Laboratory Procedure Manual

Analytes: ***Profile of 30 fatty acids - Arachidic, Arachidonic, Capric, Docosahexaenoic, Docosanoic, Docosapentaenoic (n3), Docosapentaenoic (n6), Docosatetraenoic, Eicosadienoic, Eicosapentaenoic, Eicosatrienoic, Eicosenoic, Lauric, Lignoceric, Linoleic, alpha-Linolenic, gamma-Linolenic, homo-gamma-Linolenic, Margaric, Myristic, Myristoleic, Nervonic, Oleic, Palmitic, Palmitoleic, Pentadecanoic, Stearic, Stearidonic, Tricosanoic, and cis-Vaccenic***

Matrix: **Plasma or Serum**

Method: **Gas Chromatography – Mass Spectrometry**

Method No: **4028.05**

Revised:

as performed by:

Fat-soluble Nutrients Laboratory
Nutritional Biomarkers Branch (NBB)
Division of Laboratory Sciences (DLS)
National Center for Environmental Health (NCEH)

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Lab Number	Analyte	SAS Label (and SI units)
FAS_H	LBXCAP	Capric acid (C10:0) (μmol/L)
	LBXLAR	Lauric acid (C12:0) (μmol/L)
	LBXMR1	Myristic acid (14:0) (μmol/L)
	LBXPEN	Pentadecanoic acid (C15:0) (μmol/L)
	LBXPM1	Palmitic acid (16:0) (μmol/L)
	LBXMGR	Margaric acid (C17:0) (μmol/L)
	LBXST1	Stearic acid (18:0) (μmol/L)
	LBXAR1	Arachidic acid (20:0) (μmol/L)
	LBXDA1	Docosanoic acid (22:0) (μmol/L)
	LBXTSA	Tricosanoic acid (C23:0) (μmol/L)
	LBXLG1	Lignoceric acid (24:0) (μmol/L)
	LBXML1	Myristoleic acid (14:1n-5) (μmol/L)
	LBXPL1	Palmitoleic acid (16:1n-7) (μmol/L)
	LBXVC1	cis-Vaccenic acid (18:1n-7) (μmol/L)
	LBXOL1	Oleic acid (18:1n-9) (μmol/L)
	LBXEN1	Eicosenoic acid (20:1n-9) (μmol/L)
	LBXNR1	Nervonic acid (24:1n-9) (μmol/L)
	LBXLNA	Linoleic acid (18:2n-6) (μmol/L)
	LBXALN	alpha-Linolenic acid (18:3n-3) (μmol/L)
	LBXGLA	gamma-Linolenic acid (18:3n-6) (μmol/L)
	LBXSD1	Stearidonic acid (C18:4n-3) (μmol/L)
	LBXED1	Eicosadienoic acid (20:2n-6) (μmol/L)
	LBXHGL	homo-gamma-Linolenic acid (20:3n-6) (μmol/L)
	LBXET1	Eicosatrienoic acid (C20:3n-9) (μmol/L)
	LBXARA	Arachidonic acid (20:4n-6) (μmol/L)
	LBXEPA	Eicosapentaenoic acid (20:5n-3) (μmol/L)
	LBXDTA	Docosatetraenoic acid (22:4n-6) (μmol/L)
	LBXDP3	Docosapentaenoic acid (22:5n-3) (μmol/L)
	LBXDP6	Docosapentaenoic acid (22:5n-6) (μmol/L)
	LBXDHA	Docosahexaenoic acid (22:6n-3) (μmol/L)

1. Summary of Test Principle and Clinical Relevance

A. Clinical Relevance

The analysis of individual plasma or serum fatty acids is important in the recognition of essential fatty acid deficiency (2) and in the differential diagnosis of inborn errors of metabolism, such as mitochondrial fatty acid oxidation disorders (3,4,5). Long-chain polyunsaturated fatty acids are essential for normal development (6). The dietary content of saturated, monounsaturated, and polyunsaturated fatty acids influence the concentration of cholesterol in low-density and high-density lipoproteins, and consequently the development of atherosclerosis (7). Regular consumption of or supplementation with omega-3 polyunsaturated fatty acids can have beneficial effects on long-term cardiovascular health due to anti-inflammatory and possibly antiarrhythmic effects (8). The goal of this method is to obtain US reference ranges for most circulating fatty acids. Public health recommendations advise increasing or decreasing the intake of various classes of fatty acids (saturated, monounsaturated, polyunsaturated) but relatively little fatty acid biomarker data exist to support these recommendations and reference range data are scarce.

B. Test Principle

Esterified fatty acids are hydrolyzed primarily from triglycerides, phospholipids and cholesteryl esters using sequential treatment with mineral acid and base in the presence of heat. Using a modification of (1), total fatty acids are hexane-extracted from the matrix (100uL serum or plasma) along with an internal standard solution containing eighteen stable isotopically-labeled fatty acids to account for recovery. The extract is derivatized with pentafluorobenzyl bromide (PFBBr) in the presence of triethylamine to form pentafluorobenzyl esters. The reaction mixture is injected onto a capillary gas chromatograph column to resolve individual fatty acids of interest from other matrix constituents. Fatty acids are detected using electron capture negative-ion mass spectrometry within 34 minutes. Eleven saturated, six monounsaturated, and thirteen polyunsaturated fatty acids (thirty fatty acids in total) are measured using selected ion monitoring. Quantitation is accomplished by comparing the peak area of the analyte in the unknown with the peak area of a known amount in a calibrator solution. Calculations are corrected based on the peak area of the internal standard in the unknown compared with the peak area of the internal standard in the calibrator solution.

Saturated	Fatty acids	Database Analyte Code	Carbon: Double bonds
1	Capric acid	CAP	C10:0
2	Lauric acid	LAR	C12:0
3	Myristic acid	MR1	C14:0
4	Pentadecanoic acid	PDE	C15:0
5	Palmitic acid	PM1	C16:0
6	Margaric acid	MRG	C17:0
7	Stearic acid	ST1	C18:0
8	Arachidic acid	AR1	C20:0
9	Docosanoic acid	DA1	C22:0
10	Tricosanoic acid	TSA	C23:0
11	Tetracosanoic acid	LG1	C24:0

Monounsaturated			
1	Myristoleic acid	ML1	C14:1n-5
2	Palmitoleic acid	PL1	C16:1n-7
3	*cis*-Vaccenic acid	VC1	C18:1n-7
4	Oleic acid	OL1	C18:1n-9
5	Eicosenoic acid	EN1	C20:1n-9
6	Nervonic acid	NR1	C24:1n-9
Polyunsaturated			
1	Linoleic acid	LNA	C18:2n-6
2	*alpha*-Linolenic acid	ALN	C18:3n-3
3	*gamma*-Linolenic acid	GLA	C18:3n-6
4	Stearidonic acid	SD1	C18:4n-3
5	Eicosadienoic acid	ED1	C20:2n-6
6	*homo-gamma*-Linolenic acid	HGL	C20:3n-6
7	Eicosatrienoic acid	ET1	C20:3n-9
8	Arachidonic acid	ARA	C20:4n-6
9	Eicosapentaenoic acid	EPA	C20:5n-3
10	Docosatetraenoic acid	DTA	C22:4n-6
11	Docosapentaenoic acid	DP3	C22:5n-3
12	Docosapentaenoic acid	DP6	C22:5n-6
13	Docosahexaenoic acid	DHA	C22:6n-3

2. Safety Precautions

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Place disposable plastic, glass, and paper (pipette tips, autosampler vials, gloves, etc.) that contact plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution prepared fresh daily or All Safe™ when work is finished.

Handle acids and bases with extreme care; they are corrosive or caustic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood. The derivitizating agent, pentafluorobenzyl bromide (PFBBr), is a combustible liquid and vapor. PFBBr can cause respiratory tract, eye, and skin burns, use extreme care when handling and change gloves after handling.

Organic solvents, such as methanol and acetonitrile, containing either acid or base are heated to ~104°C for complete hydrolysis. This step should be done in an oven designed for volatile organic solvents.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDS) for these chemicals are readily accessible as hard copies in the lab. If needed, MSDS for other chemicals can be viewed at http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html

3. Computerization; Data System Management

A. During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.
B. The raw data files from the mass spectrometer are collected using the instrument software and stored on the instrument workstation. The raw data files are reviewed on the instrument workstation and results files are created. Results are typically generated by auto-integration, but may require in some cases manual integration. The data file folders containing the results files are transferred to the CDC network. The results file (including analyte and internal standard names, peak areas, retention times, sample dilution factor, data file name, acquisition time, etc.) is imported into a LIMS database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See **4028_SOP Computerization & Data Management** for a step-by-step description of data transfer, review and approval.
C. Files stored on the CDC network are automatically backed up nightly by ITSO support staff.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

A. For best results, a fasting sample should be obtained.
B. Specimens for fatty acids analysis may be fresh or frozen plasma or serum.
C. A 0.5-mL sample of plasma or serum is required to allow for repeat analyses; a volume of 100uL is required per analysis.
D. The appropriate amount of plasma or serum is dispensed into a Nalge 2.0-mL cryovial or other plastic screw-capped vial labeled with the specimen ID.
E. Specimens collected in the field are frozen and then shipped on dry ice by overnight carrier. Frozen samples are stored at -70^{o}C.
F. Specimens should arrive frozen. Specimens stored long term ≤ -20^{o}C are rejected (≥ 6months).
G. Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The protocol discusses in general collection and transport of specimens and the special equipment required. If there is more than one test of interest in the specimen and it needs to be divided, the appropriate amount of serum or plasma should be transferred into a Nalge cryovial labeled with a new specimen ID linked to the particpant's ID; avoid cross-contamination.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

Prepare all solutions, samples and standards with HPLC-grade solvents and reagents. Use Class A volumetric glassware in all cases. Perform all steps involving concentrated acids, bases, and organic solvents in a chemical fume hood. Though each reagent preparation specifies a total volume of reagent prepared, these directions may be scaled up or down to prepare larger or smaller quantities if desired.

(1) Acetonitrile: 6N Hydrochloric acid, (90:10, v:v)

Add 100mL of 6N HCl to 900mL of acetonitrile and mix. Store at room temperature. Prepare fresh when crystals appear.

CAUTION!!! HCl is corrosive. Wear acid-resistant gloves, safety glasses (face shields are available if desired), lab coat and/or apron.

(2) Methanol: 10N Sodium Hydroxide, (90:10, v:v)

Add 100mL of 10N sodium hydroxide to 900mL with methanol and mix. Store at room temperature. Prepare fresh when crystals appear.

CAUTION!!! NaOH is caustic. Wear base-resistant gloves, safety glasses (face shields are available if desired), lab coat and/or apron.

(3) Derivitizing Solution – Prepare fresh daily a 7% pentafluorobenzyl bromide and 10% triethylamine in acetonitrile solution as shown in the table below.

# samples	Pentafluorobenzyl Bromide (PFBBr)	Acetonitrile (ACN)	Triethylamine (TEA)
< 20	140 uL	1.86 mL	200 uL
< 30	210 uL	2.79 mL	300 uL
< 40	280 uL	3.72 mL	400 uL
< 50	350 uL	4.65 mL	500 uL
< 60	420 uL	5.58 mL	600 uL
< 70	490 uL	6.51 mL	700 uL
< 80	560 uL	7.44 mL	800 uL
< 90	630 uL	8.37 mL	900 uL
< 100	700 uL	9.30 mL	1 mL

CAUTION!!! PFBBr is a combustible liquid. Wear chemical-resistant gloves, safety glasses (face shields are available if desired), lab coat and\or apron. Remove gloves, wash hands, and replace with new gloves after handling/pipetting PFBBr.

B. Standards Preparation

(1) Individual stock standard solutions are prepared in triplicate: 5 fatty acids that were included in the calibration solutions were not reportable because none of the QC has concentrations that were above the limit of detection (LOD).

	Fatty Acid Name	Analyte Code	FW	Target Stock Conc (mM)	Target Amount in 5.0 mL Toluene (g)
1	*alpha*-Linolenic	ALN	278.48	50	0.07
2	Arachidic	AR1	312.54	25	0.039
3	Arachidonic	ARA	304.52	250	0.381
4	Capric	CAP	172.26	10	0.017*
5	Caprylic	CL1	144.21	50	0.036
6	Docosanoic	DA1	340.59	25	0.043
7	Docosadienoic	DD1	336.55	10	0.017
8	Docosenoic	DE1	338.59	25	0.042
9	Docosahexaenoic	DHA	328.57	75	0.123
10	Docosapentaenoic n-3	DP3	330.57	25	0.041
11	Docosapentaenoic n-6	DP6	330.57	25	0.041
12	Docosatrienoic	DT1	334.5	10	0.017
13	Docosatetraenoic	DTA	332.57	75	0.125
14	Eicosadienoic	ED1	308.53	25	0.039
15	Eicosenoic	EN1	310.54	25	0.039
16	Eicosapentaenoic	EPA	302.52	50	0.076
17	*gamma*-Linolenic	GLA	278.48	75	0.104
18	*homo-gamma*-Linolenic	HGL	306.53	125	0.192
19	Hexacosanoic	HSA	396.7	10	0.02
20	Lauric	LAR	200.32	50	0.05
21	Tetracosanoic	LG1	368.64	15	0.028
22	Linoleic	LNA	280.48	500	0.701
23	Myristoleic	ML1	226.38	75	0.085
24	Myristic	MR1	228.38	100	0.114
25	Margaric	MRG	270.45	10	0.014
26	Nervonic	NR1	366.63	50	0.092
27	Oleic	OL1	282.48	500	0.706
28	Pentadecanoic	PDE	242.4	10	0.012
29	Palmitoleic	PL1	254.43	125	0.159
30	Palmitic	PM1	256.43	500	0.641
31	Stearidonic	SD1	276.4	2	0.00276
32	Stearic	ST1	300.48	150	0.225
33	Tricosanoic	TSA	354.62	10	0.018
34	*cis*-Vaccenic	VC1	282.48	100	0.141

*this stock solution prepared in 10mL instead of 5mL

(a) Weigh all materials into labeled 16- x 100-mm screw-top culture tubes.
(b) After weighing materials, calculate volume of toluene to be added based on actual amount weighed: actual weight/target weight * 5mL). Cap and mix by gentle inversion. If necessary sonicate stock solutions until analyte is in solution.
(c) Note: Eicosatrienoic acid is added directly to Intermediate Stock 1

(2) Purity Check for Individual Stock Standard Solutions – in addition to obtaining manufacturer purity information

(a) Aliquot 50uL of each individual stock standard solution to a 13 x 100mm culture tube
(b) Add 100uL of Derivitizing solution (7% pentafluorobenzyl bromide and 10% triethylamine in acetonitrile solution; preparation shown in section 6.a.3 Reagent preparation)
(c) Wait 15 minutes for derivitization to occur
(d) Add 1mL hexane to each tube and mix
(e) Transfer hexane to labeled GCMS vial and run in SIM and Scan modes on GCMS
(f) After analysis and data review, for each stock standard solution sum peak areas of all known peaks. Divide peak area (of analyte of interest) by the sum of the peak areas (within analyte of interest chromatogram) to obtain percent purity. Percent purity should be taken to account for all analytes when assigning calibration values.

(3) Composition of Intermediate Stock 1 (prepared in 250-mL volumetric cylinder)

	Fatty Acid Name	Analyte Code	Target Stock Conc (mM)	Set used in preparation of Intermediate Stock 1	mL to be added to prepare Intermediate Stock 1
1	Arachidic	AR1	25	A	2.5
2	Capric (decanoic)	CAP	10	A	3.125
3	Caprylic (octanoic)	CL1	50	A	1.25
4	13, 16 Docosadienoic	DD1	10	A	3.125
5	Docosenoic	DE1	25	A	1.25
6	Docosapentaenoic n6	DP6	25	A	3.75
7	13, 16, 19 Docosatrienoic	DT1	10	A	3.125
8	Docosatetraenoic	DTA	75	A	1.25
9	11-14, Eicosadienoic	ED1	25	A	2.5
10	*gamma* Linolenic	GLA	75	A	2.5
11	*homo-gamma* Linolenic	HGL	125	A	2.5
12	Hexacosanoic (cerotic)	HSA	10	A	3.125
13	Lauric (dodecanoic)	LAR	50	A	3.75
14	Myristoleic	ML1	75	A	1.25
15	Nervonic	NR1	50	A	2.5
16	11-Eicosenoic	EN1	25	C	2.5
Mix 4.5 mL each set into a 20mL scintillation vial, then take specified volume from the mixture to prepare Intermediate Stock 1					
17	*alpha* Linolenic	ALN	50	B	7.5
			50	C	
18	Arachidonic	ARA	250	B	6.25
			250	C	
19	Docosanoic	DA1	25	B	5
			25	C	
20	Docosahexaenoic	DHA	75	B	6.25
			75	C	
21	Docosapentaenoic n3	DP3	25	B	6.25
			25	C	
22	Myristic	MR1	100	B	6.25
			100	C	
23	Pentadecanoic	PDE	10	B	6.25
			10	C	
24	Tricosanoic	TSA	10	B	6.25
			10	C	
25	*cis*-Vaccenic	VC1	100	B	5
			100	C	

Equal volumes from each set was added to the volumetric flask					
	Fatty Acid Name	**Analyte Code**	**Target Stock Conc (mM)**	**Set used in preparation of Intermediate Stock 1**	**mL to be added to prepare Intermediate Stock 1**
26	Tetracosanoic (lignoceric)**	LG1	15	B	6.25
			15	C	
27	Eicosapentaenoic	EPA	50	A	9
			50	B	
			50	C	
28	Linoleic	LNA	500	A	12.5
			500	B	
			500	C	
29	Margaric (heptadecanoic)	MRG	10	A	9.375
			10	B	
			10	C	
30	Oleic	OL1	500	A	12.5
			500	B	
			500	C	
31	Palmitoleic	PL1	125	A	12.5
			125	B	
			125	C	
32	Palmitic	PM1	500	A	12.5
			500	B	
			500	C	
33	Stearic	ST1	150	A	12.5
			150	B	
			150	C	
34	Stearidonic	SD1	2	A	15.648
			2	B	
			2	C	
35	5Z, 8Z, 11Z-Eicosatrienoic acid	ET1	10mg/mL Cayman stock	**direct from Cayman stock materials**	1.92

(4) Standards 2-6 and Intermediate Stock 2 solution are prepared from the Intermediate Stock 1 solution as follows:
   (a) Standard 6 (Std6): Add 100 mL of Intermediate Stock 1 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.
   (b) Standard 5 (Std5): Add 60 mL of Intermediate Stock 1 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.
   (c) Standard 4 (Std4): Add 30 mL of Intermediate Stock 1 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.
   (d) Intermediate Stock 2 solution: Add 20 mL of Intermediate Stock 1 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.
   (e) Standard 3 (Std3): Add 125 mL of Intermediate Stock 2 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.
   (f) Standard 2 (Std2): Add 12.5 mL of Intermediate Stock 2 to a 250-mL volumetric flask, then fill to the mark with Toluene. Mix by Inversion.

(5) The blank (Std0) is prepared by adding Toluene directly to labeled amber GC vials containing fused glass inserts.

aliquot 225uL final standard solutions into appropriately labeled amber calibrator vials

Table of Target Concentrations for Calibrators

	Fatty Acid	Code	Std6 (uM)	Std5 (uM)	Std4 (uM)	Std3 (uM)	Std2 (uM)
1	*alpha*-Linolenic	ALN	600	360	180	60	6
2	Arachidic	AR1	100	60	30	10	1
3	Arachidonic	ARA	2500	1500	750	250	25
4	Capric	CAP	50	30	15	5	0.5
5	Caprylic	CL1	100	60	30	10	1
6	Docosanoic	DA1	200	120	60	20	2
7	13,16- Docosadienoic	DD1	50	30	15	5	0.5
8	Docosenoic	DE1	50	30	15	5	0.5
9	Docosahexaenoic	DHA	750	450	225	75	7.5
10	Docosapentaenoic n-3	DP3	250	150	75	25	2.5
11	Docosapentaenoic n-6	DP6	150	90	45	15	1.5
12	13,16,19-Docosatrienoic	DT1	50	30	15	5	0.5
13	Docosatetraenoic	DTA	150	90	45	15	1.5
14	11,14-Eicosadienoic	ED1	100	60	30	10	1
15	11-Eicosenoic	EN1	100	60	30	10	1
16	Eicosapentaenoic	EPA	720	432	216	72	7.2
17	5Z, 8Z, 11Z-Eicosatrienoic acid	ET1	100	60	30	10	1
18	*gamma*-Linolenic	GLA	300	180	90	30	3
19	*homo-gamma*-Linolenic	HGL	500	300	150	50	5
20	Hexacosanoic	HSA	50	30	15	5	0.5
21	Lauric	LAR	300	180	90	30	3
22	Tetracosanoic	LG1	150	90	45	15	1.5
23	Linoleic	LNA	10000	6000	3000	1000	100
24	Myristoleic	ML1	150	90	45	15	1.5
25	Myristic	MR1	1000	600	300	100	10
26	Margaric	MRG	150	90	45	15	1.5
27	Nervonic	NR1	200	120	60	20	2
28	Oleic	OL1	10000	6000	3000	1000	100
29	Pentadecanoic	PDE	100	60	30	10	1
30	Palmitoleic	PL1	2500	1500	750	250	25
31	Palmitic	PM1	10000	6000	3000	1000	100
32	Stearidonic	SD1	50	30	15	5	0.5
33	Stearic	ST1	3000	1800	900	300	30
34	Tricosanoic	TSA	100	60	30	10	1
35	*cis*-Vaccenic	VC1	800	480	240	80	8

(6) Internal standard solution – individual stock solutions are prepared so that each will yield the following final concentrations in the final solution. If the deuterated form cannot be obtained, then the 13C form will also work just be sure to use the correct FW when calculating the amount needed to yield the target concentration and for the instrument method. The final concentration of the mixed internal standard solution should be calculated (including correction for purity).

	Internal Standard Acid Name	Label	Analyte Code	Target (uM)
1	Arachidic	d-39	AR1_IS	25
2	Capric	d-3	CAP_IS	15
3	Caprylic	d-3	CL1_IS	35
4	Docosanoic	d-4	DA1_IS	60
5	Docosahexaenoic	d-5	DHA_IS	125
6	Eicosapentaenoic	d-5	EPA_IS	40
7	Lauric	d-3	LAR_IS	50
8	Lignoceric	d-4	LG1_IS	50
9	Linoleic	13C	LNA_IS	3500
10	Myristic	d-27	MR1_IS	100
11	Margaric	d-3	MRG_IS	15
12	Oleic	13C	OL1_IS	2100
13	Pentadecanoic	d-3	PDE_IS	20
14	Palmitic	d-31	PM1_IS	2700
15	Stearic	d-35	ST1_IS	700
16	*alpha*-Linolenic	d-14	ALN _IS	65
17	Arachidonic	d-8	ARA_IS	800
18	Palmitoleic	d-14	PL1_IS	200

C. Preparation of Quality Control Materials

Normal serum or plasma (650uL) containing 3.33g BHT in methanol/L serum is aliquotted into 2.0-mL Nalgene cryovials, capped, and frozen. The QC pools are stored at -70°C.

Means plus range limits for all pools are established by analyzing duplicates for at least 20 runs. QC pools are prepared by analyzing numerous serum or plasma samples and selecting and blending the ones which best match the following criteria: if possible, each level should fall within lower 1/3, middle 1/3 and upper 1/3 of the US population reference range values for key fatty acids (Palmitic, Oleic, Linoleic, Stearic and Arachidonic).

