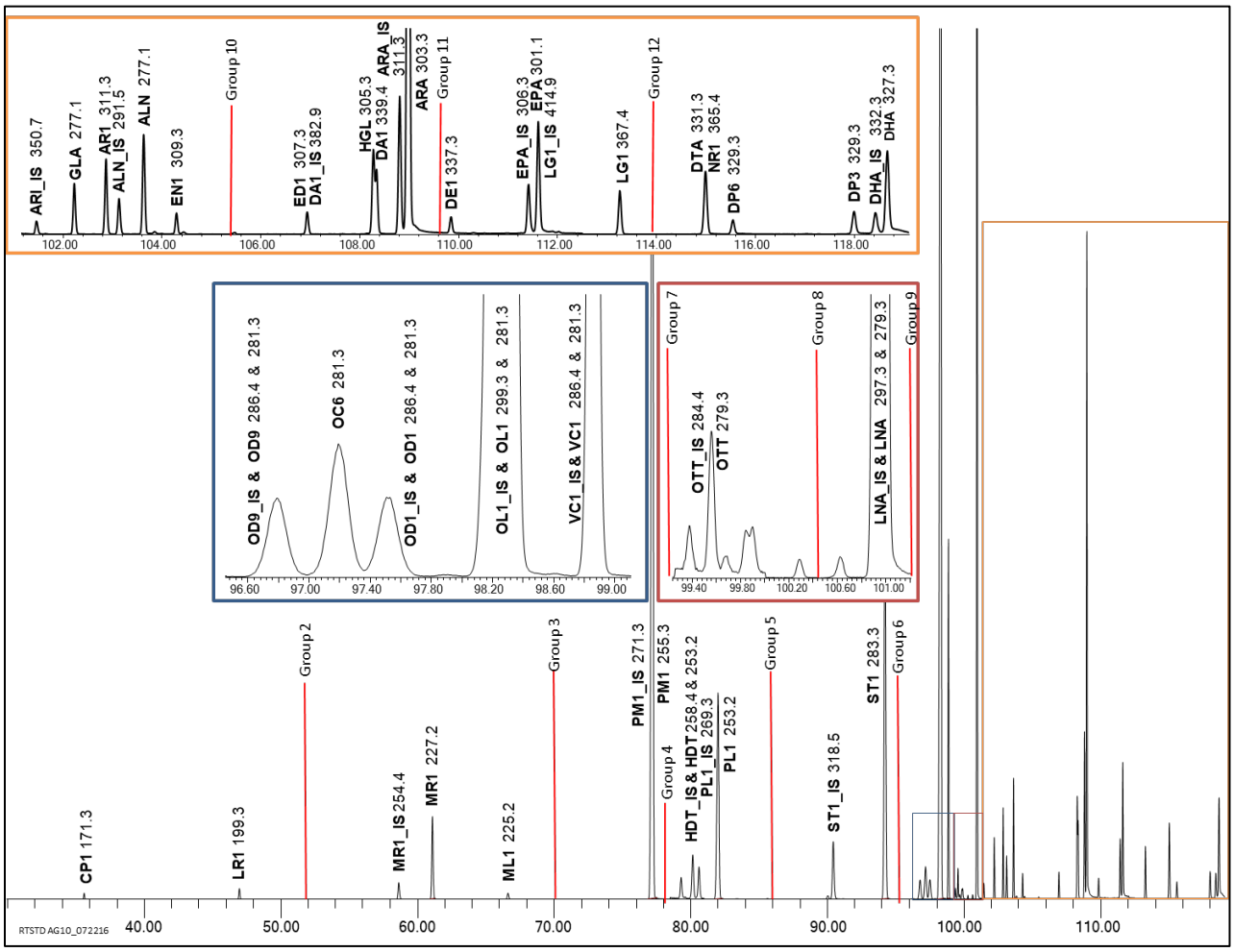
MS Source : 230 C maximum 300 C
MS Quad : 150 C maximum 200 C

Appendix 7. Retention Time Standard And Low QC Sample Chromatograms

Retention Time Standard Chromatogram (Total Iron Chromatogram)



Low QC Chromatogram
(Total Iron Chromatogram)