D. Other Materials

With some exceptions, a material listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed. In the case of standards, internal standards, chemicals and reagents, the chemical and/or isotopic purity of the substitute must meet or exceed that of the listed product. In the case of the GC column, equivalent performance must be demonstrated experimentally in accordance with DLS policies and procedures.

(1) General Supplies

(a) Pyrex Screw caps with teflon liners 415\15 (Corning, Inc., Corning, NY)
(b) 16- x 100-mm Pyrex Disposable screw caps culture tubes (Corning, Inc.)
(c) 13- x 100-mm Pyrex culture tubes (Corning, Inc.)
(d) 13- x 100-mm Pyrex Disposable screw cap culture tubes (Corning, Inc.)
(e) Pyrex Screw caps with teflon liners 415\13 (Corning, Inc., Corning, NY)
(f) 2.0-mL Polypropylene cryovials (Nalgene Company, Rochester, NY)
(g) 6” Disposable glass Pasteur pipettes (Kimble Glass, Vineland, NJ)
(h) Blue tips (50-1000 uL) for Eppendorf pipette (Brinkmann Instruments Inc., Westbury, NY)
(i) Yellow tips (2-200 uL) for Eppendorf pipette (Brinkmann Instruments Inc.)
(j) Combitip Plus (0.5 mL) for Eppendorf repeater pipette (Brinkmann Instruments Inc.)
(k) Combitip Plus (5.0 mL) for Eppendorf repeater pipette (Brinkmann Instruments Inc.)
(l) Combitip Plus (50 mL) for Eppendorf repeater pipette (Brinkmann Instruments Inc.)
(m) 10mL glass serological disposable pipettes, sterile (7077-10N; Fisher Scientific, Fair Lawn, NJ)
(n) 5mL glass serological disposable pipettes, sterile (7077-5N; Fisher Scientific, Fair Lawn, NJ)
(o) Rainin Positive Displace tips (C-1000, C-250, C-50 and C-25, Rainin)
(p) Hamilton high volume (1mL) tips without filter (cat. no. 235904, Hamilton, Reno, NV)
(q) Hamilton standard volume (300uL) tips without filter (cat. no. 235902, Hamilton, Reno, NV)
(r) 20mL Disposable glass scintillation vials with caps
(s) GC vials and caps (C4000-2W and C4000-53B; Fisher Scientific, Fair Lawn, NJ)
(t) Various glass beakers, graduated cylinders and glass bottles, class A glassware

(2) GCMS supplies

(a) Helium, ultrapure (>99.99% purity) (Air Products, Inc., Atlanta, GA)
(b) Methane, ultrapure (>99.99% purity) (Air Products, Inc., Atlanta, GA)
(c) J&W DB-23 capillary GC column, 60.0m x 0.25mm x 0.25μm (Agilent, Wilmington, DE)
(d) Thermo Trace Gold TG-POLAR GC Column, 60.0m x 0.25mm x 0.25μm (Thermo, West Palm Beach, FL)
(e) Split/splitless liner (19251-60540, Agilent, Wilmington, DE)
(f) Liner o-ring (5188-5365, Agilent, Wilmington, DE)
(g) Split gold seal disk (5182-9652, Agilent, Wilmington, DE)
(h) Silver washer (5061-5869, Agilent, Wilmington, DE)
(i) Inlet Ferrule, 0.4mm 15%/85% graphite/vespel (5181-3323, Agilent, Wilmington, DE)
(j) Column nut for GC capillaries (5181-8830, Agilent, Wilmington, DE)
(k) Ferrule, 0.4mm 15%/85% graphite/vespel (5062-3508, Agilent, Wilmington, DE)
(l) Column nut for MS interface (05988-20066, Agilent, Wilmington, DE)
(m) Lens insulator, 597X MSD (G1370-20530, Agilent, Wilmington, DE)

(n) 73CI Repeller (G1999-20432, Agilent, Wilmington, DE)
(o) CI Filament for the 5973 and 5975 MSD (G1099-80053, Agilent, Wilmington, DE)
(p) ALS Syringe, 10ul tapered, fixed needle (5181-3360, Agilent, Wilmington, DE)
(q) Advanced Green 11mm septa (5183-4759, Agilent, Wilmington, DE)
(r) 4mL Wash vial with fill markings and cap (5182-0551, Agilent, Wilmington, DE)
(s) Diffusion caps for 4 mL wash vials (07673-40180, Agilent, Wilmington, DE)
(t) Various Swagelok fittings (Agilent or equivalent, Wilmington, DE)
(u) Copper tubing cutter (Agilent or equivalent, Wilmington, DE)

(3) Chemicals
(a) Methanol, HPLC grade, # AH230-4 (Burdick & Jackson, Muskegan, MI)
(b) Acetonitrile, HPLC grade, Acros # UN1648 (Fisher Scientific, Pittsburgh, PA)
(c) Hexanes, HPLC grade, # UN1208 (Tedia Company Inc, Fairfield, OH)
(d) Toluene, # T-323-4 (Fisher Scientific, Fair Lawn, NJ)
(e) 10N Sodium Hydroxide, #SS255-1 (Fisher Scientific, Fair Lawn, NJ)
(f) Pentafluorobenzyl bromide (Pierce, Rockford, IL)
(g) 6N Hydrochloric acid, # SA56-1 (Fisher Scientific, Suwanee, GA)
(h) Triethylamine, # W639-07 (J.T. Baker, Phillipsburg, NJ)

(4) Standards
(a) *alpha*-Linolenic (Nu-Chek Prep, U-62A)
(b) Arachidic (Nu-Chek Prep, N-20A)
(c) Arachidonic (Nu-Chek Prep, U-71A)
(d) Capric (decanoic) (Nu-Chek Prep, N-10A)
(e) Caprylic (octanoic) (Nu-Chek Prep, N-8A)
(f) Docosanoic (Nu-Chek Prep, N-22A)
(g) 13,16- Docosadienoic (Nu-Chek Prep, U-81A)
(h) Docosenoic (Nu-Chek Prep, U-79A)
(i) Docosahexaenoic (Nu-Chek Prep, U-84A)
(j) Docosapentaenoic n-3 (Nu-Chek Prep, U-101)
(k) Docosapentaenoic n-6 (Nu-Chek Prep, U-102)
(l) 13,16,19-Docosatrienoic (Nu-Chek Prep, U-82A)
(m) Docosatetraenoic (Nu-Chek Prep, U-83A)
(n) 11,14-Eicosadienoic (Nu-Chek Prep, U-68A)
(o) 11-Eicosenoic (Nu-Chek Prep, U-66A)
(p) Eicosapentaenoic (Nu-Chek Prep, U-99A)
(q) *gamma*-Linolenic (Nu-Chek Prep, U-63A)
(r) *homo-gamma*-Linolenic (Nu-Chek Prep, U-69A)
(s) Hexacosanoic (cerotic) (Cayman Chemical, 13354)
(t) Lauric (dodecanoic) (Nu-Chek Prep, N-12A)
(u) Tetracosanoic (Nu-Chek Prep, N-24A)
(v) Linoleic (Nu-Chek Prep, U-59A)
(w) Myristoleic (Nu-Chek Prep, U-36A)

(x) Myristic (Nu-Chek Prep, N-14A)
(y) Margaric (heptadecanoic) (Nu-Chek Prep, N-17A)
(z) Nervonic (Nu-Chek Prep, U-88A)
(aa) Oleic (Nu-Chek Prep, U-46A)
(bb) Pentadecanoic (Nu-Chek Prep, N-15A)
(cc) Palmitoleic (Nu-Chek Prep, U-40A)
(dd) Palmitic (Nu-Chek Prep, N-16A)
(ee) Stearidonic (Cayman Chemical, 90320)
(ff) Stearic (Nu-Chek Prep, N-18A)
(gg) Tricosanoic (Nu-Chek Prep, N-23A)
(hh) *cis*-Vaccenic (Nu-Chek Prep, U-48A)
(ii) 5Z, 8Z, 11Z-Eicosatrienoic (Cayman, 90190)

(5) Isotopically Labeled Standards
(a) Arachidic, d-39 (C/D/N Isotopes, D-1617)
(b) Capric, d-3 (C/D/N Isotopes, D-4021)
(c) Caprylic, d-3 (C/D/N Isotopes, D-3992)
(d) Docosanoic, d-4 (Isosciences , custom synthesis)
(e) Docosahexaenoic, d-5 (Isosciences, custom synthesis)
(f) Eicosapentaenoic, d-5 (Isosciences, custom synthesis)
(g) Lauric, d-3 (C/D/N Isotopes, D-4027)
(h) Lignoceric, d-4 (Isosciences, custom synthesis)
(i) Linoleic, 13C (Isosciences, custom synthesis)
(j) Myristic, d-27 (Cambridge Isotopes, I1-7220D)
(k) Margaric, d-3 (C/D/N Isotopes, D-5255)
(l) Oleic, 13C (Isosciences, custom synthesis)
(m) Pentadecanoic, d-3 (C/D/N Isotopes, D-5258)
(n) Palmitic, d-31 (Cambridge Isotopes, I1-10006B)
(o) Stearic, d-35 (Cambridge Isotopes, I1-7911A)
(p) *alpha*-Linolenic, d-14 (Cayman, custom synthesis)
(q) Arachidonic, d-8 (Cayman and/or Isosciences, custom synthesis)
(r) Palmitoleic, d-14 (Cayman, custom synthesis)

E. Instrumentation

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.) a product listed herein may be substituted with equivalent product from a different manufacturer provided that it meets or exceeds the specifications of the product listed. In the case of analysis instrumentation (e.g., GC components, mass spectrometer) equivalent performance must be demonstrated experimentally in accordance with *DLS Policies and Procedures Manual* if a product substitution is made. Equivalent performance must also be demonstrated in accordance with DLS policies and procedures when multiple analysis systems are used in parallel, even if they are of the exact same type.

(1) Agilent GC-MS (Agilent, Wilmington, DE)
    (a) GC model: 7890A or 7890B
    (b) Autosampler model:7683A or 7693
    (c) Mass Spectrometer model:5975C or 5977A
    (d) Software model: Mass Hunter GCMS B.07.01
(2) Thermo Scientific FREAS Mechanical Convention Oven model 625S (Thermo, Marietta, OH)
(3) Speedvac Plus SC110 A (Savant Instrument Co., Farmingdale, NY)
(4) Mixer Type 16700 Model # M16715 (Barnstead International, Dubuque, IA)
(5) Magnetic stirrer (Baxter Scientific Products, Stone Mountain, GA)
(6) Eppendorf micropipette (Brinkmann Instruments Inc., Westbury, NY)
(7) Eppendorf repeater pipette (Brinkmann Instruments Inc., Westbury, NY)
(8) Rainin Pos-D positive displacement pipettes various sizes (Rainin)
(9) Mettler Toledo AG 104 balance (Mettler Instrument Corp., Hightstown, NJ)
(10) Hamilton Starlet 8-channel with auto-load arm (Hamilton, Reno, NV)
    (a) Various carriers (sample, reagent, and tip)

7. Calibration and Calibration Verification Procedures

A. Method Calibration

At the beginning of each run, an equilibration sample (typically the highest calibrator from current run) is run to determine that the retention times and analyte responses are within expected limits.

Five calibration standards plus a reagent blank are prepared and extracted along with the samples. The calibration curve is injected at the beginning of the analytical run. Following the completion of the analytical run, calibration data are calculated using MassHunter software (see calculation section at the end of Section 8 for analyte specific calibration curve information). Each chromatographic peak is reviewed for correct delineation of the baseline and proper retention. Fatty acid concentrations in unknown samples are calculated from their respective calibration curves. The calculated concentrations for the calibration solutions must fall within 15% of the target values, with the exception of low concentration calibrators that are approaching the LOD. All calibrators are typically prepared within a few percent (mean 3%; range -11 to 33%) of the targeted concentrations after correcting for purity, lot-to-lot correction, and calibration verification.

Calibration verification is conducted at least twice a year. For details, see **4028_SOP Calibration Verification Fatty Acids**.

This method uses toluene as the matrix for the calibrators. Matrix based calibration was tested by comparing the average slope and intercept parameters of three 10-point calibration curves prepared using stripped serum (Aalto Scientific, Ltd., Carlsbad, CA) with three 10-point toluene-based calibration curves. A ≤ 5% difference in the average calibration curve slope was observed between serum and toluene-based calibrations for 29/35 analytes. For the remaining analytes

the percent difference ranged from 6-8%. For these analytes showing > 5% difference, the differences observed (toluene vs serum) were of a similar magnitude to slope variability observed within and between individual calibration curves of a particular matrix.

Serum- or plasma-based reference materials are not available for all fatty acids. NIST, NIH and CDC are working toward an inter-laboratory quality assurance program for various fatty acids in biological matrices. There are four NIST SRM materials available with certified and reference values for the majority of the fatty acids measured in this method.

NIST SRM	No. Certified Values	No. Reference Values
SRM 1950	8	19
SRM 2378-1	12	17
SRM 2378-2	12	17
SRM 2378-3	12	17

External proficiency testing programs currently do not exist for the majority of the fatty acids analyzed in our method. An in-house proficiency testing program has been developed and is conducted at least twice a year. For details, see **4028_SOP for In-House Proficiency Testing.** For general information on the handling, analysis, review, and reporting of proficiency testing materials see **NBB_SOP Proficiency Testing Procedure.**

Results from a series of in-house ruggedness testing experiments designed to assess accuracy changes when certain experimental parameters are varied are presented in **Appendix B**.

Method comparison results are presented in **Appendix C**.

B. Instrument Calibration
Gas chromatograph-mass spectrometer
The calibration of the mass spectrometer is scheduled on an annual basis as part of a preventative maintenance program and is performed whenever the system is vented for routine maintenance.
The tuning and mass calibration of the quadrupole of the mass spectrometer is performed using PFDTD (Fluoroether E-3) and running the instrument in CI autotune. Please refer to the User's Manual and the **4028_SOP Agilent GCMS calibration** for additional details.

C. Hamilton Microlab Starlet Calibration Verification
Twice a year a Hamilton service engineer performs preventative maintenance including volume verification at 10uL and 1000uL.
A volume verification of the various steps of the method can also be performed either gravimetrically (e.g., using pre-weighed sample vessels) or photometrically (e.g., using a microplate reader and a suitable chromophore) by the user. Precision should be equal or better than that obtained using manual pipettes.

D. Pipettes (air displacement and positive displacement)
Pipettes are calibrated or calibration is verified on a semi-annual basis.

E. Balances
Balances are calibrated annually and verified as needed using calibrated weights.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries
(1) Sample ID numbers must be scanned into the computer if they are barcoded.
(2) Allow frozen plasma or serum, quality control plasma or serum, and standards to reach ambient temperature, then sonicate for 15 minutes and vortex each sample individually or as a set prior to aliquotting. Visually check each sample for unusual specimen color or debris/precipitate.
(3) Set up Excel run sheet containing sample IDs prior to starting sample preparation. This will be used later to build a sequence to run the samples and also used to keep track of any problems that may occur during the sample preparation.
(4) A typical run consists of 6 calibrators (includes a blank), 3 QC samples (first set), 38-75 patient samples, 3 QC samples (second set).

Sample Preparation Note: If necessary a combination of manual and Hamilton automated sample preparation and extraction could be done: i.e. manual sample preparation can be done then use the Hamilton Extraction method for the liquid-liquid extraction or Hamilton sample preparation can be used with the Manual Extraction method for the liquid-liquid extraction. For a detailed step-by-step description of the manual and Hamilton Microlab Starlet sample preparation see **4028_SOP Sample Preparation**.

B. Sample preparation
(1) Set-up and label one 16- x 100-mm screw cap culture tube and one 13- x 100-mm glass tube per sample. All steps are done in the fume hood except when items are in the oven.
(2) Add 100uL of serum or plasma to 16- x 100-mm screw cap culture tube.
(3) Add 100uL of internal standard mixture.
(4) Add 2mL of acetonitrile: 6N hydrochloric acid (90:10, v:v).
(5) Cap each tube tightly using Teflon-lined caps.
(6) Heat samples in oven for 45 minutes at 104^{o}C.
(7) Remove samples from oven and allow samples to cool. If noticeable evaporation has taken place in any sample, then add additional acetonitrile (without hydrochloric acid) to bring back up to the approximate volume.
(8) Add 2mL of a solution of methanol:10N sodium hydroxide (90:10, v:v).
(9) Recap tightly with the same teflon caps and heat in oven for 45 minutes at 104^{o}C.
(10) Remove samples from oven and allow samples to cool. If noticeable evaporation has taken place in any sample, then add additional methanol (without sodium hydroxide) to bring back up to the approximate volume.
(11) Re-acidify samples by addition of 350uL of 6N HCl. If Hamilton liquid-liquid extraction method will be used, then use a Pasteur pipette and tubing attached to the lab airline to blow off the vapors that are created after the addition of the acid to protect the Hamilton pipetting heads from corrosion.
(12) Proceed with either manual liquid-liquid extraction (step C) or Hamilton liquid-liquid extraction steps (step D).

C. Manual Double Liquid-Liquid Extraction
   (1) Add 3mL of hexane to samples; cap each tube tightly; mix by inversion for 20 seconds.
   (2) Allow time for layers to separate.
   (3) Using a clean glass Pasteur pipette carefully draw up the top (organic) layer and place it into a 13 x 100mm tube. Avoid the bottom (aqueous) layer.
   (4) Repeat steps 1) – 3) for double extraction. Proceed to sample preparation for GC-MS (step e.).

D. Hamilton Automated Triple Liquid-Liquid Extraction
   (1) The Hamilton Microlab Starlet can be used for a triple liquid-liquid extraction of the samples.
   (2) A brief description of the triple extraction is as follows:
      (a) 3mL of hexane added to all samples
      (b) after mixing 2mL of the hexane extract is transferred to 13 x 100mm culture tubes
      (c) 2mL of hexane added to all samples
      (d) after mixing 2mL of the hexane extract is transferred to 13 x 100mm culture tubes
      (e) 2mL of hexane added to all samples
      (f) after mixing 2mL of the hexane extract is transferred to 13 x 100mm culture tubes

E. Sample Preparation for GC-MS
   (1) Dry down samples in the Speedvac (@ 45°C). This takes approximately 45 minutes to an hour and should not be stopped as long there is a liquid residue. Restart if liquid residue remains.
   (2) Prepare fresh daily a 7% pentafluorobenzyl bromide and 10% triethylamine in acetonitrile solution (as shown in the table in section 6.a section 3). Add 100uL of the derivitizing solution to each tube.
   (3) Allow the solution to react for 15 minutes at room temperature.
   (4) Reconstitute residue with 1.0 mL of hexane.
   (5) Transfer the reconstituted sample (avoiding the bottom) using a clean Pasteur pipette to a labeled GC-MS autosampler vial containing a glass insert, then immediately cap, and place on autosampler tray for injection.

F. GCMS Instrument Preparation
   An Agilent GCMS system is used to quantitate dietary fatty acids in extracted serum or plasma.
   (1) GC preparation
      (a) Septum should be changed prior to each run
      (b) Typically the liner should be changed every 2-3 runs
      (c) Fill toluene and hexane wash vials; rinsing well with the respective solvent prior to filling
      (d) Empty waste vials from autosampler
      (e) Verify syringe is moving freely (remove from arm to gauge stickiness; replace with new syringe, if sticky)
      (f) Load autosampler vials into appropriate positions according to sequence.
   (2) Typical Instrument Method (oven ramps, inlet temperatures, and split ratio are adjusted as needed)
      (a) Oven: 230°C for 0 min, then 5°C/min to 234°C for 7 min; then 1°C/min to 250°C for 3 min
      (b) Front inlet: injector temperature: 240°C; initial pressure: 47.377 psi; total flow: 105 mL/min; septum purge flow: 3 mL/min; gas saver: on at 2.0 min with a gas saver flow of 20.0 mL/min; gas type: Helium; split ratio: 50:1.
      (c) MSD transfer line: initial temperature: 250°C.
      (d) Injector: solvent A and B washes (pre and post injection): 5 each at 8uL; sample washes: 3 at 8uL; sample pumps: 2; injection volume: 1.0-uL; syringe size: 10.0-uL.
      (e) 3.5 min solvent delay
      (f) EMV mode relative
      (g) CI flow rate: 40
      (h) Capillary column: TG-POLAR; maximum temperature: 275°C; nominal length: 60.0 m; nominal diameter: 0.25mm; nominal film thickness: 0.25 μm; mode: constant flow at 2mL/min
      (i) Outlet pressure: vacuum

Table of Selected Ion Monitoring (SIM) masses

	No.	Fatty Acid	Analyte code	SIM (M/Z)	Internal Standard used for quantitation
Segment 1	1	Caprylic-d3	CL1_IS	146.2	
	2	Caprylic	CL1	143.2	CL1_IS
	3	Capric-d3	CAP_IS	174.2	
	4	Capric	CAP	171.2	CAP_IS
	5	Lauric-d3	LAR_IS	202.3	
	6	Lauric	LAR	199.3	LAR_IS
Segment 2	7	Myristic-d27	MR1_IS	254.5	
	8	Myristic	MR1	227.4	MR1_IS
	9	Myristoleic	ML1	225.3	MR1_IS
	10	Pentadecanoic-d3	PDE_IS	244.3	
	11	Pentadecanoic	PDE	241.3	PDE_IS
	12	Palmitic-d31	PM1_IS	286.6	
	13	Palmitic	PM1	255.45	PM1_IS
	14	Palmitoleic-d14	PL1_IS	267.4	
	15	Palmitoleic	PL1	253.45	PL1_IS
segment 3	16	Margaric-d3	MRG_IS	272.3	
	17	Margaric	MRG	269.3	MRG_IS
	18	Stearic-d35	ST1_IS	318.6	
	19	Stearic	ST1	283.4	ST1_IS
	20	13C-Oleic	OL1_IS	299.5	
	21	Oleic	OL1	281.5	OL1_IS
	22	*cis*-Vaccenic	VC1	281.5	OL1_IS
Segment 4	23	13C- Linoleic	LNA_IS	297.5	
	24	Linoleic	LNA	279.4	LNA_IS
	25	*alpha*-linolenic-d14	ALN_IS	291.4	
	26	*gamma*-Linolenic	GLA	277.4	ALN_IS
	27	*alpha*-Linolenic	ALN	277.4	ALN_IS
	28	Arachidic-d39	AR1_IS	350.3	
	29	Stearidonic	SD1	275.1	AR1_IS
	30	Arachidic	AR1	311.4	AR1_IS
Segment 5	31	11-Eicosenoic	EN1	309.4	AR1_IS
	32	5Z, 8Z, 11Z-Eicosatrienoic	ET1	305.3	AR1_IS
	33	11,14-Eicosadienoic	ED1	307.4	AR1_IS
	34	*homo-gamma*-Linolenic	HGL	305.4	ALN_IS
	35	Arachidonic-d8	ARA_IS	311.4	
	36	Arachidonic	ARA	303.4	ARA_IS
Segment 6	37	Docosanoic-d4	DA1_IS	343.5	
	38	Docosanoic	DA1	339.4	DA1_IS
	39	Docosenoic	DE1	337.4	DA1_IS
	40	Tricosanoic	TSA	353.4	DA1_IS
	41	13,16-Docosadienoic	DD1	335.3	DA1_IS
	42	Eicosapentaenoic-d5	EPA_IS	306.4	
	43	Eicosapentaenoic	EPA	301.4	EPA_IS
Segment 7	44	Docosahexaenoic-d5	DHA_IS	332.4	
	45	Docosatetraenoic	DTA	331.4	DHA_IS
	46	Docosapentaenoic n-6	DP6	329.4	DHA_IS
	47	13, 16, 19 Docosatrienoic	DT1	333.3	DHA_IS
	48	Docosapentaenoic n-3	DP3	329.4	DHA_IS
	49	Docosahexaenoic	DHA	327.4	DHA_IS
	50	Tetracosanoic-d4	LG1_IS	371.5	
	51	Tetracosanoic	LG1	367.4	LG1_IS
	52	Nervonic	NR1	365.4	LG1_IS
	53	Hexacosanoic	HSA	395.4	LG1_IS

G. Processing and reporting a run

1) The Agilent MassHunter software is used to review/process a run. A LIMS database is used for additional levels of data review by the analyst, project lead, QA officer, and supervisor and for data reporting.
2) For a detailed step-by-step description of chromatography review, see **4028_SOP Processing and reporting a run**.
   (a) Reviewing the chromatography
      - When the run is finished acquiring the data, the data is reviewed in MassHunter Quantitative Analysis. Chromatograms for each fatty acid and stable isotope labeled standard are checked for retention times, peak shapes, peak separation, intensity and/or potential interferences.
   (b) Quantitation and integration of the completed data file
      - Generate a batch table using auto integration.
      - Review integrations and make any necessary integration corrections either using the manual or auto integration option. Auto integration is preferred over manual integration.
      - Print the results for each analyte as a PDF to allow future review and documentation (routine procedure) or print hardcopies (exception).
      - Save the results in an excel file to import into the LIMS database.
      - Import the results file into the LIMS database for further data review.
   (c) The Agilent MassHunter software is used to review/process a run. A LIMS database is used for additional levels of data review by the analyst, project lead, QA officer, and supervisor and for data reporting.

Calculations

The MassHunter software performs all calculations using linear or quadratic regression of the peak area response of the extracted calibration solutions versus their nominal concentrations. Fatty acid concentrations in unknown samples are calculated using the regression parameters. Calculations are based on the single analysis of five (Std2 – Std6) standard concentrations according to the following formula: Concentration = Response factor (amount/area ratio) x peak area ratio x multiplier (dilution factor). Note: Twice a year calculations are based on the single analysis of six standard concentrations to fulfill CDC requirements for expanded calibration for ML1 and SD1 results that are lower than Std2, but above LOD. The following table lists calibration curve type and weighting associated with each analyte:

	Fatty Acid Name	Analyte Code	Curve Type	Weighting
1	*alpha*-Linolenic	ALN	linear	$1/x^2$
2	Arachidic	AR1	linear	$1/x^2$
3	Arachidonic	ARA	linear	$1/x^2$
4	Capric	CAP	quadratic	$1/x^2$
5	Caprylic	CL1	linear	$1/x$
6	Docosanoic	DA1	linear	$1/x^2$
7	13, 16 Docosadienoic	DD1	linear	$1/x^2$
8	Docosenoic	DE1	quadratic	$1/x$
9	Docosahexaenoic	DHA	linear	$1/x^2$
10	Docosapentaenoic n-3	DP3	linear	$1/x^2$
11	Docosapentaenoic n-6	DP6	linear	$1/x^2$
12	13, 16, 19 Docosatrienoic	DT1	quadratic	$1/x^2$
13	Docosatetraenoic	DTA	quadratic	$1/x^2$
14	11-14, Eicosadienoic	ED1	quadratic	$1/x^2$
15	11-Eicosenoic	EN1	quadratic	$1/x^2$
16	Eicosapentaenoic	EPA	quadratic	$1/x^2$
17	5Z, 8Z, 11Z-Eicosatrienoic	ET1	quadratic	$1/x^2$
18	*gamma*-Linolenic	GLA	linear	$1/x^2$

19	*homo-gamma*-Linolenic	HGL	quadratic	$1/x^2$
20	Hexacosanoic	HSA	linear	$1/x^2$
21	Lauric	LAR	quadratic	$1/x^2$
22	Tetracosanoic	LG1	linear	$1/x^2$
23	Linoleic	LNA	linear	$1/x^2$
24	Myristoleic	ML1	quadratic	$1/x^2$
25	Myristic	MR1	quadratic	$1/x^2$
26	Margaric	MRG	quadratic	1/x
27	Nervonic	NR1	quadratic	$1/x^2$
28	Oleic	OL1	linear	$1/x^2$
29	Pentadecanoic	PDE	linear	$1/x^2$
30	Palmitoleic	PL1	quadratic	$1/x^2$
31	Palmitic	PM1	quadratic	$1/x^2$
32	Stearidonic	SD1	quadratic	1/x
33	Stearic	ST1	quadratic	$1/x^2$
34	Tricosanoic	TSA	linear	$1/x^2$
35	*cis*-Vaccenic	VC1	quadratic	$1/x^2$

H. System Maintenance (other than daily maintenance)

(1) Trim the column: as needed (~ every 2 months).
(2) Source cleaning: as needed (determined by running an NCI autotune to look for elevated background peaks).
(3) Replacement of the gas tanks: helium tank approximately every 3 months or when the tank pressure falls below ~500PSI and the methane tank approximately once per year or when the tank pressure falls below ~500PSI.

I. CDC Modifications

This document represents the fourth official method for the CDC lab for measuring 35 Fatty Acids.

9. **Reportable Range of Results**

The reportable range of results for each fatty acid is between the lowest and highest standards whose approximate values are shown in the table below:

	Fatty Acid	Code	Lowest Standard (uM)	Highest Standard (uM)
1	*alpha*-Linolenic	ALN	6.53	653
2	Arachidic	AR1	0.90	89.5
3	Arachidonic	ARA	23.4	2337
4	Capric	CAP	0.44	43.9
5	Caprylic	CL1	0.48	48.1
6	Docosanoic	DA1	1.83	183
7	13,16- Docosadienoic	DD1	0.43	43.0
8	Docosenoic	DE1	0.58	57.5
9	Docosahexaenoic	DHA	7.88	788
10	Docosapentaenoic n-3	DP3	2.49	249
11	Docosapentaenoic n-6	DP6	1.59	159
12	13,16,19-Docosatrienoic	DT1	0.43	42.8
13	Docosatetraenoic	DTA	1.48	148
14	11,14-Eicosadienoic	ED1	0.98	97.7
15	11-Eicosenoic	EN1	1.00	100
16	Eicosapentaenoic	EPA	7.15	715
17	5Z, 8Z, 11Z-Eicosatrienoic acid	ET1	0.64	64.3
18	*gamma*-Linolenic	GLA	2.93	293
19	*homo-gamma*-Linolenic	HGL	4.62	462
20	Hexacosanoic	HSA	0.37	36.7
21	Lauric	LAR	2.33	233
22	Tetracosanoic	LG1	1.47	147
23	Linoleic	LNA	82.7	8274
24	Myristoleic	ML1	1.42	142
25	Myristic	MR1	10.1	1012
26	Margaric	MRG	1.50	150
27	Nervonic	NR1	1.95	195
28	Oleic	OL1	85.0	8496
29	Pentadecanoic	PDE	0.96	95.7
30	Palmitoleic	PL1	25.1	2514
31	Palmitic	PM1	87.4	8742
32	Stearidonic	SD1	0.47	46.9
33	Stearic	ST1	25.0	2505
34	Tricosanoic	TSA	0.98	97.6
35	*cis*-Vaccenic	VC1	7.42	742

Fatty acids in grey text (CL1, DD1, DE1, DT1, and HSA) are being monitored but not reported. Samples with fatty acid concentrations exceeding the highest calibrator are re-analyzed after appropriate dilution with smaller serum or plasma volume. There is no known maximum acceptable dilution for the majority of fatty acids. When possible, avoid small volume pipetting and minimize use of serial dilutions when generating diluted samples. Diluted results for Arachidic, Docosanoic, Docosatetraenoic, Nervonic, Stearidonic, and *cis*-Vaccenic acid are not reported due to poor linear agreement between diluted and un-diluted results (**see Appendix A - dilution linearity**).

10. **Quality Control (QC) Procedures**

A. Blind Quality Controls

Blind QC specimens can be inserted into the mix of patient specimens. These QC specimens are often prepared at two levels that would be encountered in patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed. Alternatively, open label blind QC specimens can be used where the analyst knows that the sample is a blind QC, but does not know what pool the sample is from. Open label blind QCs are only used if one can choose from at least 6 different pools and the analyte concentrations are similar to those found in patient samples.

B. Bench Quality Controls

In general, three bench QC pools are prepared by blending units of serum or plasma to achieve low, intermediate, and high levels of key fatty acids (Palmitic, Oleic, Linoleic, Stearic and Arachidonic). QC pools are aliquoted into 200-300 2-mL vials and stored at -70^{o}C. Generally, a vial of each pool is thawed before every assay and duplicate aliquots of each QC pool are prepared for analysis in the same manner as patient samples. QC samples are analyzed as part of each run (pre- and post- unknowns).

The results from the pools are checked after each run. The system is declared “in control” if the results pass the following tests:
Multi-rule quality control system: quality control rules for three QC pools per run

1) If all three QC run means are within $2S_m$ limits and individual results are within $2S_i$ limits, accept the run
2) If 1 of the 3 QC run means is outside a $2S_m$ limit – reject run if:
    a. 1_{3s}: Any of the three QC results are outside the 3S limit
    b. 2_{2s}: Two of the three QC results in the run are outside the 2S limit (same side of mean)
    c. 10_x: Ten sequential QC results (across pools and across runs) are on the same side of the mean.
3) If one of the six QC individual results is outside a 2 S_i limit – reject run if:
    a. Outlier – One individual result is beyond the characterization mean + 4 S_i or
    b. R_{4s}: Sequential QC results (either within the run or across runs) are outside the 2S limit on the opposite sides of the mean

S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements)
S_m = Standard deviation of the run means (the limits are shown on the chart)
S_w = Within-run standard deviation (the limits are not shown on the chart)

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts (9). No results for a given analyte are to be reported from an analytical run that has been declared “out of control” for that analyte as assessed using bench QC. The initial limits are established by analyzing QC pool material in 20 consecutive runs and then are reevaluated as needed. When necessary, limits are updated to include more runs. QC results are stored in the LIMS database.

C. Sample QC Criteria

Each individual sample result is checked against established sample QC criteria limits to assure data quality. The method uses the following sample QC criteria:

- Calibrator values within 15% of target
- Calibration curve $R^2 > 0.985$
- Blank is less than lowest standard
- Individual ISTD area within 3SD from the mean ISTD of the run

For details, see **4028_SOP Sample QC Criteria.**

11. **Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria**

A. Look for sample preparation errors, e.g., analyst forgot or under-pipetted the isotope-labeled standard or derivitizing agent; or any sample preparation problems, e.g. vial evaporation during heating.

B. Check the calibration of the pipettes.

C. Check to make sure that the hardware is functioning properly. Make sure the MS is tuned properly, and the gas velocity is as required. Check the autosampler to make sure the injections are being made as programmed.

D. If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.

E. Do not report analytical results for runs not in statistical control.

12. **Limitations of Method; Interfering Substances and Conditions**

A. Due to the complexity of this assay, not all analytes being monitored are found in the quality control pools. At this time 5 fatty acids (Caprylic, Docosadienoic, Docosenoic, Docosatrienoic, and Hexacosanoic) are non-reportable because we lack QC materials with concentrations greater than the LOD. We continue to collect data for non-reportable fatty acids in order to assess whether some individuals have measurable concentrations, but will not report any analyte that is not in the quality control pools.

B. The most common causes of imprecision are intermittently inaccurate micropipettors and pipetting errors.

C. Stock standards, stable isotope labeled standards and specimens should be mixed thoroughly by vortexing before pipetting.

D. Handling stocks and internal standards in step-wise sequential manner will minimize the chances of cross-contaminations.

E. Also changing of gloves after preparations of stock and working standards and internal standards is recommended to avoid any contamination.

F. This method has also undergone a series of in-house ruggedness testing experiments designed to assess how much method accuracy changes when certain experimental parameters are varied. A total of five parameters judged to most likely affect the accuracy of the method have been identified and tested. Testing generally consisted of performing replicate measurements on a test specimen with the selected parameter set at a value substantially lower and higher

than that specified in this method while holding all other experimental variables constant. **The ruggedness testing findings for this method are presented in Appendix B.** Please refer to Chapter 21 of the *DLS Policies and Procedures Manual* for further information on ruggedness testing.

13. **Reference Ranges (Normal Values)**

The reference ranges in fasted, noninstitutionalized civilians aged ≥20y from the cross-sectional 2003–2004 National Health and Nutrition Examination Survey for 24 of the 35 fatty acids analyzed in this method are tabulated below (data for new fatty acids are not yet available).

Analyte Code	Geometric mean (uM)	5th (uM)	50th (uM)	95th (uM)	Sample Size
ALN	63.1	30	61.4	137	1,801
AR1	23.4	16.2	23.2	33.6	1,757
ARA	776	484	789	1,180	1,807
DA1	69.3	45.2	69.5	102	1,739
DE1	3.44	0.712	3.62	10.3	1,604
DHA	125	61.1	121	277	1,808
DP3	41.6	24.5	41.4	72.7	1,808
DP6	19.6	9.8	19.3	39	1,808
DTA	25	14.4	24.7	43	1,808
ED1	21.2	12.4	20.9	36.9	1,805
EN1	13.6	7.56	13.3	25.9	1,805
EPA	42.1	17.1	40.9	113	1,806
GLA	46.9	20.2	49	100	1,795
HGL	151	87.5	151	262	1,806
LG1	54	35.3	53.8	80.9	1,743
LNA	3,450	2,370	3,430	4,980	1,806
ML1	6.57	1.79	6.5	23.9	1,808
MR1	119	48.1	116	308	1,796
NR1	74.9	49.8	75	116	1,696
OL1	2,100	1,220	2,070	3,850	1,798
PL1	217	84	213	563	1,805
PM1	2,710	1,690	2,630	4,710	1,805
ST1	692	471	684	1,040	1,806
VC1	146	83.6	143	262	1,762

14. **Critical Call Results (“Panic Values”)**

There are no known critical call values for fatty acids.

15. **Specimen Storage and Handling During Testing**

Specimens are allowed to reach room temperature during preparation. The unused portion of the patient specimen is returned to frozen storage (typically -70°C, but not > -40°C) as soon as possible. Once the derivatized samples have been completed, they are placed into the autosampler tray. If necessary, derivatized samples can be stored at -20°C or -70°C for a few days or weeks until chromatographed, but must be brought to room temperature prior to injection.

16. **Alternate Methods for Performing Test of Storing Specimens if Test System Fails**

Because the analysis of fatty acids is inherently complex and challenging, there are no acceptable alternative methods of analysis. If the analytical system fails, we recommend that the extracted and/or derivatized specimens be stored at -70°C until the analytical system is restored to functionality. All specimens should be brought to room temperature prior to chromatographic analysis.

17. **Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)**

Test results that are reported to the collaborating agency at a frequency and using a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through electronic mail or via FTP through the internet.

18. **Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking**

A LIMS database is used to track specimens and store results for all studies.

We recommend that records, including related QA\QC data, be maintained for 10 years after completion of studies. Only numerical identifiers should be used (e.g., Sample ID); all personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies are retained for at least 1 year after results have been reported and may then be returned or discarded at the request of the principal investigator. Very little residual material will be available after NHANES analyses are completed, however residual serum is retained for at least 2 year after results have been publicly released; at that point, samples with sufficient volume (>0.2 mL) are returned to NHANES and samples with insufficient volume may be routinely autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens are stored in a freezer at -70^{o}C. The specimen ID is read by a barcode reader attached to the computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the export file containing the electronic copy of the results is loaded in the LIMS database, and the analytical results are linked to the LIMS database by ID number. The analyst is responsible for keeping records of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. In general, these are documented using codes in the LIMS.

19. Method Performance Documentation

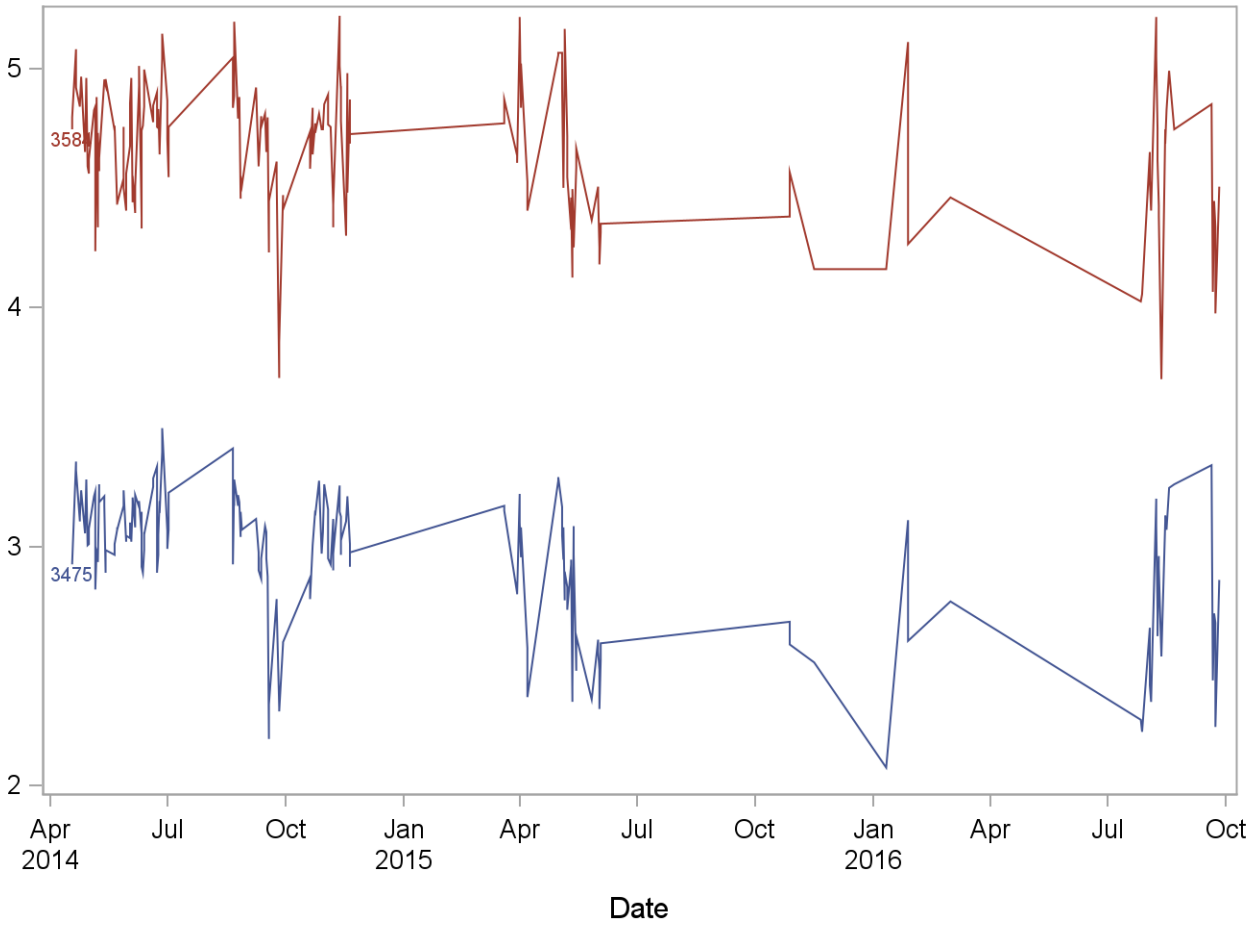
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in **Appendix A** of this method documentation. **The signatures of the branch chief and director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

20. Summary Statistics and QC Graphs

Please see follow page

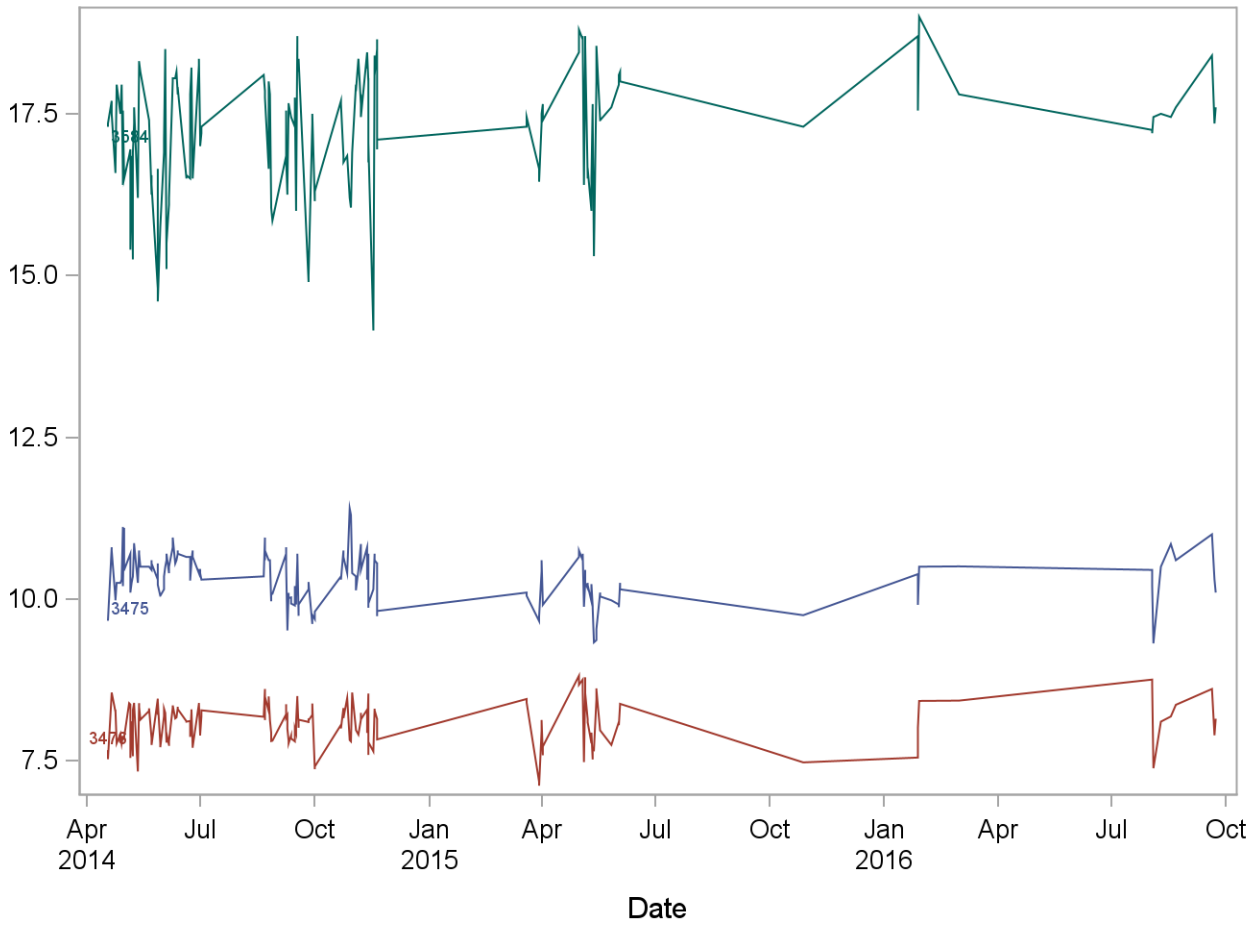
2013-2014 Summary Statistics and QC Chart
LBXCAP (Capric acid (C10:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	193	18APR14	26SEP16	2.9543	0.2797	9.5
3584	193	18APR14	26SEP16	4.6650	0.2849	6.1