Appendix 8. List of Compounds Tested for Interference

Number	Analyte	Shorthand	m/z
1	2-hydroxy-decanoic acid		187.3
2	undecanoic acid	C11:0	185.9
3	dodecanoic acid	C12:0	199.3
4	tridecanoic acid	C13:0	213.4
5	12-tridecenoic acid	C13:1n-1	211.4
6	12-methyl-tetradecanoic acid		241.4
7	13-methyl-tetradecanoic acid		241.4
8	pentadecanoic acid	C15:0	241.4
9	trans-10-pentadecenoic acid	C15:1n-5t	239.4
10	14-methyl-pentadecanoic acid		255.4
11	cis-10-pentadecenoic acid	C15:1n-5c	239.4
12	14-pentadecenoic acid	C15:1n-1	239.4
13	9R,10S-methylene-hexadecanoic acid		267.4
14	15-methyl-hexadecanoic acid		269.5
15	2-hydroxy-hexadecanoic acid		271.4
16	heptadecanoic acid	C17:0	269.5
17	trans-10-heptadecenoic acid	C17:1n-7t	267.5
18	cis-9, cis-12-hexadecadienoic acid	C16:2n-4c,7c	251.4
19	cis-10-heptadecenoic acid	C17:1n-7c	267.5
20	9S,10R-methylene-octadecanoic acid		295.5
21	trans-6-octadecenoic acid	C18:1n-12t	281.5
22	2-hydroxy-dodecanoic acid		215.3
23	3-hydroxy-dodecanoic acid		215.3
24	cis-6, cis-9, cis-12, 15-hexadecatetraenoic acid	C16:4n-1,4c,7c,10c	247.4
25	nonadecanoic acid	C19:0	297.5
26	cis-9, trans-12-octadecadienoic acid	C18:2n-6t,9c	279.5
27	cis-11, cis-14-octadecadienoic acid	C18:2n-4c,7c	279.5
28	trans-9, cis-12-octadecadienoic acid	C18:2n-6c,9t	279.5
29	trans-7-nonadecenoic acid	C19:1n-12t	295.5
30	trans-10-nonadecenoic acid	C19:1n-9t	295.5
31	2-hydroxy-tetradecanoic acid		243.4
32	cis-7-nonadecenoic acid	C19:1n-12c	295.5
33	trans-9, trans-12, trans-15-octadecatrienoic acid	C18:3n-3t,6t,9t	277.5
34	cis-10-nonadecenoic acid	C19:1n-9c	295.5
35	cis-9, trans-12, trans-15-octadecatrienoic acid	C18:3n-3t,6t,9c	277.5
36	trans-9, cis-12, trans-15-octadecatrienoic acid	C18:3n-3t,6c,9t	277.5
37	trans-9, trans-12, cis-15-octadecatrienoic acid	C18:3n-3c,6t,9t	277.5
38	cis-9, cis-12, trans-15-octadecatrienoic acid	C18:3n-3t,6c,9c	277.5
39	cis-9, trans-12, cis-15-octadecatrienoic acid	C18:3n-3c,6t,9c	277.5
40	cis-9, cis-11, cis-14-octadecatrienoic acid	C18:3n-4c,7c,9c	277.5
41	trans-9, cis-12, cis-15-octadecatrienoic acid	C18:3n-3c,6c,9t	277.5
42	cis-5-eicosenoic acid	C20:1n-15c	309.5
43	cis-10, cis-13-nonadecadienoic acid	C19:2n-6c,9c	293.5
44	trans-11-eicosenoic acid	C20:1n-9t	309.5
45	cis-8-eicosenoic acid	C20:1n-12c	309.5
46	cis-6, cis-9, cis-12, cis-15-octadecatetraenoic acid	C18:4n-3c,6c,9c,12c	275.5
47	3-hydroxy-tetradecanoic acid		243.4
48	12-hydroxy-cis-9-octadecenoic acid		297.5
49	12-hydroxy-trans-9-octadecenoic acid		297.5
50	cis-12-heneicosenoic acid	C21:1n-9c	323.6
51	cis-11, cis-14, cis-17-eicosatrienoic acid	C20:3n-3c,6c,9c	305.5
52	trans-13-docosenoic acid	C22:1n-9t	337.6
53	cis-12, cis-15-heneicosadienoic acid	C21:2n-6c,9c	321.6
54	cis-11-docosenoic acid	C22:1n-11	337.6
55	cis-13-docosenoic acid	C22:1n-9c	337.4
56	tricosanoic acid	C23:0	353.6
57	cis-13, cis-16-docosadienoic acid	C22:2n-6c,9c	335.6
58	cis-14-tricosenoic acid	C23:1n-9c	351.6
59	cis-13, cis-16, cis-19-docosatrienoic acid	C22:3n-3c,6c,9c	333.6
60	pentacosanoic acid	C25:0	381.7
61	hexacosanoic acid	C26:0	395.7
62	octacosanoic acid	C28:0	423.7
63	nonacosanoic acid	C29:0	437.8

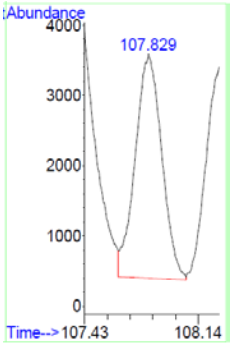
Appendix 9. Integration guidelines

Consistent and accurate integration of the fatty acid and internal standard peaks is a critical component of this method. Guidance is provided for peaks where manual integration may be needed to ensure accuracy and reliability of test results.