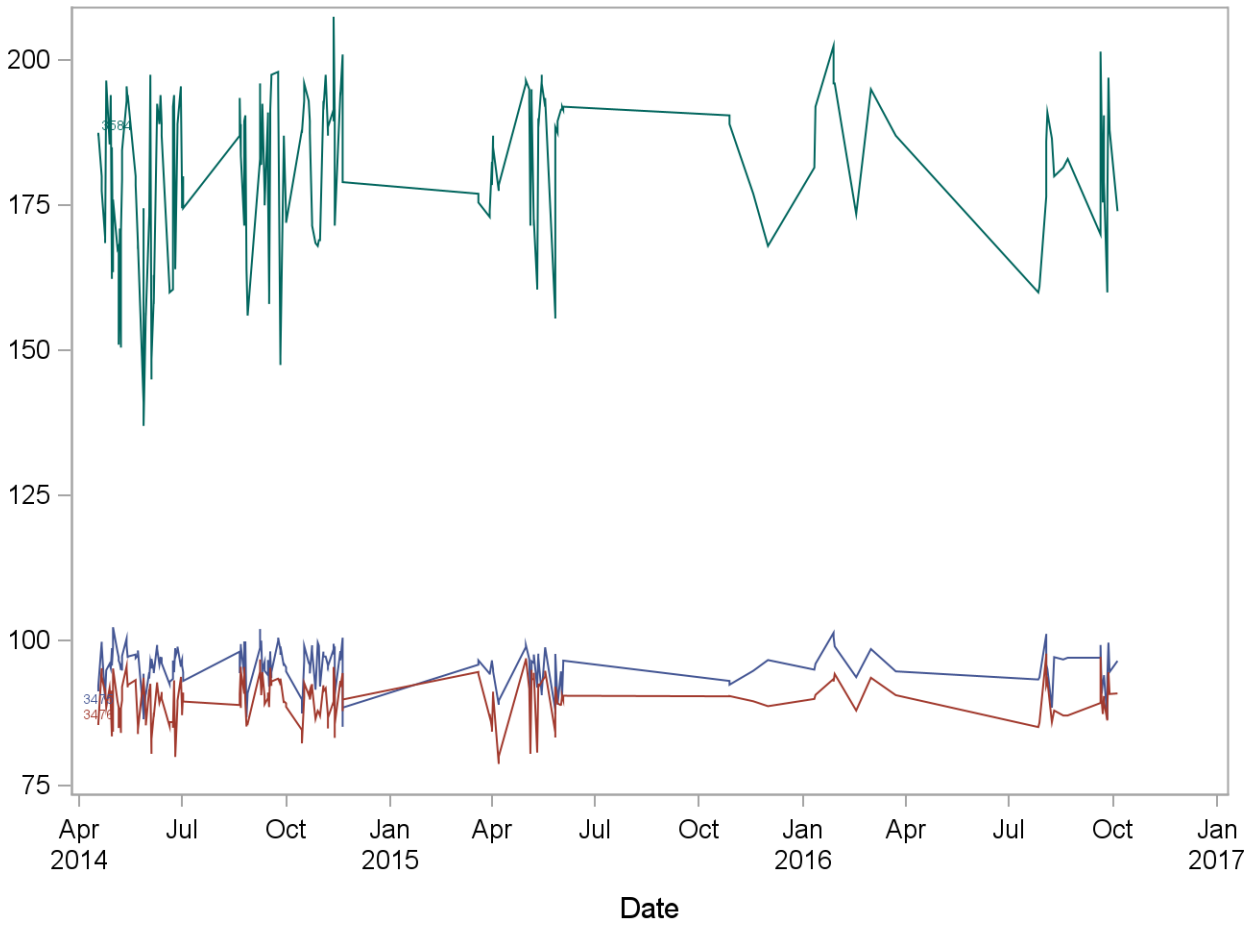
2013-2014 Summary Statistics and QC Chart
LBXLAR (Lauric acid (C12:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	180	18APR14	23SEP16	10.3318	0.3897	3.8
3476	180	18APR14	23SEP16	8.0832	0.3357	4.2
3584	180	18APR14	23SEP16	17.2525	0.9531	5.5



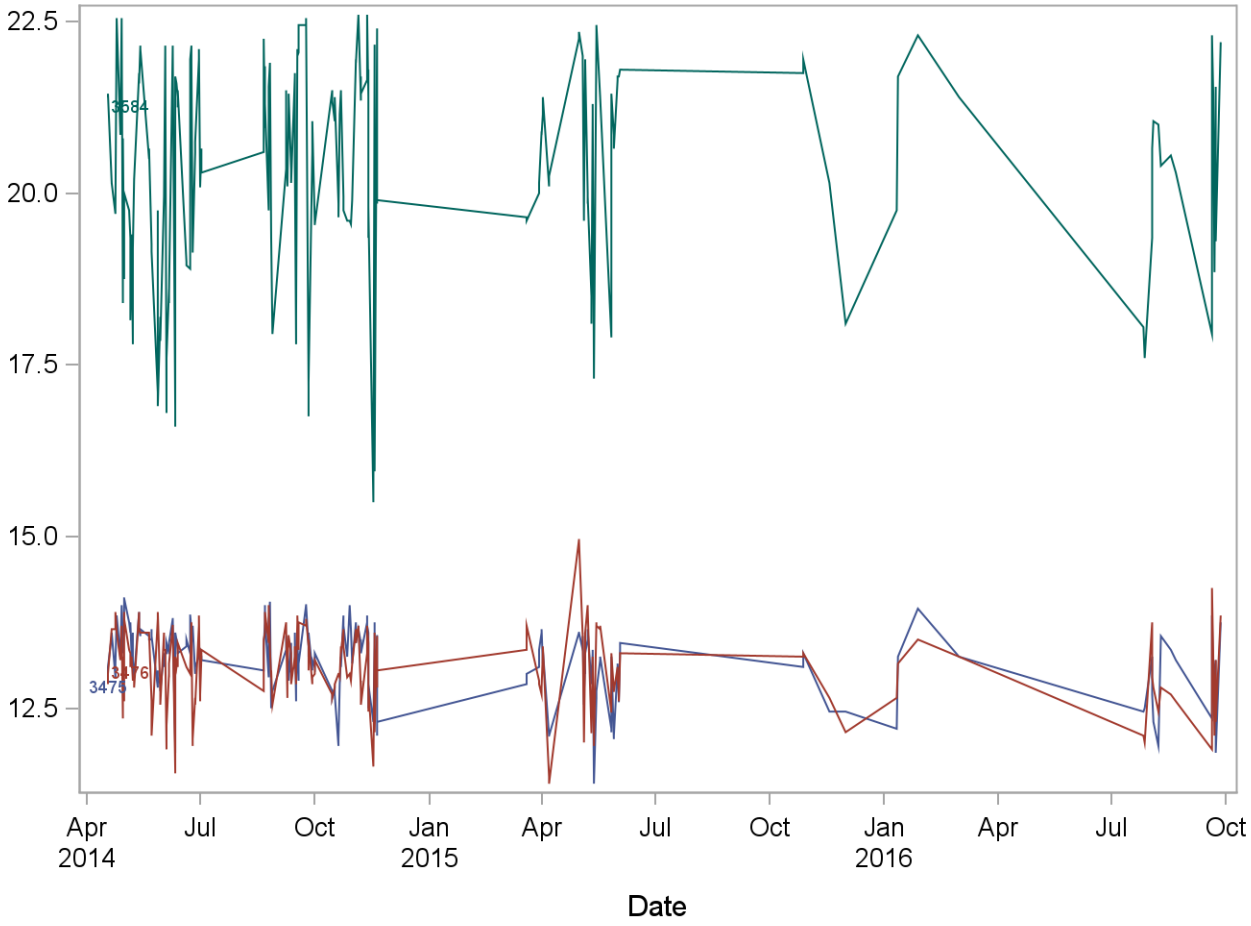
2013-2014 Summary Statistics and QC Chart
LBXMR1 (Myristic acid (14:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	214	18APR14	05OCT16	95.52	3.47	3.6
3476	214	18APR14	05OCT16	89.92	3.90	4.3
3584	214	18APR14	05OCT16	180.66	13.84	7.7



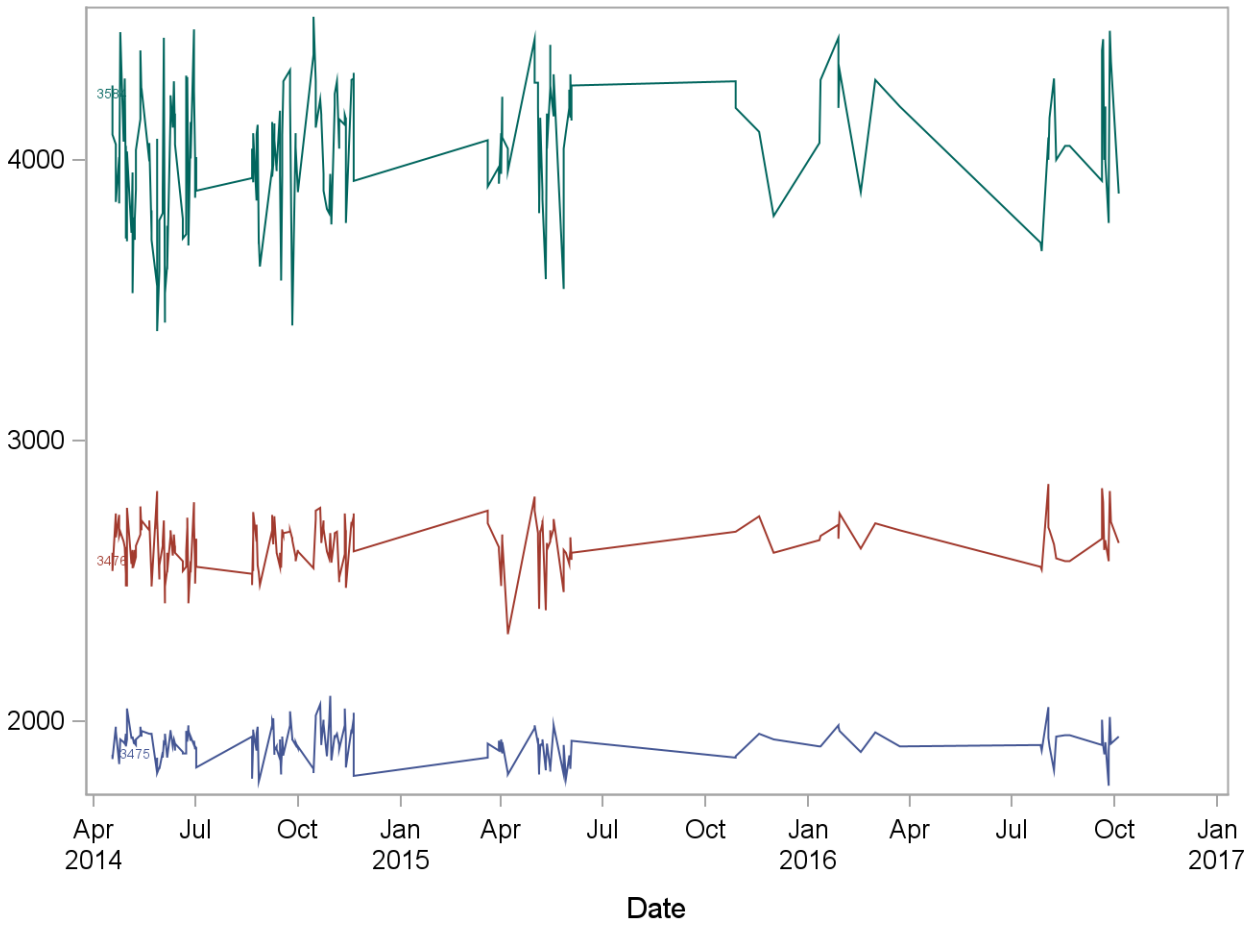
2013-2014 Summary Statistics and QC Chart
LBXPEN (Pentadecanoic acid (C15:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	214	18APR14	27SEP16	13.187	0.525	4.0
3476	214	18APR14	27SEP16	13.121	0.592	4.5
3584	214	18APR14	27SEP16	20.402	1.557	7.6



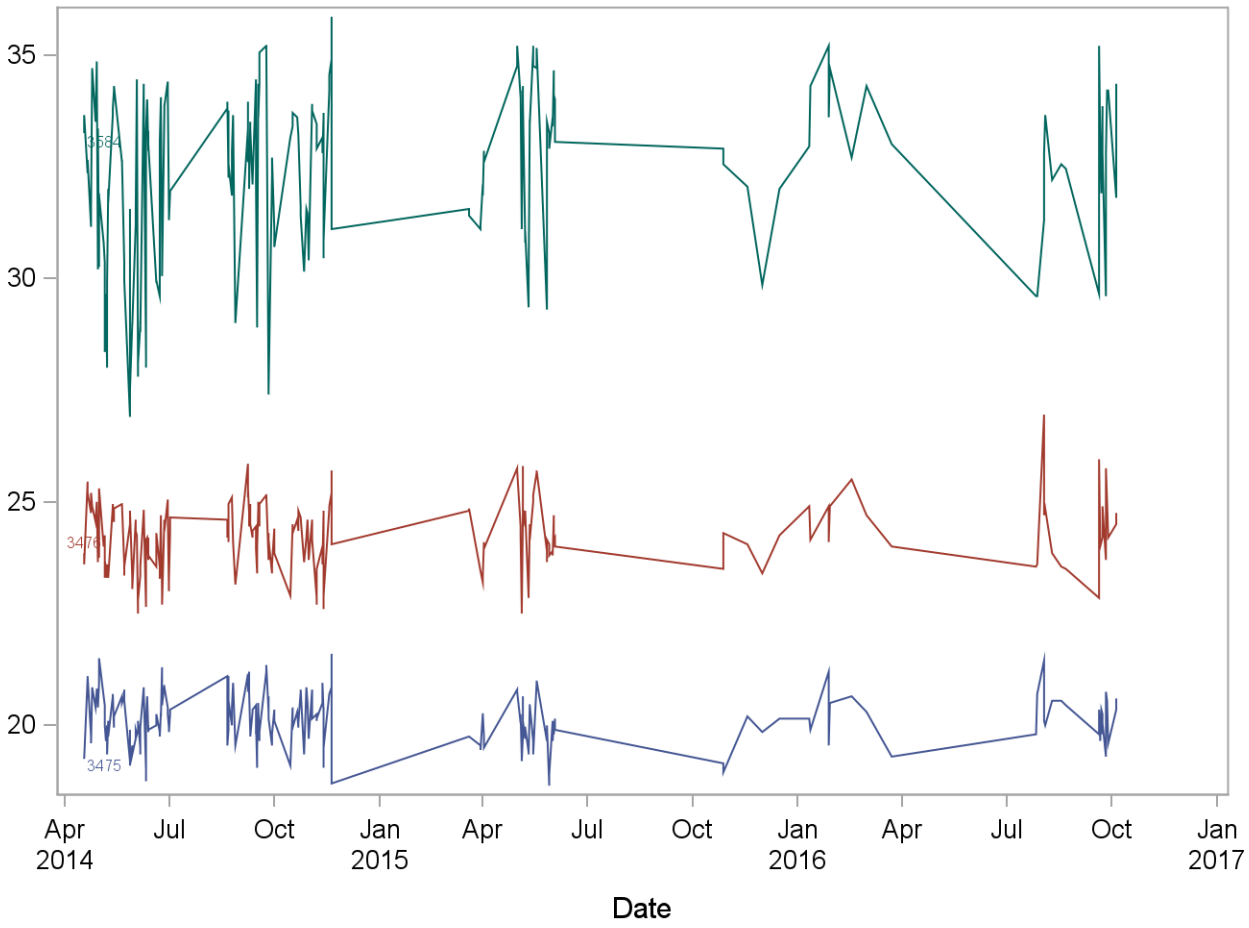
2013-2014 Summary Statistics and QC Chart
LBXPM1 (Palmitic acid (16:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	213	18APR14	05OCT16	1918.0	58.6	3.1
3476	213	18APR14	05OCT16	2622.5	93.2	3.6
3584	213	18APR14	05OCT16	4018.9	239.1	5.9



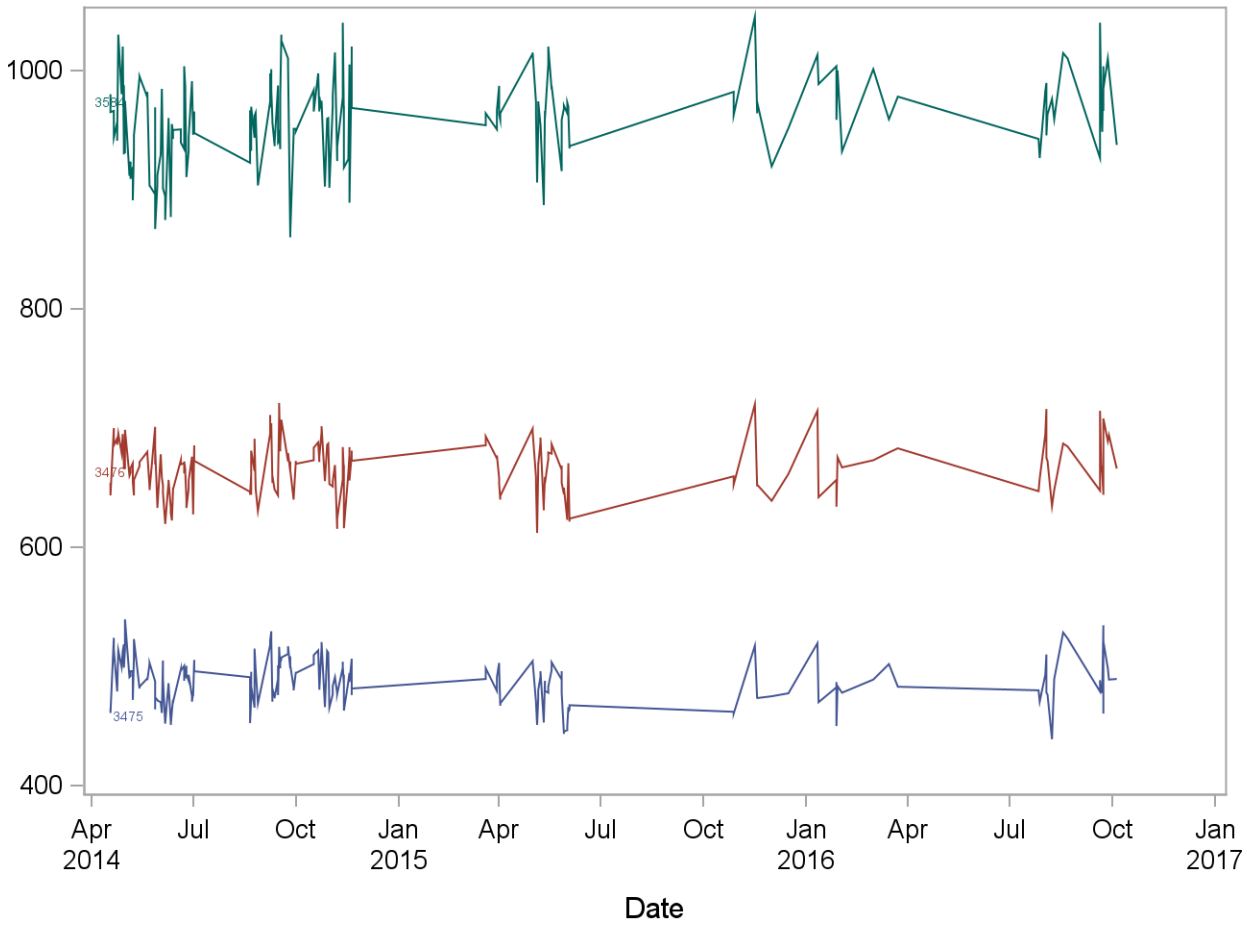
2013-2014 Summary Statistics and QC Chart
LBXMGR (Margaric acid (C17:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	212	18APR14	05OCT16	20.149	0.598	3.0
3476	212	18APR14	05OCT16	24.245	0.790	3.3
3584	212	18APR14	05OCT16	32.298	1.953	6.0



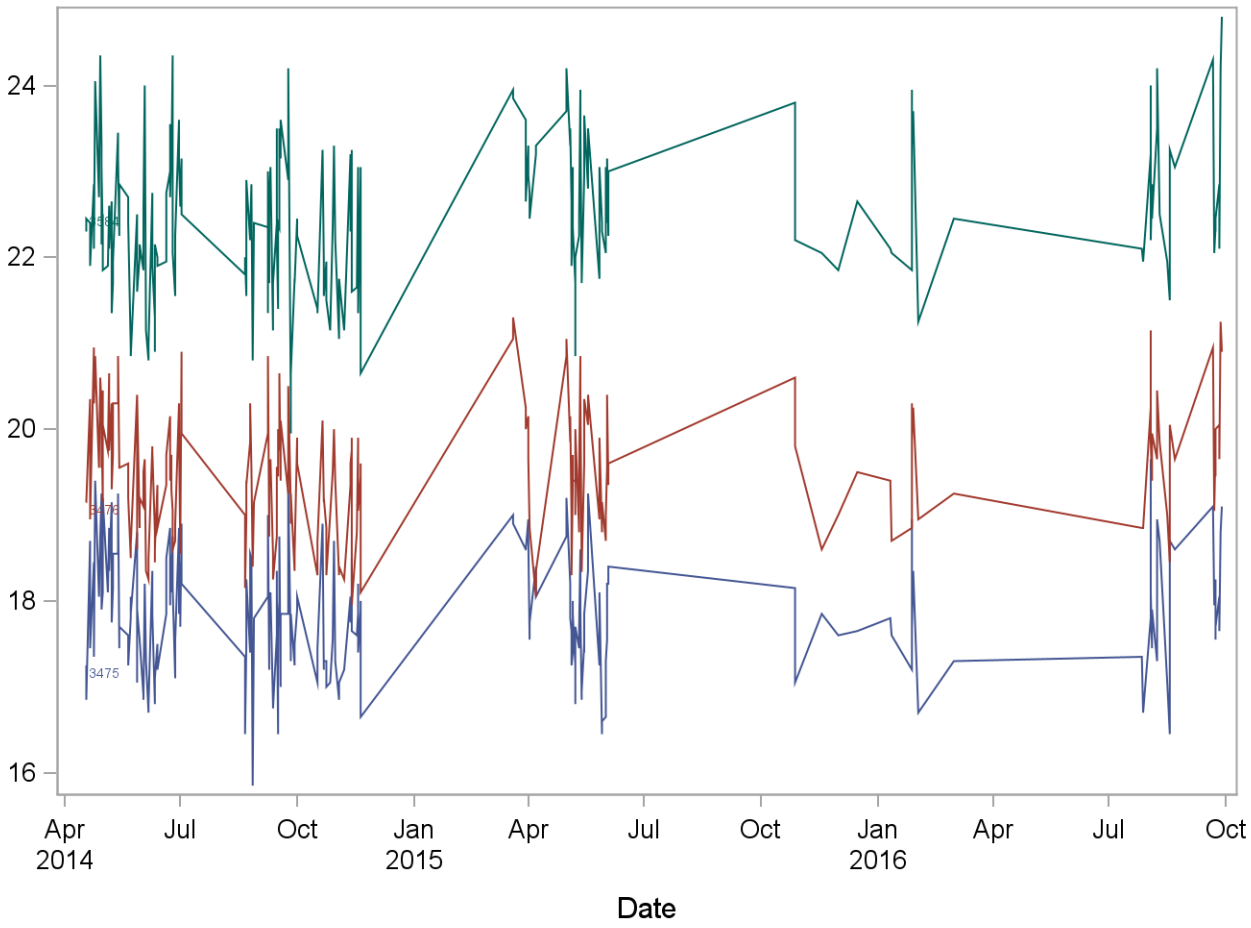
2013-2014 Summary Statistics and QC Chart
LBXST1 (Stearic acid (18:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	209	18APR14	05OCT16	488.0	20.0	4.1
3476	209	18APR14	05OCT16	665.6	23.0	3.5
3584	209	18APR14	05OCT16	958.9	36.5	3.8



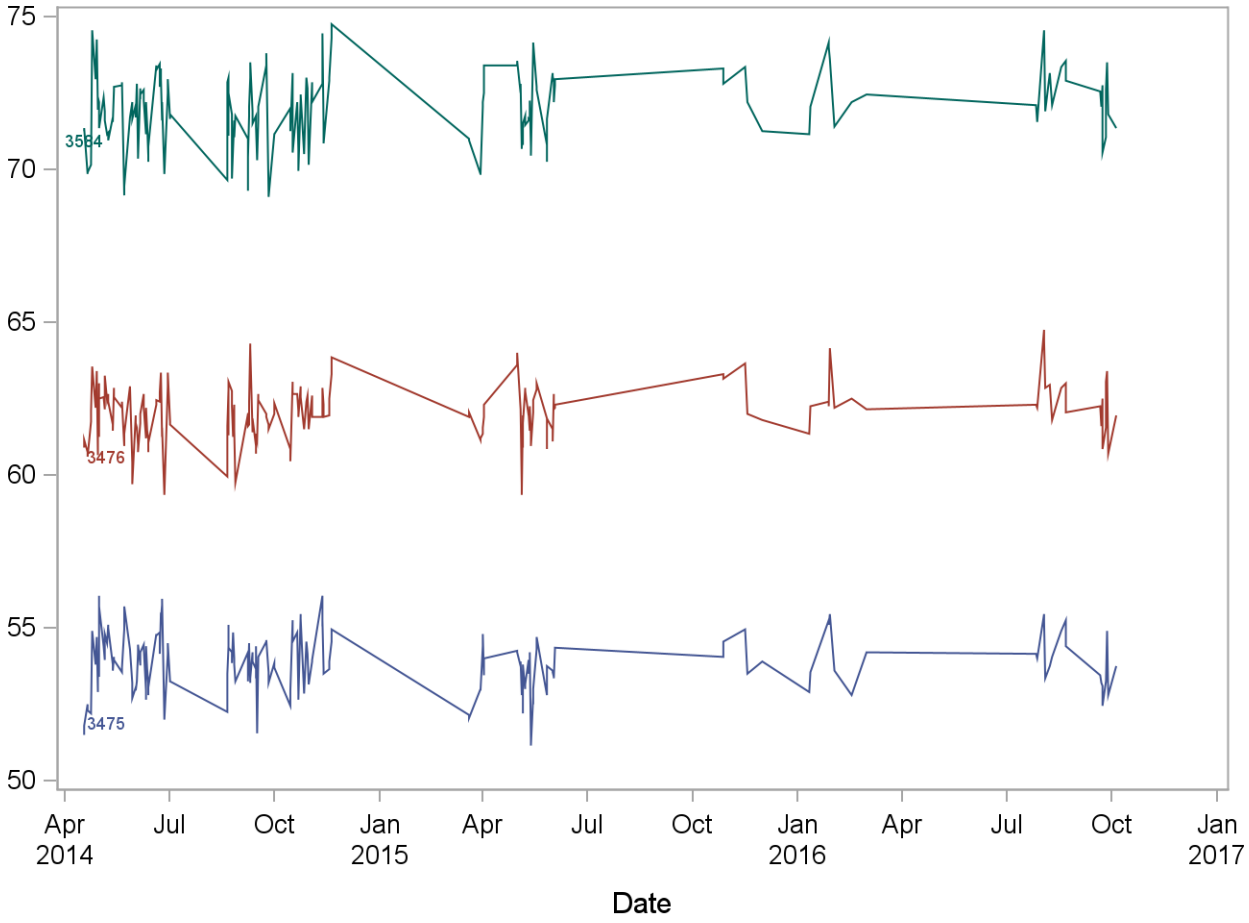
2013-2014 Summary Statistics and QC Chart
LBXAR1 (Arachidic acid (20:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	209	18APR14	28SEP16	17.857	0.713	4.0
3476	209	18APR14	28SEP16	19.497	0.742	3.8
3584	209	18APR14	28SEP16	22.464	0.874	3.9



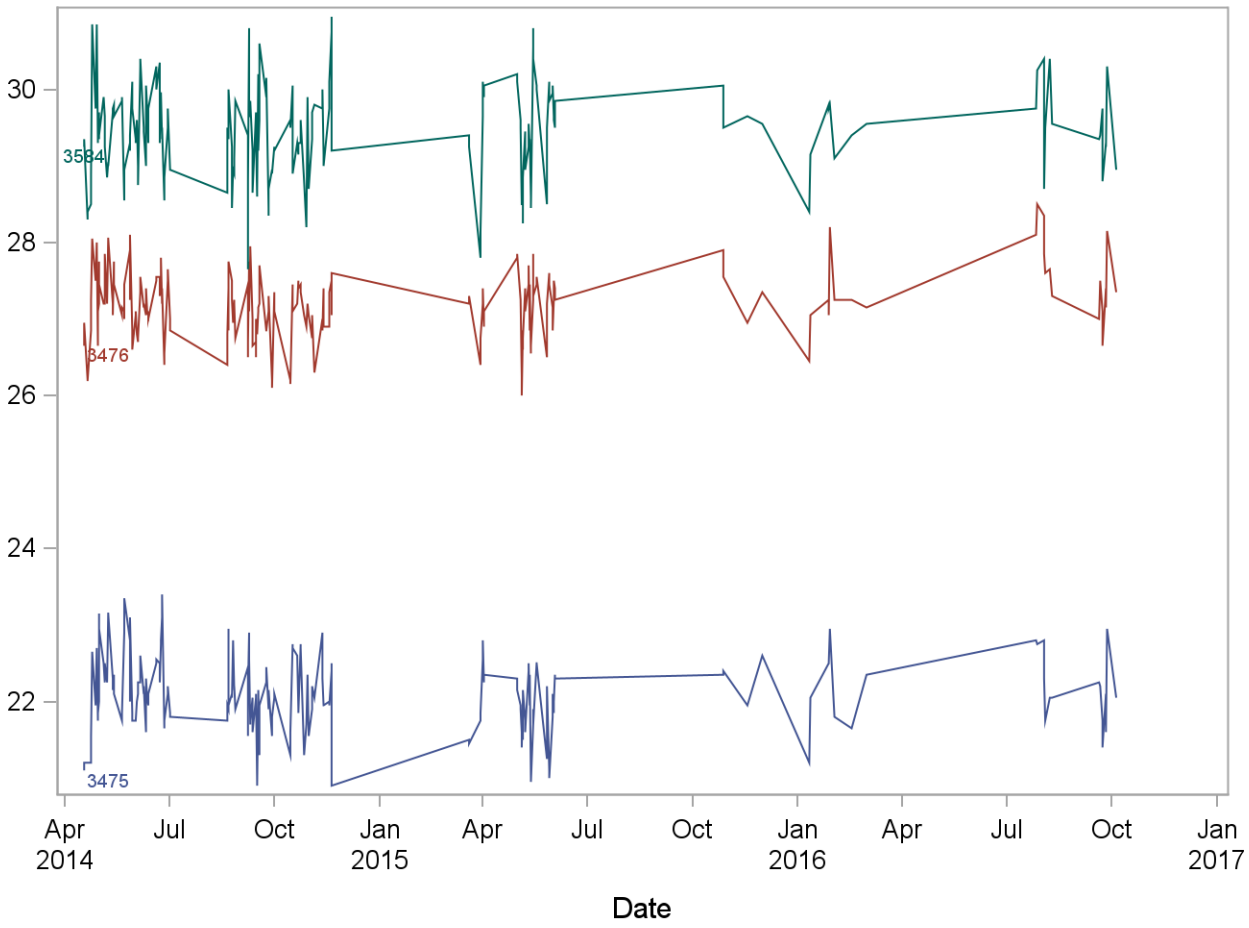
2013-2014 Summary Statistics and QC Chart
LBXDA1 (Docosanoic acid (22:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	192	18APR14	05OCT16	53.834	0.963	1.8
3476	192	18APR14	05OCT16	62.034	0.924	1.5
3584	192	18APR14	05OCT16	71.955	1.215	1.7



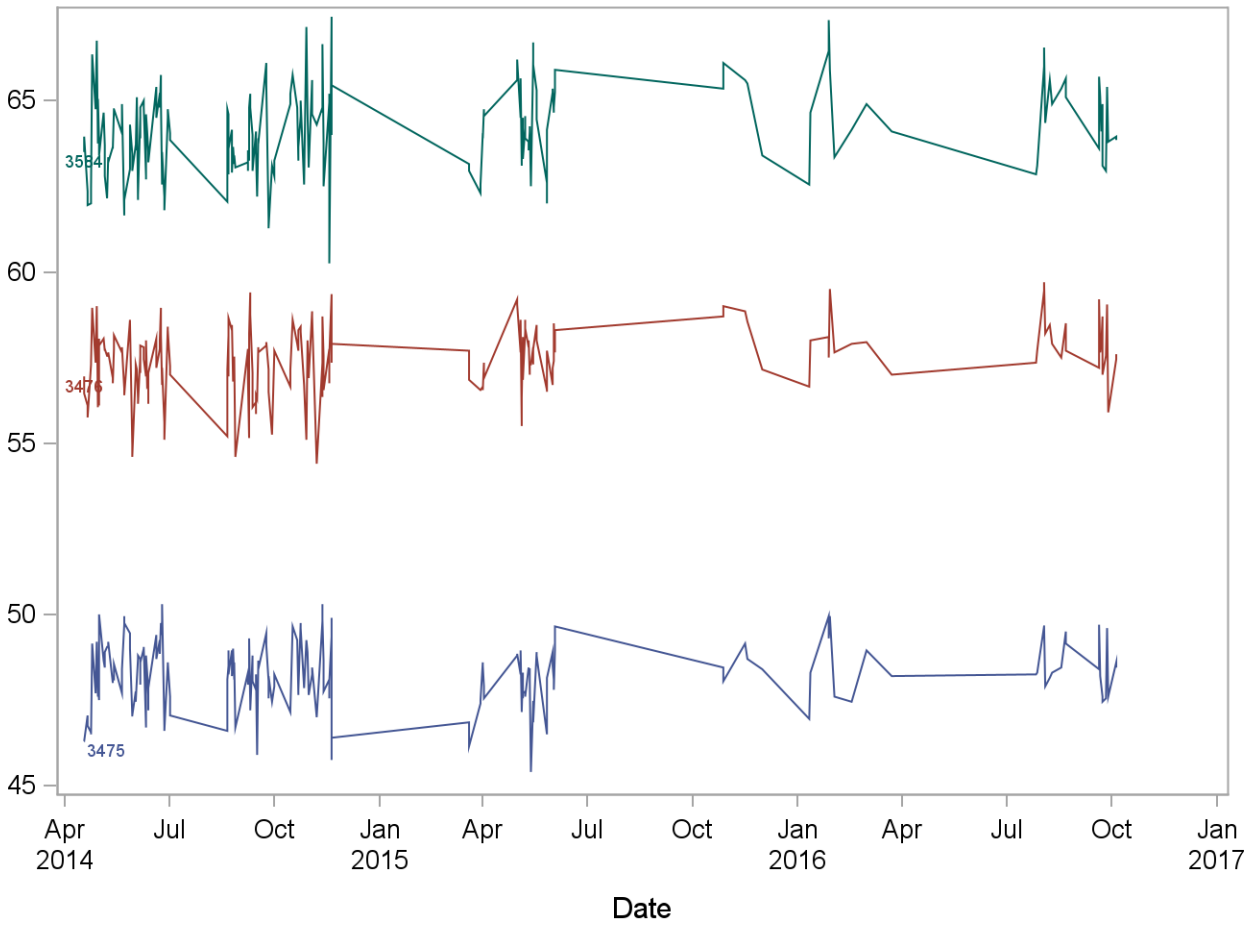
2013-2014 Summary Statistics and QC Chart
LBXTSA (Tricosanoic acid (C23:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	200	18APR14	05OCT16	22.080	0.509	2.3
3476	200	18APR14	05OCT16	27.183	0.453	1.7
3584	200	18APR14	05OCT16	29.433	0.602	2.0



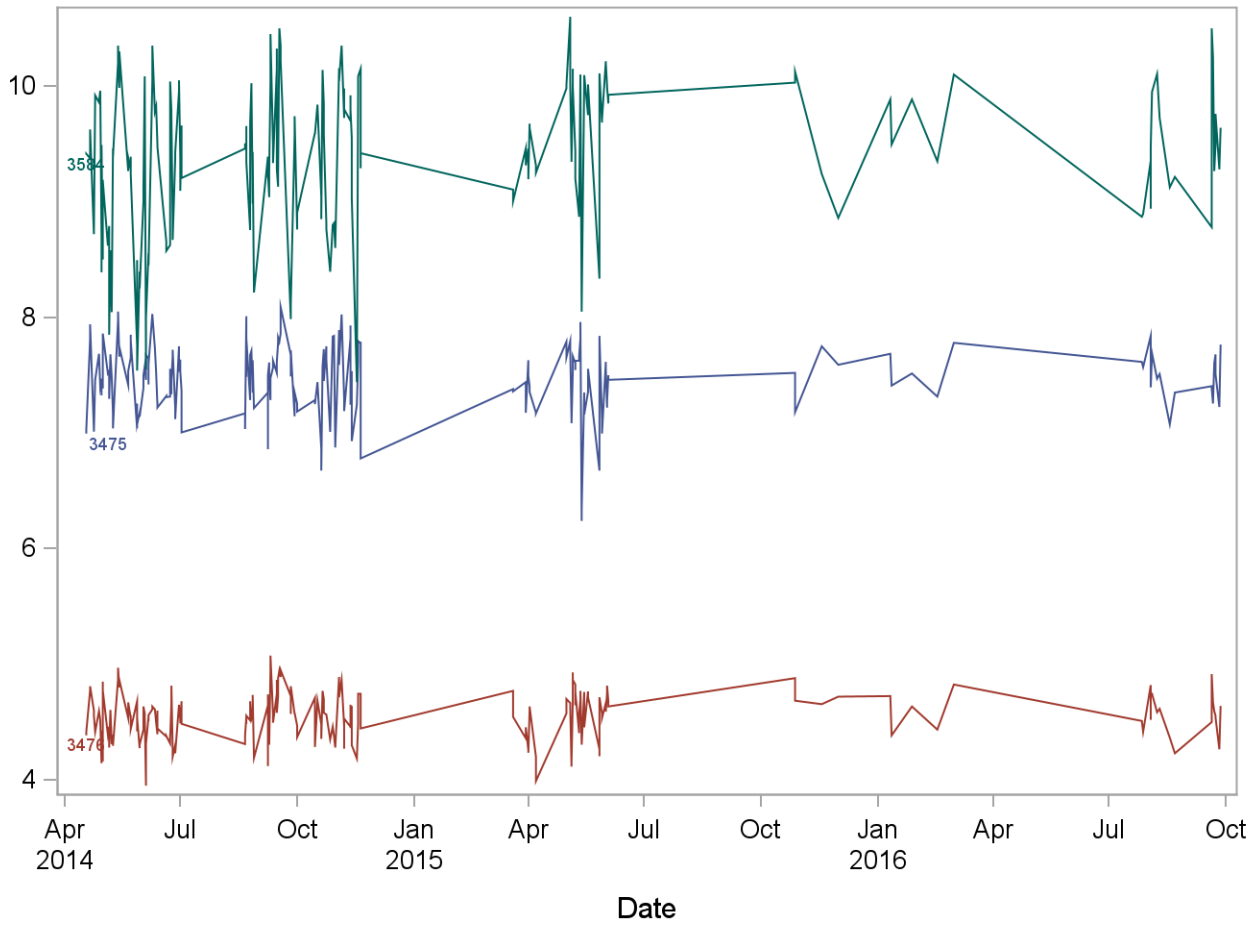
2013-2014 Summary Statistics and QC Chart
LBXLG1 (Lignoceric acid (24:0))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	201	18APR14	05OCT16	48.242	0.961	2.0
3476	201	18APR14	05OCT16	57.436	1.000	1.7
3584	201	18APR14	05OCT16	64.114	1.285	2.0



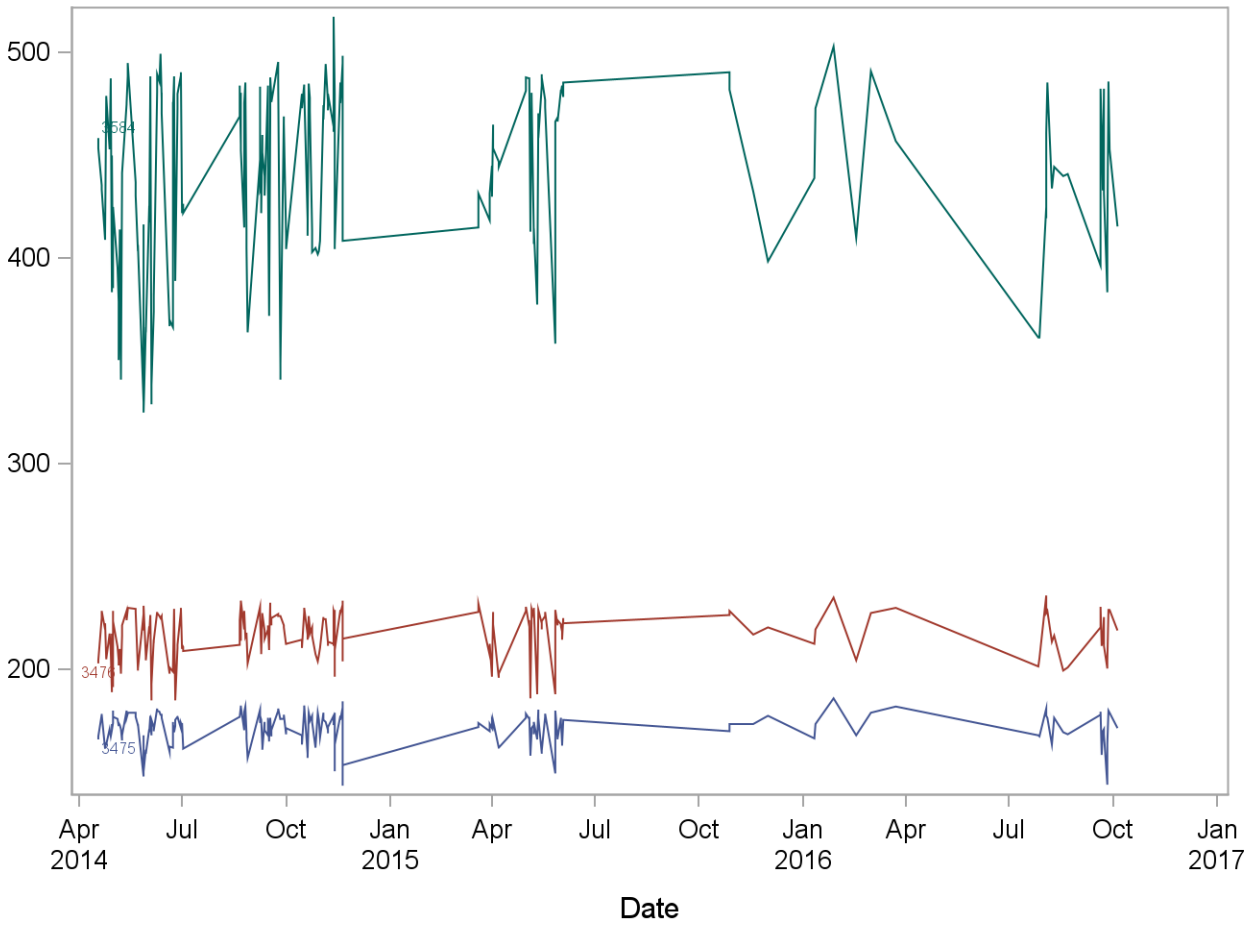
2013-2014 Summary Statistics and QC Chart
LBXML1 (Myristoleic acid (14:1n-5))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	208	18APR14	27SEP16	7.4571	0.3088	4.1
3476	208	18APR14	27SEP16	4.5490	0.2075	4.6
3584	208	18APR14	27SEP16	9.3869	0.6535	7.0



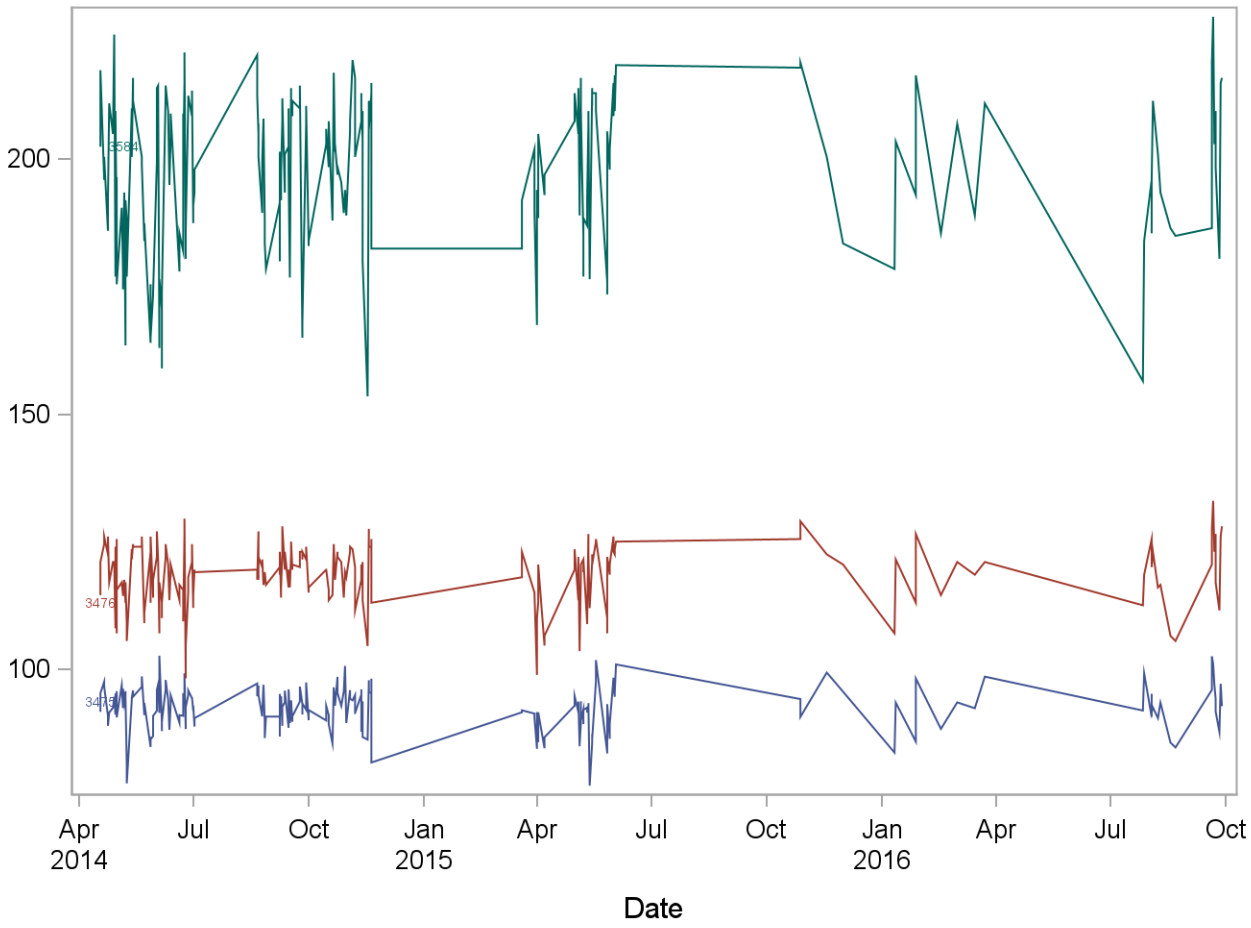
2013-2014 Summary Statistics and QC Chart
LBXPL1 (Palmitoleic acid (16:1n-7))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	213	18APR14	05OCT16	171.57	7.71	4.5
3476	213	18APR14	05OCT16	216.81	12.08	5.6
3584	213	18APR14	05OCT16	440.54	43.78	9.9