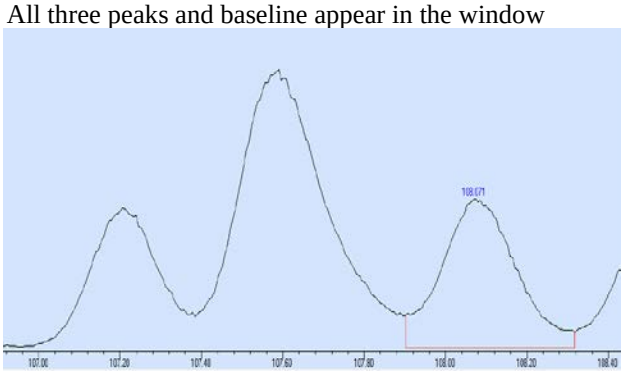
The figures below are example Total Iron Chromatogram (TIC) signals illustrating some integration considerations for this method.

Two or more peaks are not baseline resolved
Isomer peaks may not always be baseline resolved, due to the large number of positional and geometric fatty acid isomers present in plasma and serum. In cases where the peaks are not baseline resolved, integration should be from valley to valley using a straight line down from the bottom of each valley to the baseline.
Elaidic acid, *trans*-vaccenic acid, and vaccenic acid peaks should appear in the same integration window. The baseline for all three should to be lowered to the lowest signal in the window. The lowest signal is usually found directly in front of elaidic acid. The baseline can be pulled straight across the integration window from this point.

Window too narrow

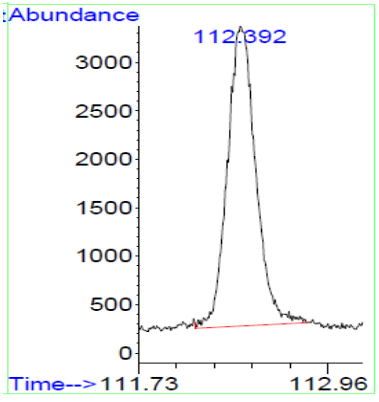


All three peaks and baseline appear in the window

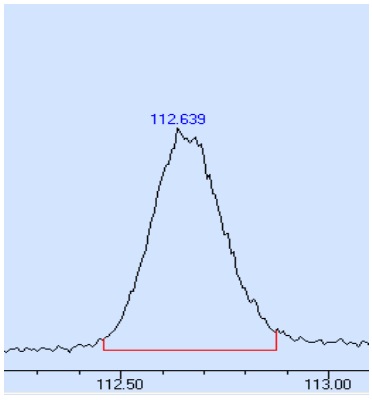


Linoelaidic acid (OTT) is in a crowded area of the chromatogram. Bracketing the peak as closely as possible, including very little of either tail is recommended, unless there is no evidence of co-eluting substances. Baseline needs to be level and set to lowest signal in the peak area.

Incorrect



Correct

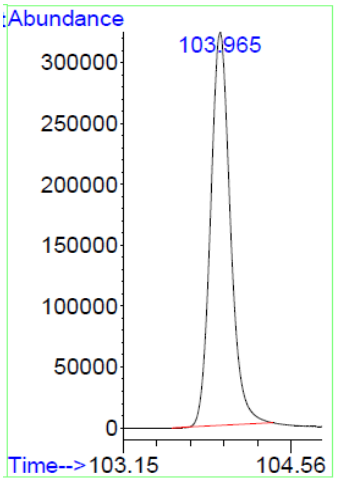


Level baseline

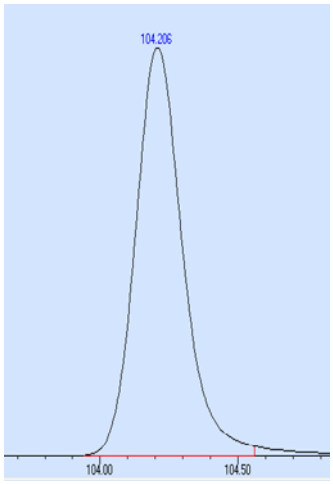
Baselines should be level and set to the lowest signal in the peak area

Example: Stearic acid (ST1)