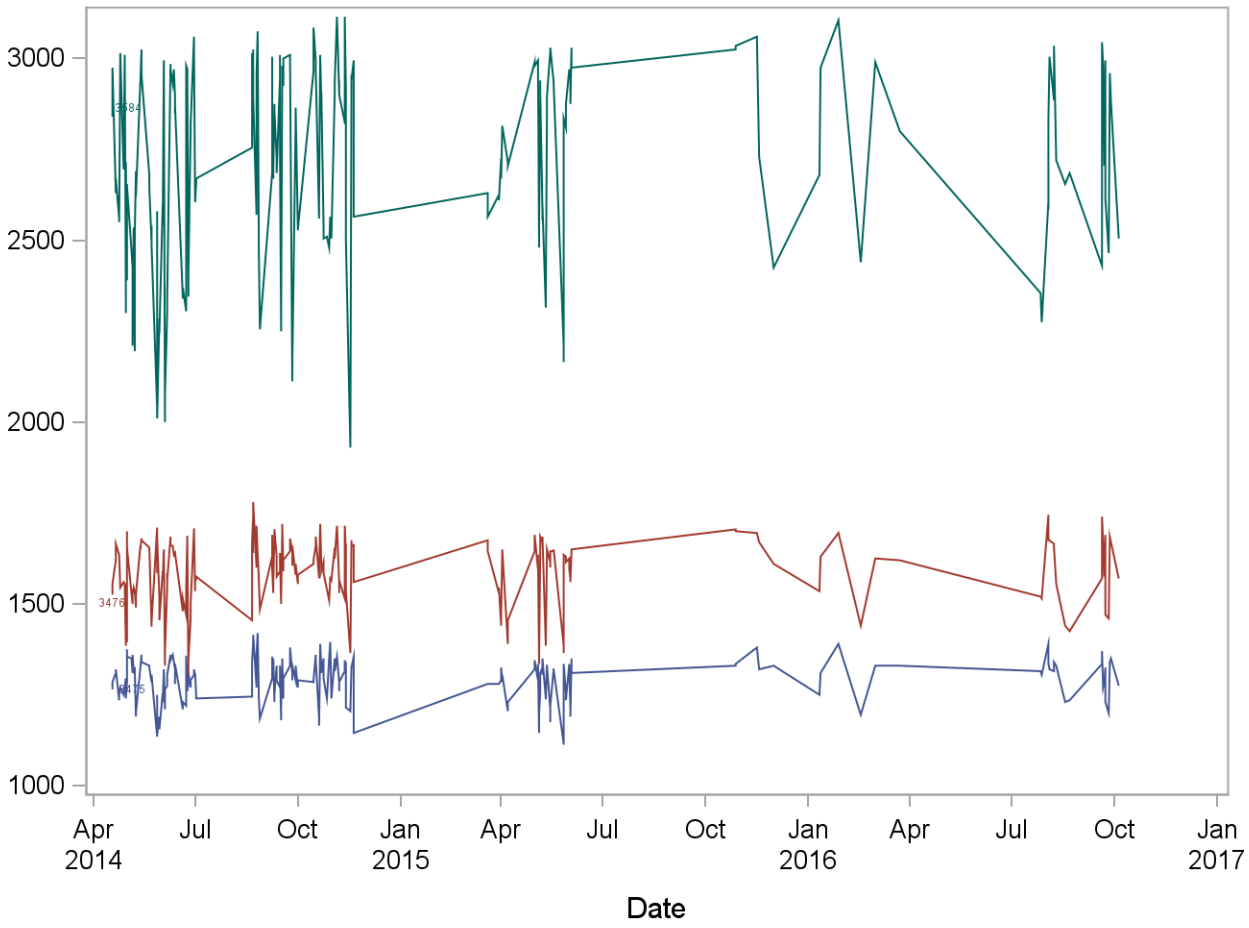
2013-2014 Summary Statistics and QC Chart
LBXVC1 (cis-Vaccenic acid (18:1n-7))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	214	18APR14	28SEP16	92.26	4.28	4.6
3476	214	18APR14	28SEP16	118.46	6.07	5.1
3584	214	18APR14	28SEP16	197.32	15.25	7.7



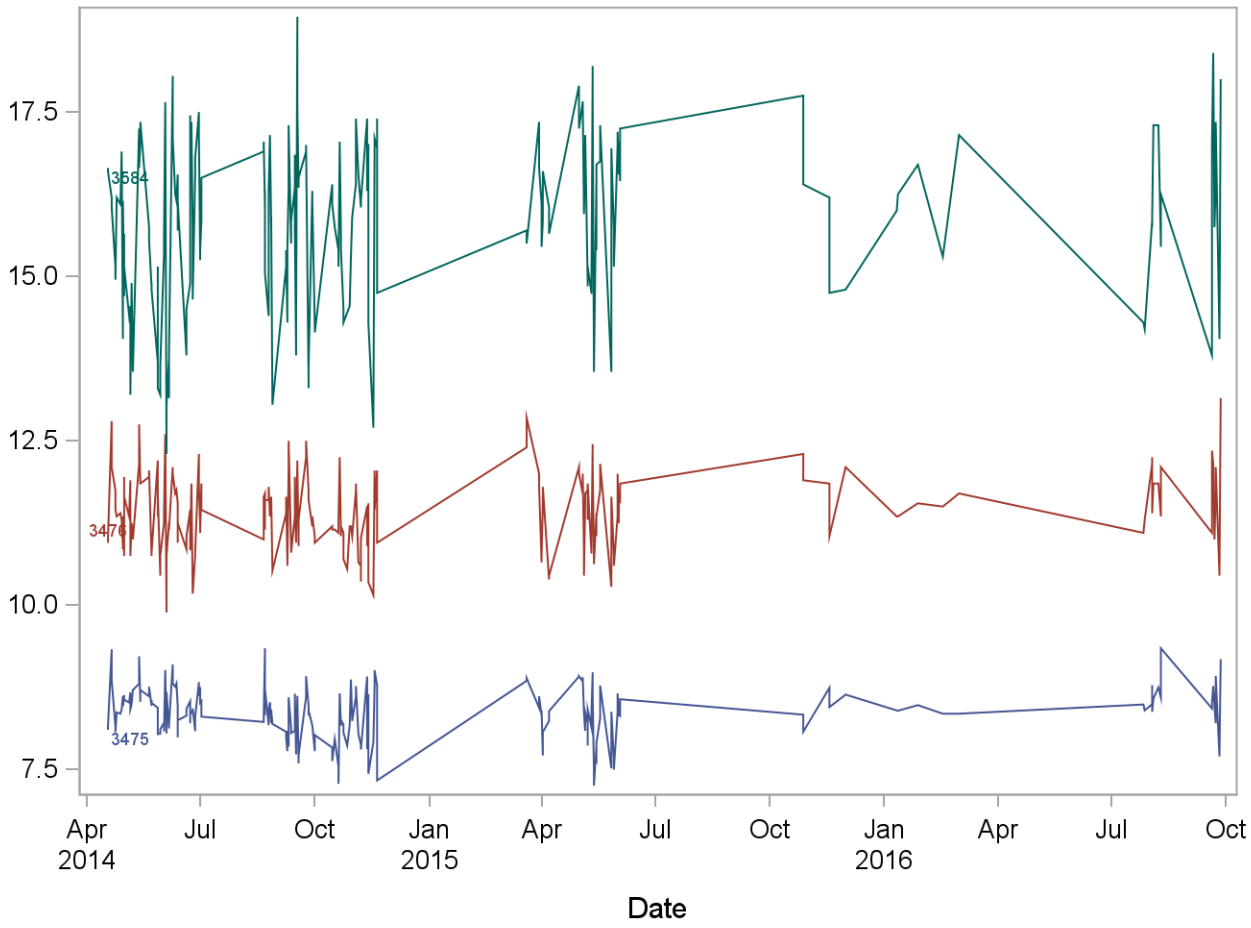
2013-2014 Summary Statistics and QC Chart
LBXOL1 (Oleic acid (18:1n-9))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	216	18APR14	05OCT16	1291.7	59.6	4.6
3476	216	18APR14	05OCT16	1588.8	94.3	5.9
3584	216	18APR14	05OCT16	2717.7	271.3	10.0



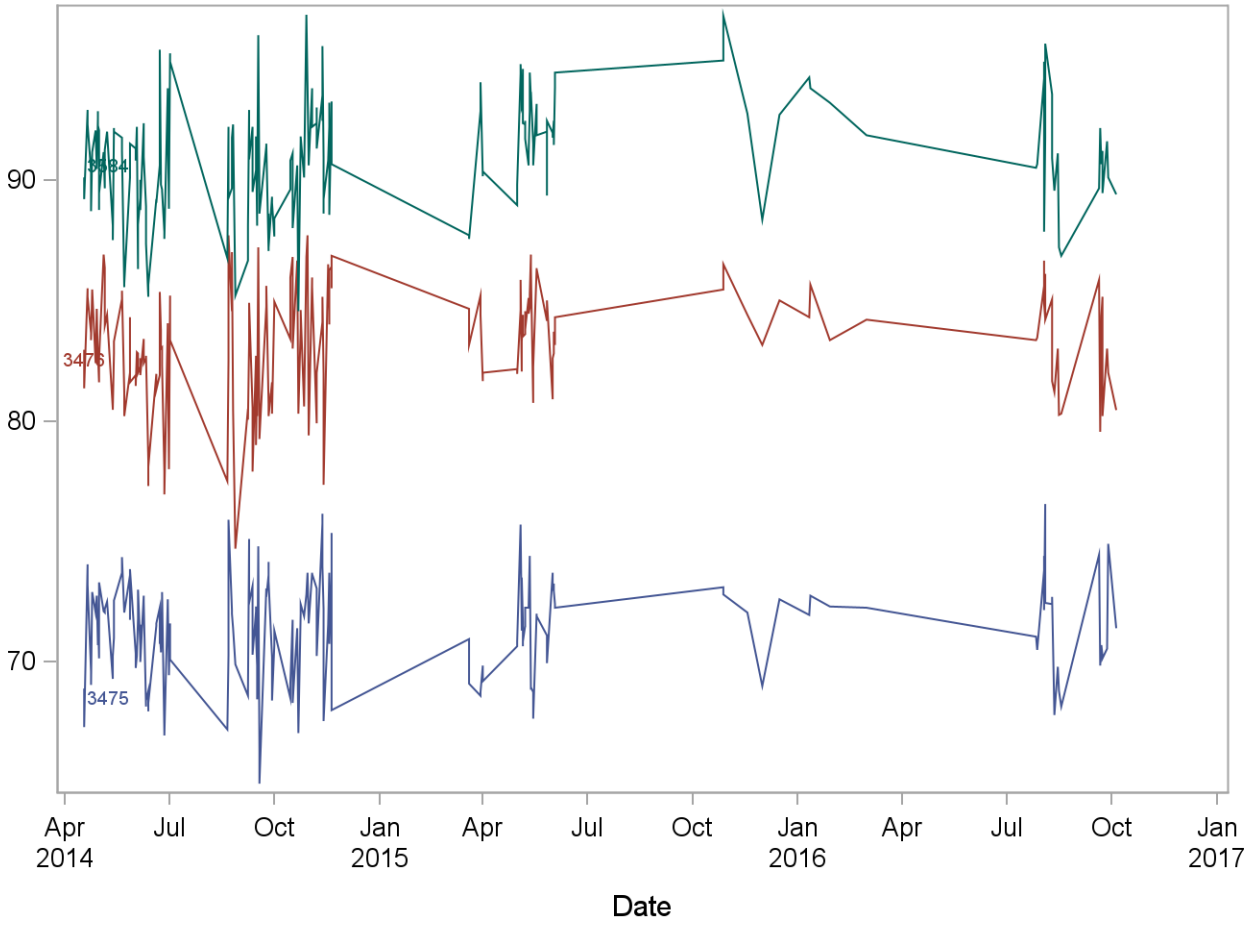
2013-2014 Summary Statistics and QC Chart
LBXEN1 (Eicosenoic acid (20:1n-9))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	207	18APR14	27SEP16	8.352	0.398	4.8
3476	207	18APR14	27SEP16	11.410	0.589	5.2
3584	207	18APR14	27SEP16	15.802	1.290	8.2



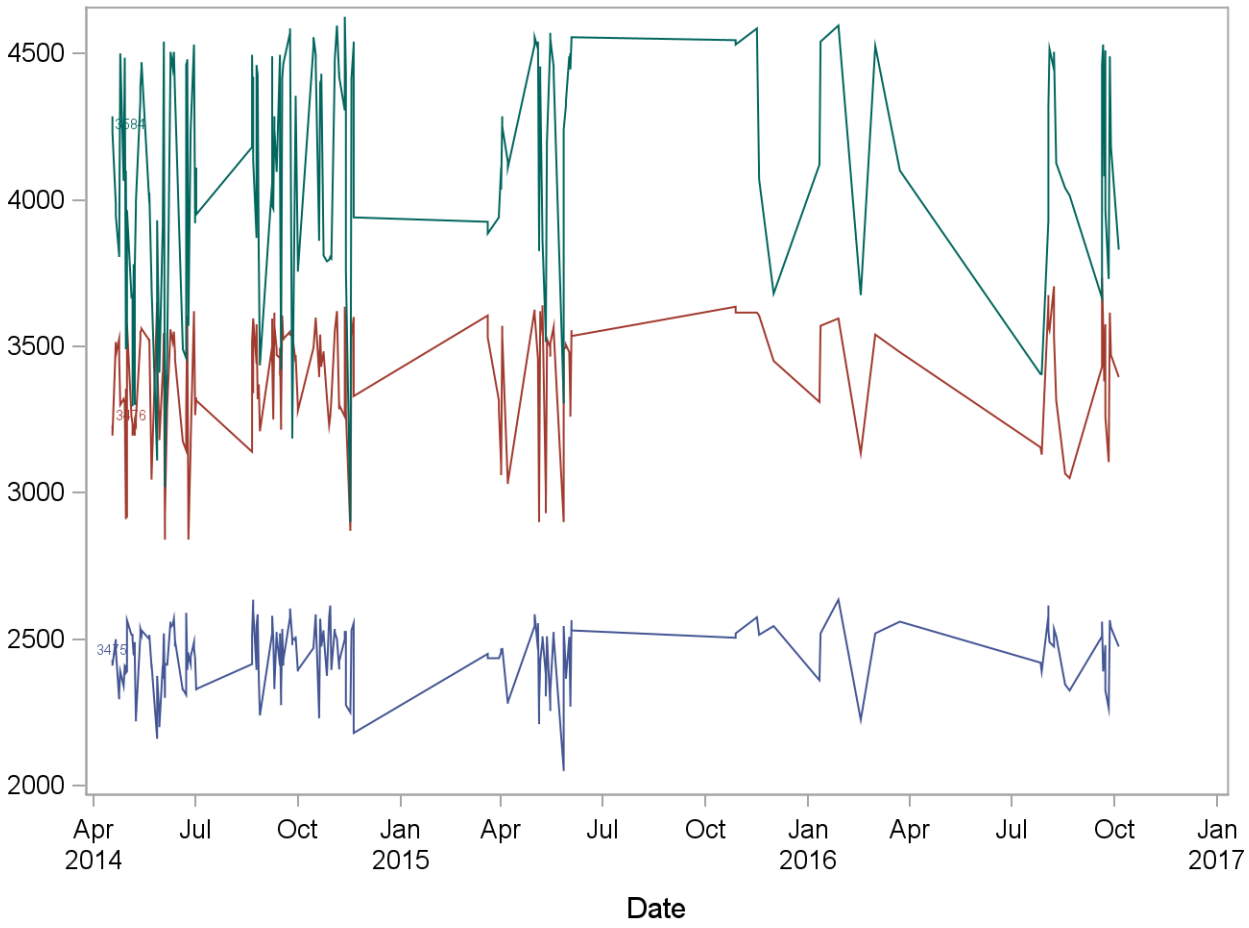
2013-2014 Summary Statistics and QC Chart
LBXNR1 (Nervonic acid (24:1n-9))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	194	18APR14	05OCT16	71.37	2.12	3.0
3476	194	18APR14	05OCT16	83.05	2.44	2.9
3584	194	18APR14	05OCT16	90.92	2.46	2.7



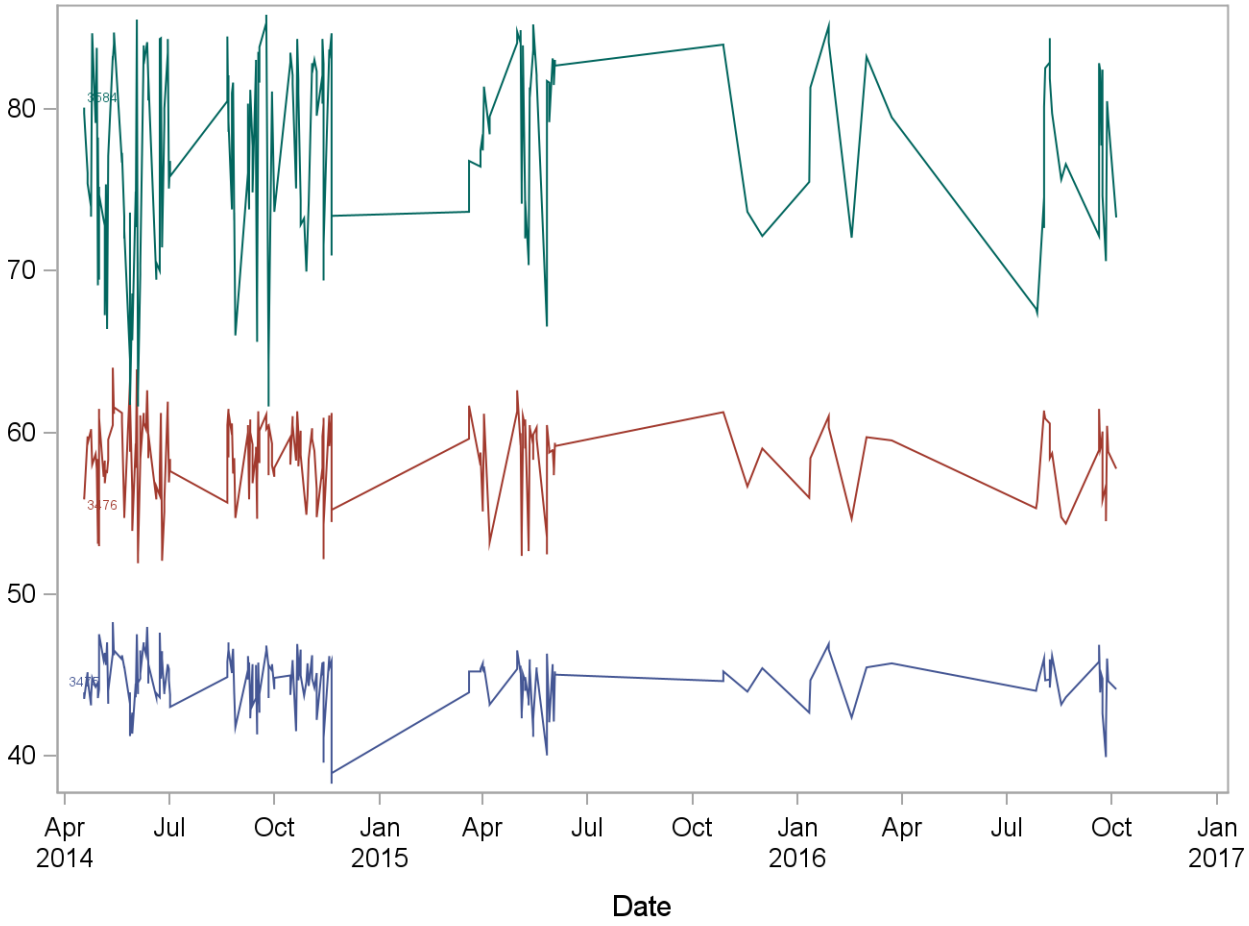
2013-2014 Summary Statistics and QC Chart
LBXLNA (Linoleic acid (18:2n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	215	18APR14	05OCT16	2443.0	110.1	4.5
3476	215	18APR14	05OCT16	3389.7	204.4	6.0
3584	215	18APR14	05OCT16	4091.4	401.8	9.8



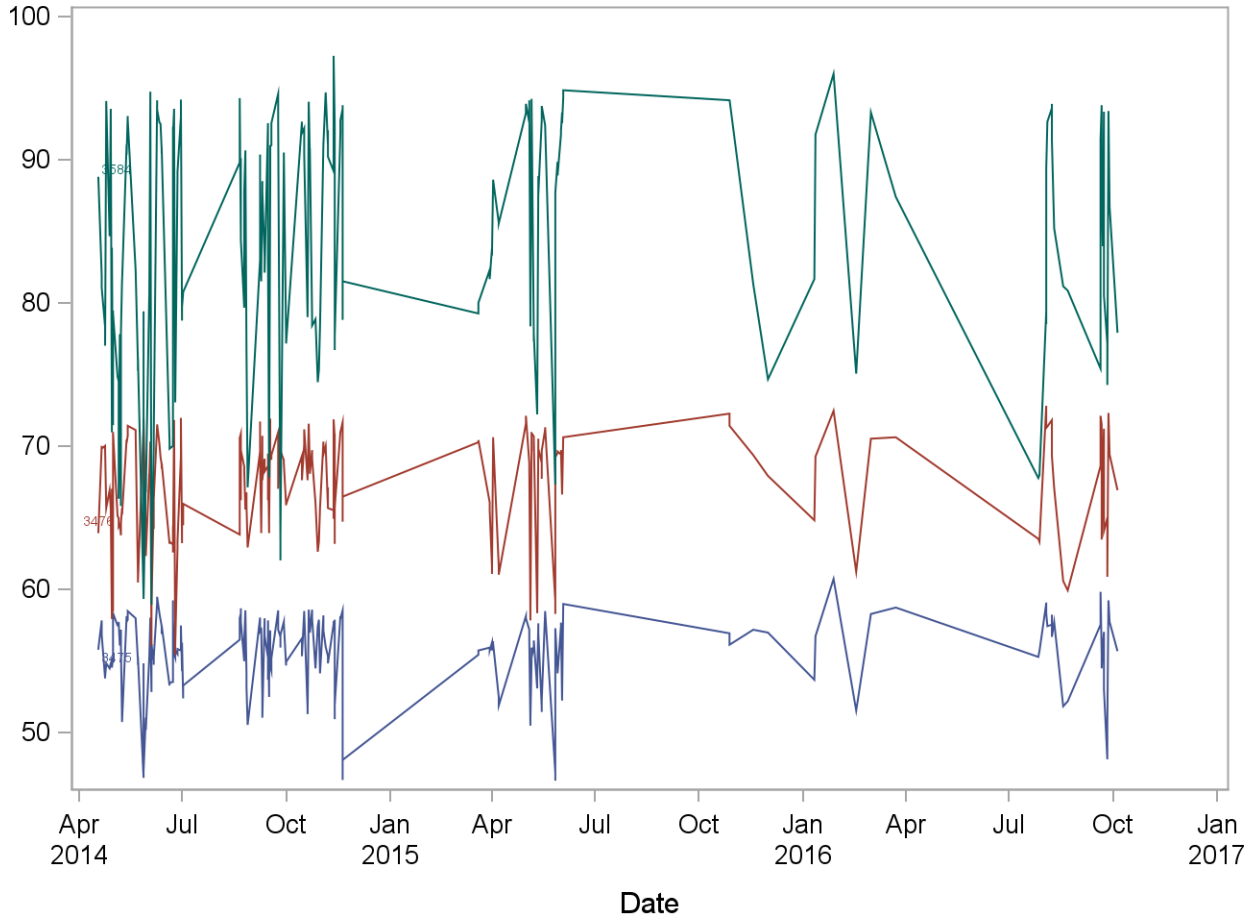
2013-2014 Summary Statistics and QC Chart
LBXALN (alpha-Linolenic acid (18:3n-3))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	215	18APR14	05OCT16	44.627	1.682	3.8
3476	215	18APR14	05OCT16	58.393	2.601	4.5
3584	215	18APR14	05OCT16	77.566	5.908	7.6



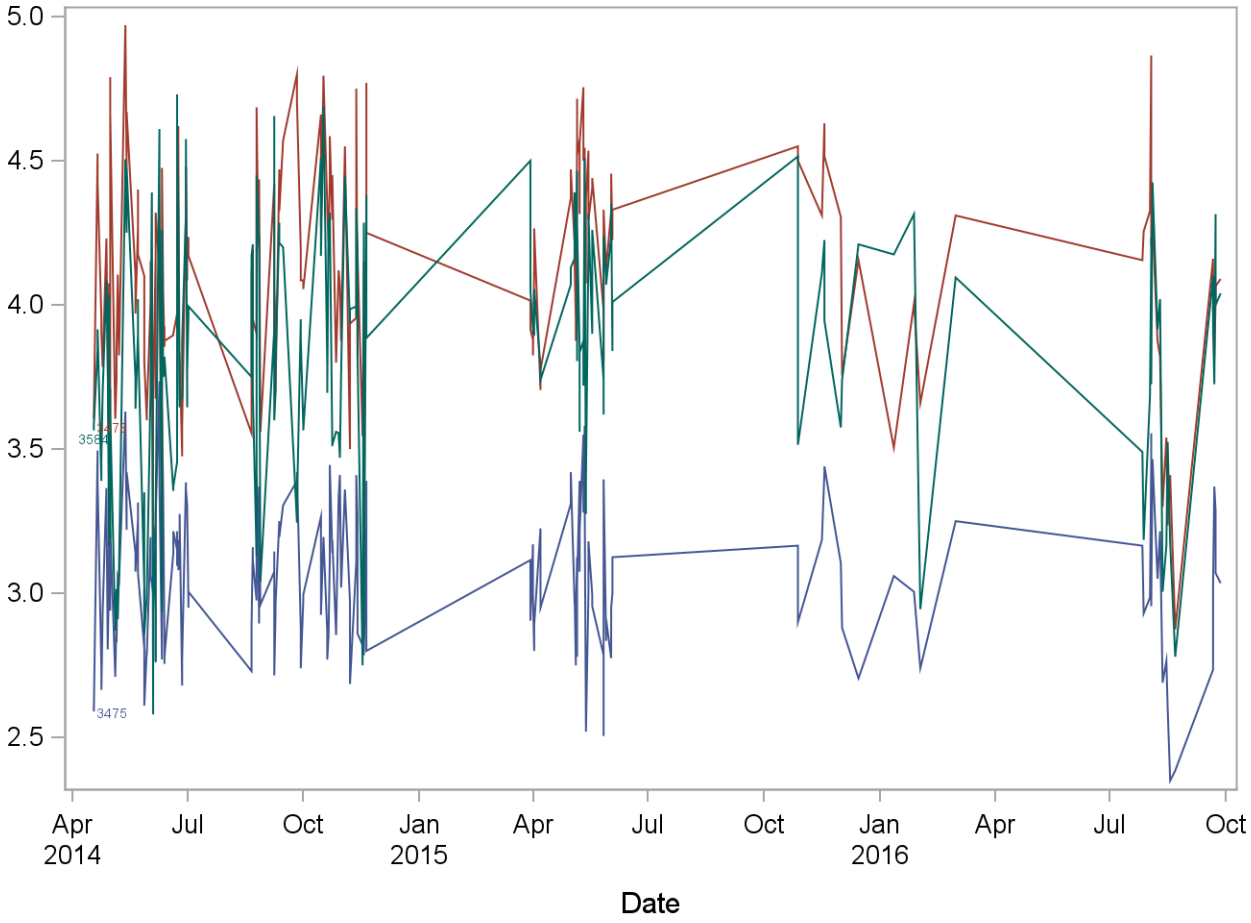
2013-2014 Summary Statistics and QC Chart
LBXGLA (gamma-Linolenic acid (18:3n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	217	18APR14	05OCT16	55.620	2.715	4.9
3476	217	18APR14	05OCT16	67.246	3.933	5.8
3584	217	18APR14	05OCT16	83.858	9.099	10.9



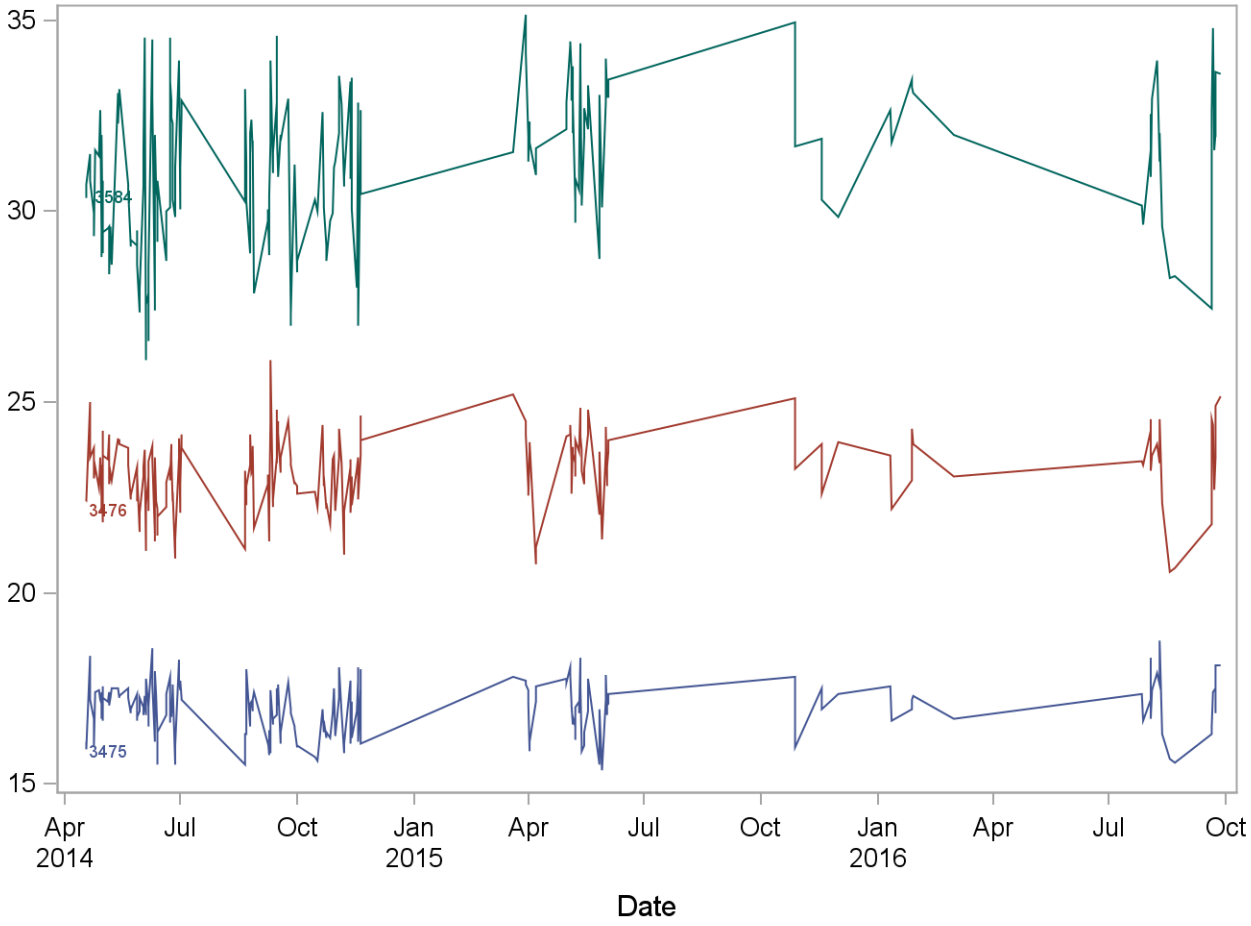
2013-2014 Summary Statistics and QC Chart
LBXSD1 (Stearidonic acid (C18:4n-3))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	196	18APR14	27SEP16	3.0603	0.2595	8.5
3476	196	18APR14	27SEP16	4.1143	0.3760	9.1
3584	196	18APR14	27SEP16	3.8336	0.4677	12.2



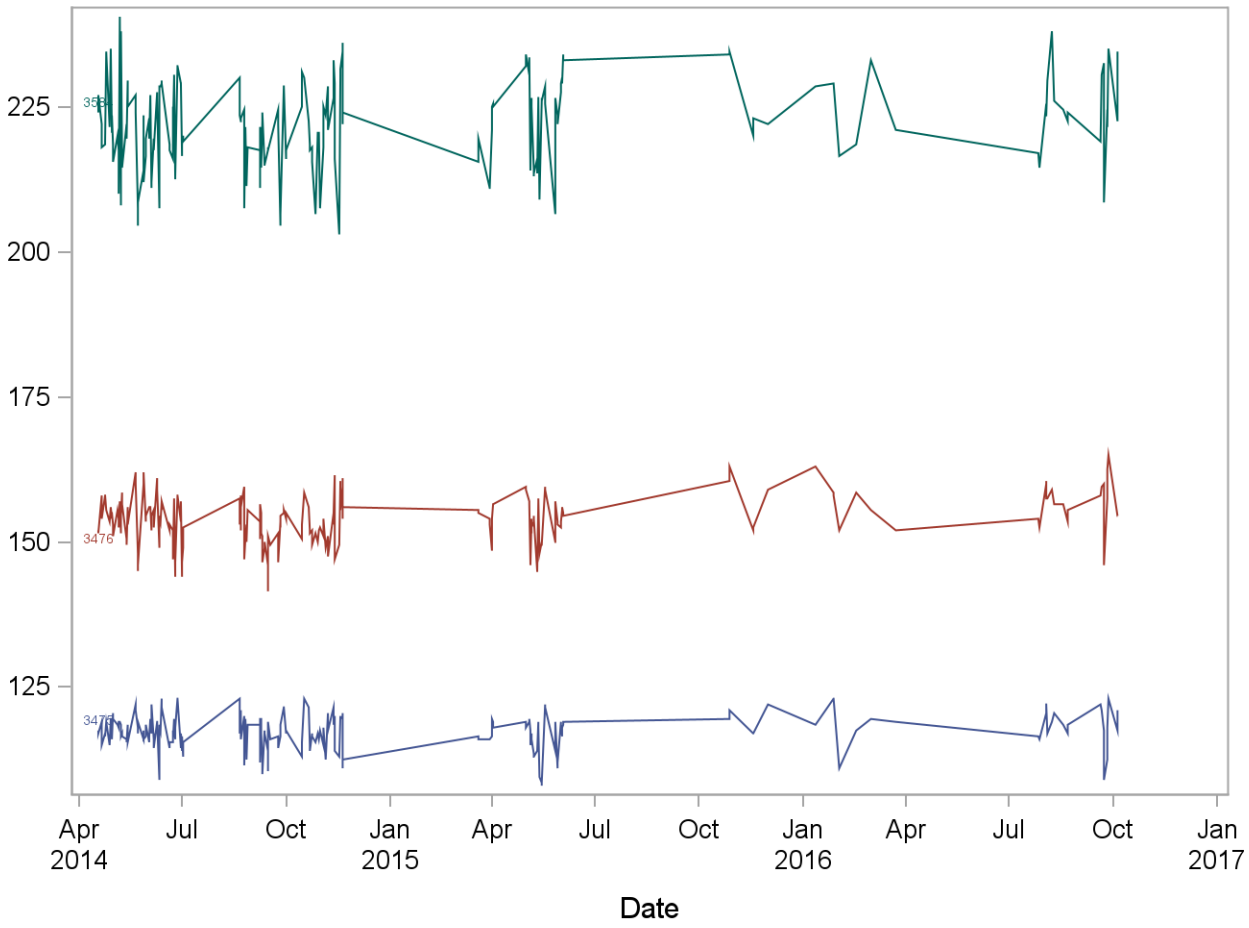
2013-2014 Summary Statistics and QC Chart
LBXED1 (Eicosadienoic acid (20:2n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	205	18APR14	27SEP16	16.954	0.687	4.1
3476	205	18APR14	27SEP16	23.195	0.965	4.2
3584	205	18APR14	27SEP16	31.185	1.930	6.2



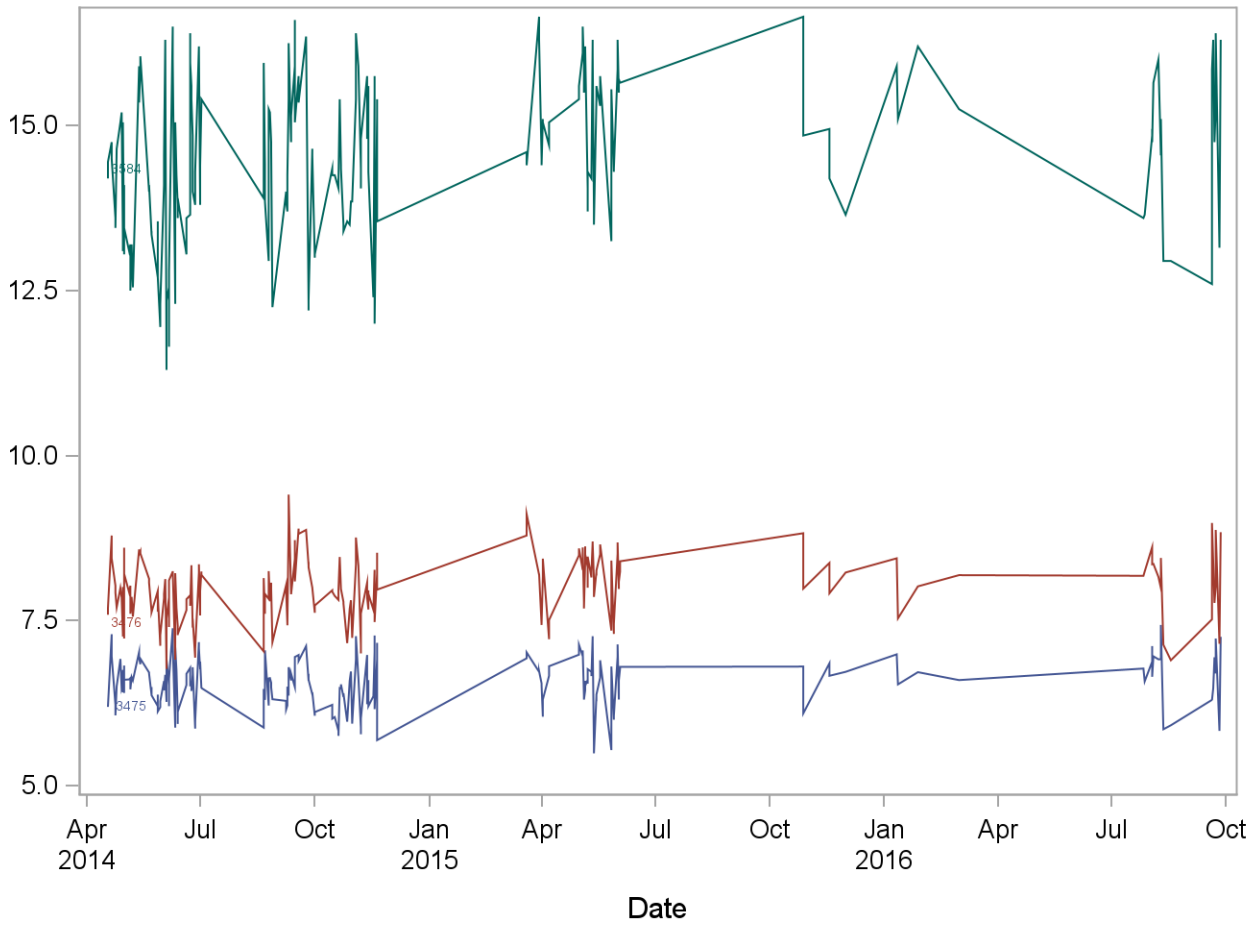
2013-2014 Summary Statistics and QC Chart
LBXHGL (homo-gamma-Linolenic acid (20:3n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	205	18APR14	05OCT16	117.229	3.143	2.7
3476	205	18APR14	05OCT16	153.990	4.250	2.8
3584	205	18APR14	05OCT16	221.907	7.686	3.5



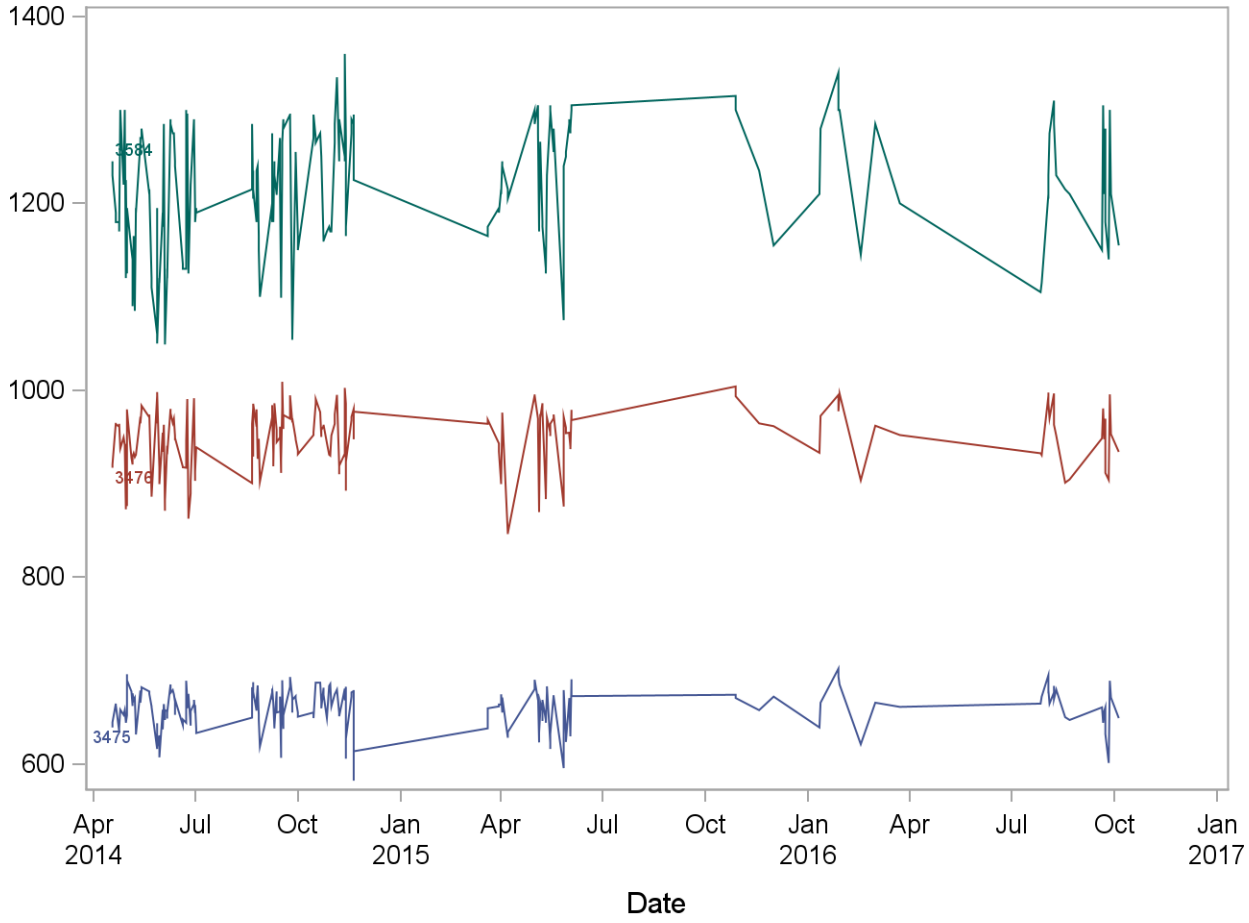
2013-2014 Summary Statistics and QC Chart
LBXET1 (Eicosatrienoic acid (C20:3n-9))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	207	18APR14	27SEP16	6.545	0.390	6.0
3476	207	18APR14	27SEP16	7.998	0.490	6.1
3584	207	18APR14	27SEP16	14.538	1.206	8.3