Incorrect



Correct

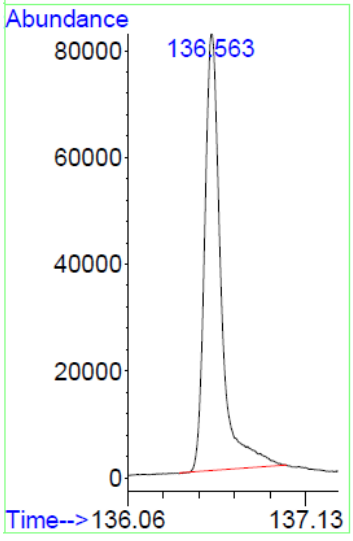


Consistent integration of tailing

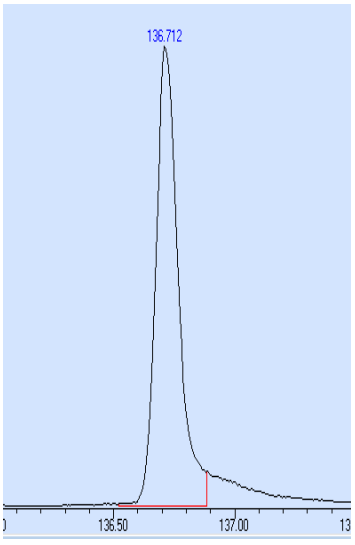
Tailing is common with late eluting peaks such as DHA since they remain on the column for an extended period. Peaks should be integrated with a flat baseline (not angled) and a consistent end time.

Example: Docosahexaenoic acid (DHA)

Incorrect



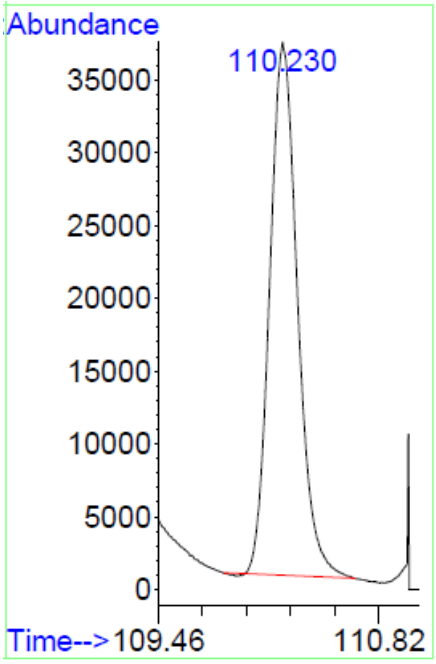
Correct



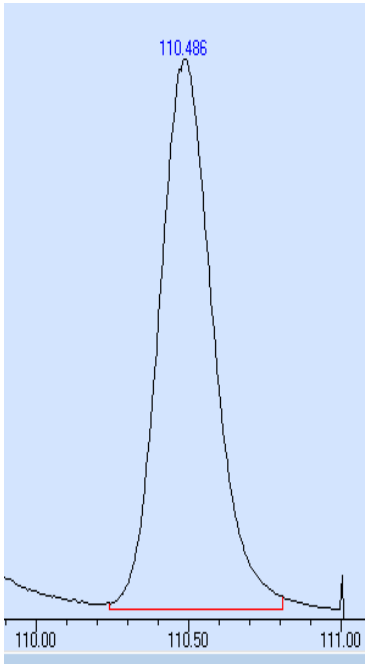
Negative peaks

Integration software occasionally integrates areas outside the peak (negative peak integration). Manual integration to remedy this error is recommended.

Incorrect



Correct



Appendix 10. Symbols, Abbreviations, Terminology

Abbreviations

ACS American Chemical Society
ASTM American Society for Testing and Material
BP Boiling Point
CDC Centers for Disease Control and Prevention
CC Calibrators
CI Chemical Ionization
CLIA Clinical Laboratory Improvement Act/Amendment
CLSI Clinical and Laboratory Standards Institute
CV Coefficient of Variation
EMV Electron Multiplier Voltage
EDTA Ethylenediaminetetraacetic Acid
FDA Food and Drug Administration
GC-MS Gas Chromatography-Mass Spectrometry
HCl Hydrochloric Acid
HIV Human Immunodeficiency Virus
HPLC High Performance Liquid Chromatography
ID Identification
IS Internal Standards
ISO International Organization for Standardization
LDL Low-density Lipoprotein
MSD Mass Selective Detector
MSDS Material Safety Data Sheets
NaOH Sodium Hydroxide
NCEH National Center of Environmental Health
NCEP National Cholesterol Education Program
OHS Occupational Health and Safety
PFB-Br Pentafluorobenzyl Bromide
PT Proficiency Testing/External Quality Assurance Testing
QA Quality Assurance
QC Quality Control
SAS Statistical Analysis Software
SD Standard Deviation
SIM Single Ion Monitoring
SAS Statistical Analysis System
TFA *trans*-fatty acid

Terminology

The terminology defined in CLIA ’88 (57 FR 7139 Subpart A Sec Sec. 493.2) is used in this document. Otherwise, the terminology described in the Clinical and Laboratory Standards Institute’s terminology database was used. The database can be accessed at: (http://www.clsi.org/Content/NavigationMenu/Resources/HarmonizedTerminologyDatabase/Harmonized_Terminolo.htm)