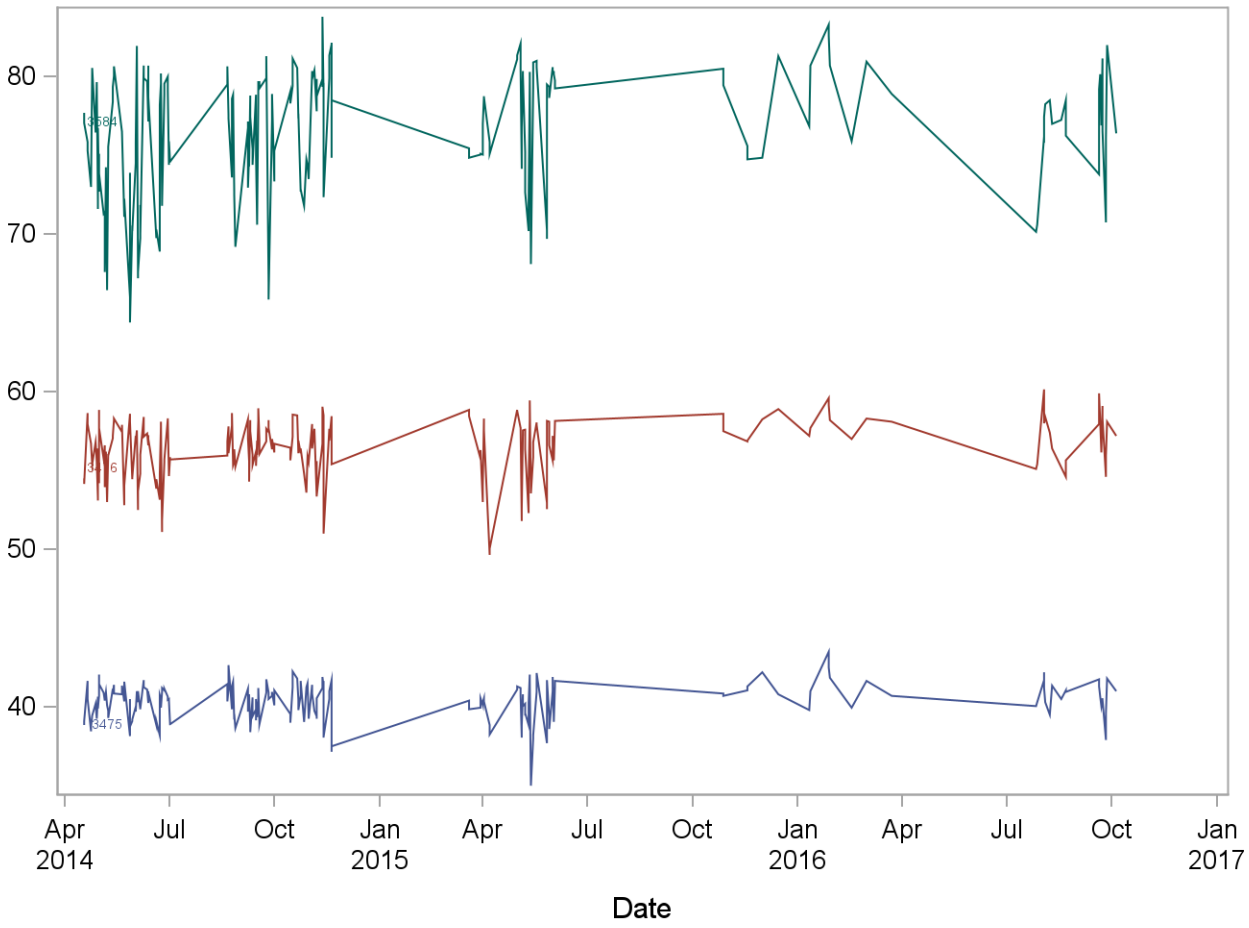
2013-2014 Summary Statistics and QC Chart
LBXARA (Arachidonic acid (20:4n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	213	18APR14	05OCT16	659.6	21.6	3.3
3476	213	18APR14	05OCT16	948.6	34.5	3.6
3584	213	18APR14	05OCT16	1215.9	68.3	5.6



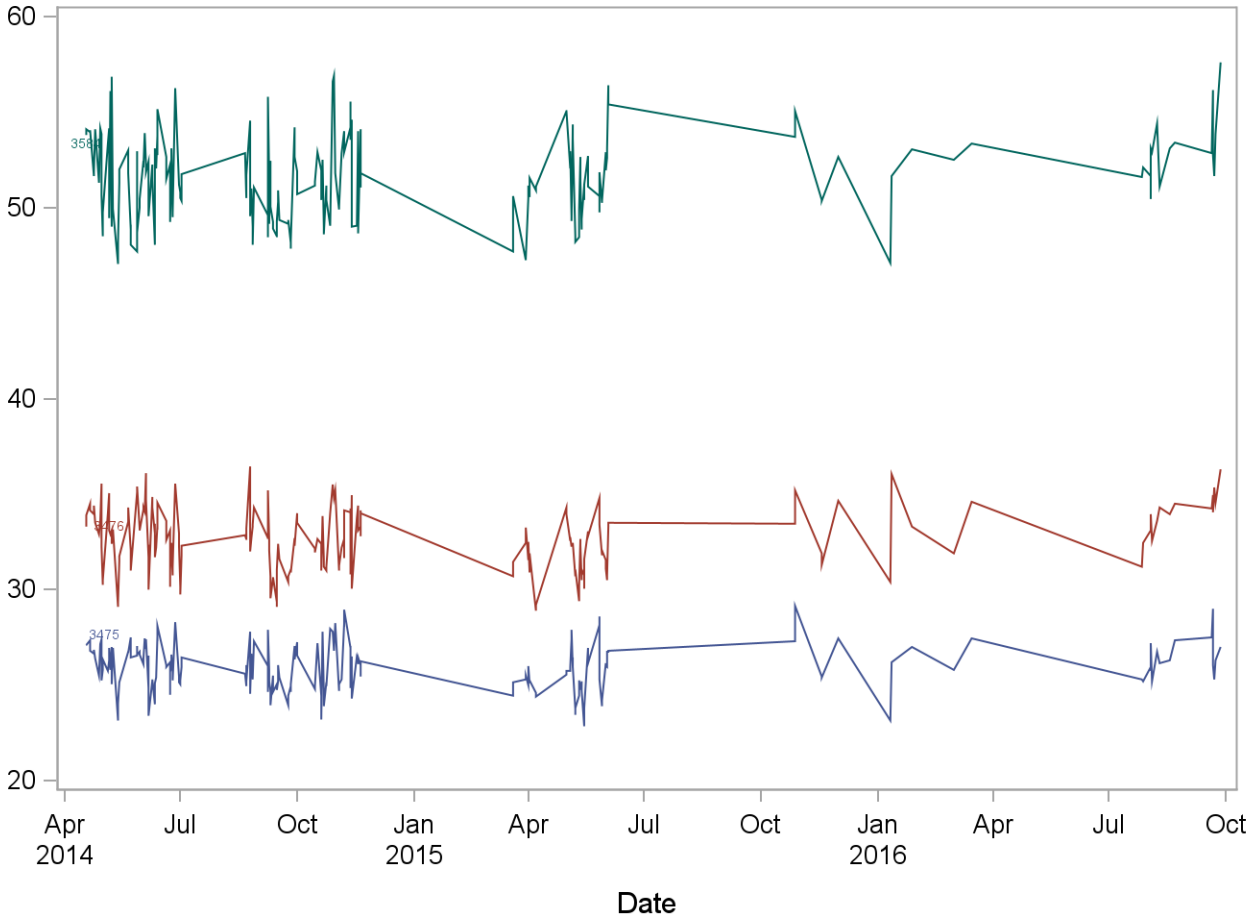
2013-2014 Summary Statistics and QC Chart
LBXEPA (Eicosapentaenoic acid (20:5n-3))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	214	18APR14	05OCT16	40.315	1.179	2.9
3476	214	18APR14	05OCT16	56.417	1.929	3.4
3584	214	18APR14	05OCT16	76.365	4.046	5.3



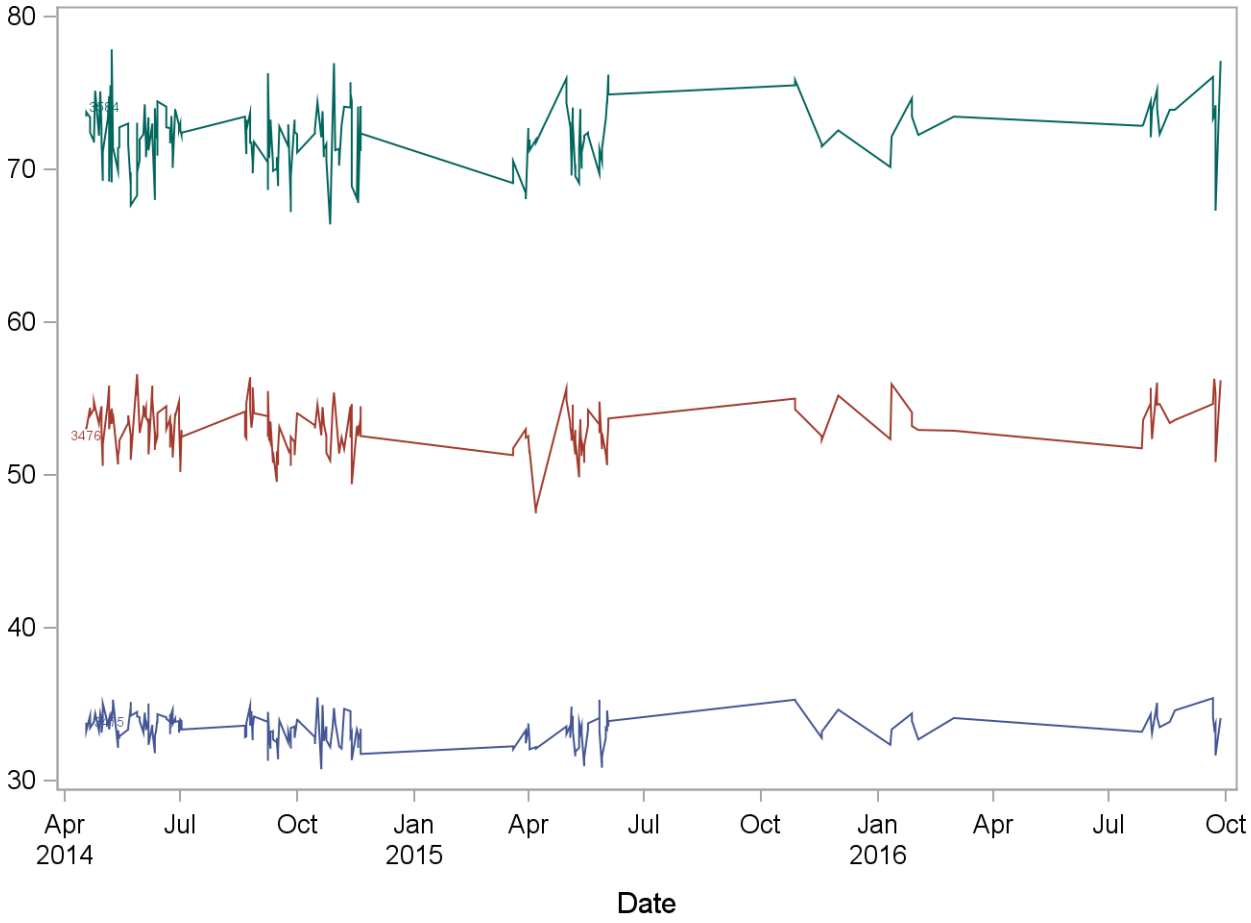
2013-2014 Summary Statistics and QC Chart
LBXDTA (Docosatetraenoic acid (22:4n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	208	18APR14	27SEP16	25.919	1.238	4.8
3476	208	18APR14	27SEP16	32.649	1.636	5.0
3584	208	18APR14	27SEP16	51.519	2.223	4.3



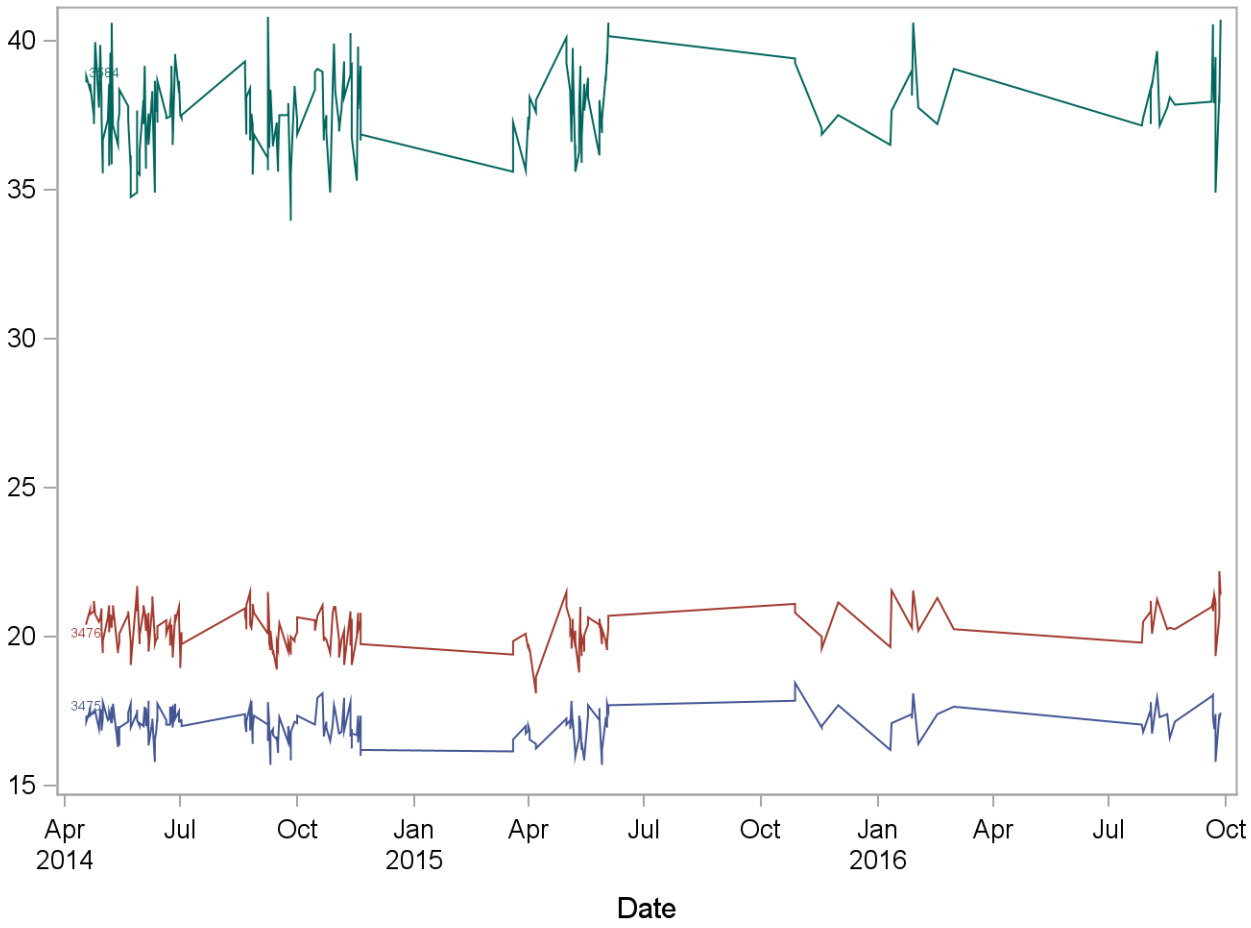
2013-2014 Summary Statistics and QC Chart
LBXDP3 (Docosapentaenoic acid (22:5n-3))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	209	18APR14	27SEP16	33.400	0.965	2.9
3476	209	18APR14	27SEP16	53.044	1.575	3.0
3584	209	18APR14	27SEP16	72.111	2.032	2.8



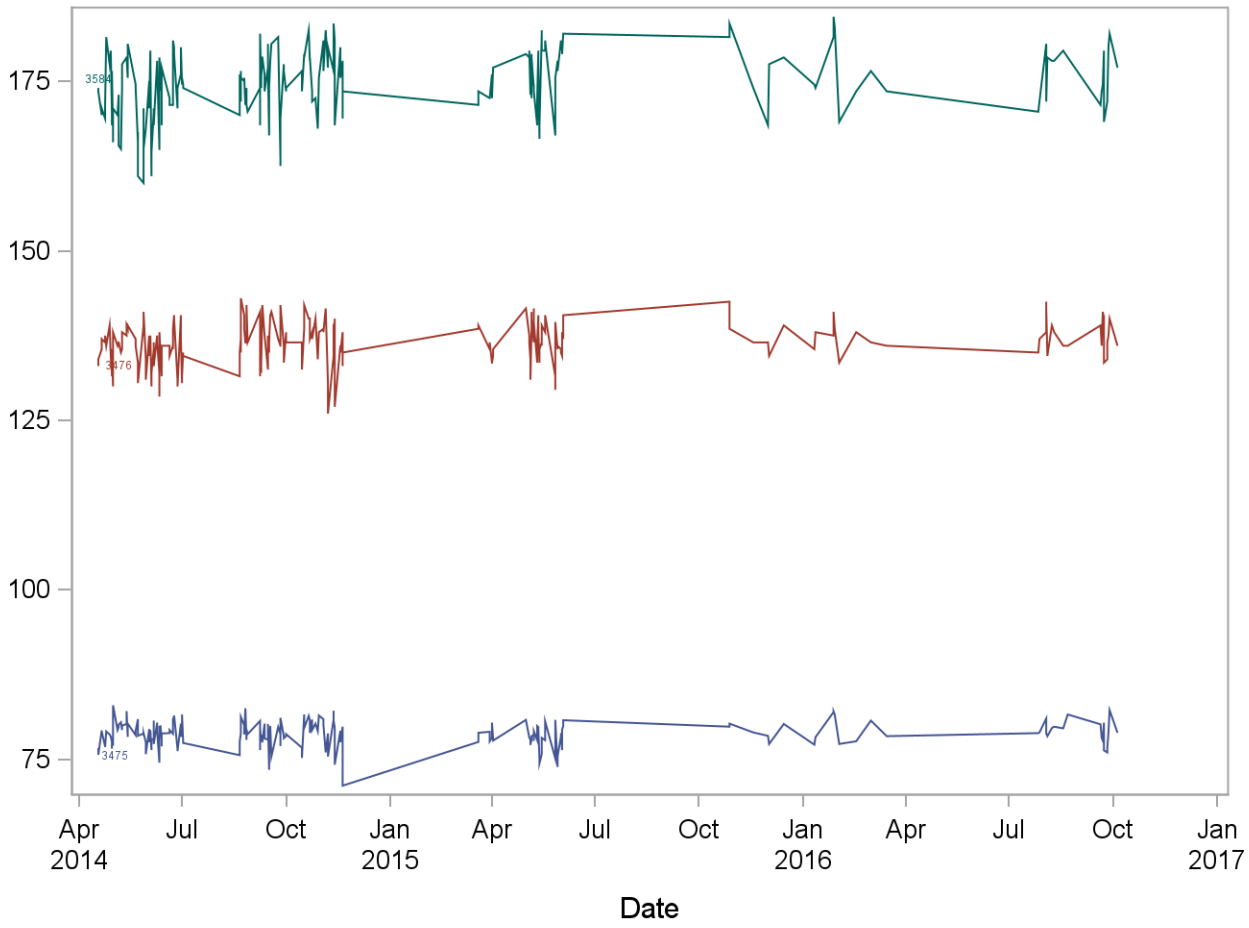
2013-2014 Summary Statistics and QC Chart
LBXDP6 (Docosapentaenoic acid (22:5n-6))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	211	18APR14	27SEP16	17.073	0.532	3.1
3476	211	18APR14	27SEP16	20.276	0.652	3.2
3584	211	18APR14	27SEP16	37.674	1.338	3.6



2013-2014 Summary Statistics and QC Chart
LBXDHA (Docosahexaenoic acid (22:6n-3))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
3475	208	18APR14	05OCT16	78.689	1.952	2.5
3476	208	18APR14	05OCT16	136.435	3.170	2.3
3584	208	18APR14	05OCT16	174.700	4.963	2.8



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Acknowledgments

We gratefully acknowledge the contributions of Leslie F McCoy, PhD who assisted in developing this methodology for the CDC Nutrition Lab.

Appendix A: Method Performance

Accuracy using Spike Recovery

1. *alpha*-Linolenic acid (C18:3n-3, ALN)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ALN**

		Sample 1: Aalto stripped serum					Sample 2: Low QC					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	25.8	30.2	29.0			19.7	18.8	19.2			
	2		33.0	26.4			0	19.9	18.9			100.0	2.8
	3		29.9	28.7				19.9	18.3				
Sample + Spike 1	1	199	243	228	230	101.0		317	292	310	97.5		
	2		228	227			298	308	323				
	3		234	218				308	310				
Sample + Spike 2	1	397	420	418	427	100.1		413	419	408	97.9		
	2		475	418			397	401	407				
	3		436	392				396	413				
Sample + Spike 3	1	662	725	765	724	105.0		514	519	509	98.7		
	2		682	740			497	494	514				
	3		754	678				483	531				

2. Arachidic acid (C20:0, AR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**AR1**

		Sample 1: Aalto stripped serum					Sample 2: Low QC					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	17.1	20.5				8.42	8.42				
	2		19.2	20.9	20.5		0	8.54	8.88	8.6		96.9	4.6
	3		22.4	22.8				8.75	8.7				
Sample + Spike 1	1		44.0	50.5				52.4	49.2				
	2	28.5	39.7	49.1	46.6	91.4	42.8	52.7	54.7	51.9	101.0		
	3		44.6	51.4				48.8	53.4				
Sample + Spike 2	1		68.8	77.7				65.0	68.1				
	2	57.1	82.7	72.4	74.1	93.9	57.1	63.2	67.8	65.6	99.8		
	3		67.0	75.8				62.9	66.4				
Sample + Spike 3	1		106	102				83.7	84.6				
	2	95.1	103	133	109	93.1	71.3	83.7	81.8	81.6	102.3		
	3		110	101				72.5	83.0				

3. Arachidonic acid (C20:4n-6, ARA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ARA**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		935	1051				292	282				
	2	0	1113	983	1028		0	299	290	289		104.2	4.7
	3		1030	1054				299	274				
Sample + Spike 1	1		1794	1703				1345	1232				
	2	690	1562	1853	1747	104.2	1035	1312	1383	1320	99.6		
	3		1697	1871				1310	1339				
Sample + Spike 2	1		2406	2412				1692	1723				
	2	1380	2616	2640	2520	108.2	1380	1658	1667	1677	100.5		
	3		2465	2583				1624	1696				
Sample + Spike 3	1		3575	3726				2051	2070				
	2	2300	3411	3720	3586	111.2	1725	1974	2057	2035	101.2		
	3		3489	3596				1947	2114				

4. Capric acid (C10:0, CAP)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**CAP**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		1.23	1.80				1.62	1.57				
	2	0	1.29	1.32	1.5		0	1.77	1.65	1.8		96.1	4.5
	3		1.60	1.55				2.52	1.80				
Sample + Spike 1	1		11.8	17.6				23.5	18.9				
	2	12.9	12.8	14.5	14.0	97.5	20.2	22.7	21.3	21.6	97.7		
	3		13.1	14.4				22.8	20.1				
Sample + Spike 2	1		26.0	24.8				28.9	27.4				
	2	26.9	27.0	31.5	26.3	92.0	26.9	28.7	26.5	27.6	95.6		
	3		25.5	22.8				27.5	26.5				
Sample + Spike 3	1		57.6	42.1				34.2	32.2				
	2	45.7	50.2	44.0	48.5	103.0	34.3	33.1	32.3	32.8	90.5		
	3		57.5	39.4				32.2	32.9				

5. Docosanoic acid (C22:0, DA1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DA1**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	54.3	61.0	60.9		0	25.5	25.0	25.4		98.5	3.2
	2		65.2	58.8				25.5	25.9				
	3		62.8	63.4				25.5	25.0				
Sample + Spike 1	1	58.2	121	123	119	100.2	87.3	110	101	108	94.2		
	2		107	128				107	111				
	3		113	123				108	109				
Sample + Spike 2	1	116	154	177	178	100.3	116	140	139	137	96.1		
	2		198	183				136	137				
	3		172	183				134	137				
Sample + Spike 3	1	194	271	234	260	102.8	146	174	168	167	97.6		
	2		276	300				168	168				
	3		255	226				157	170				

6. Docosahexaenoic acid (C22:6n-3, DHA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DHA**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	89.8	92.1	93.0		0	36.7	35.2	36.4		98.9	2.6
	2		93.9	96.4				36.2	36.5				
	3		89.0	97.2				38.2	35.3				
Sample + Spike 1	1	242	333	341	341	102.6	363	403	360	392	98.1		
	2		337	354				391	405				
	3		337	344				405	390				
Sample + Spike 2	1	484	561	548	569	98.4	484	524	525	507	97.2		
	2		528	629				501	487				
	3		564	585				501	502				
Sample + Spike 3	1	806	885	987	911	101.5	605	639	621	615	95.7		
	2		877	947				599	608				
	3		891	878				595	629				

7. Docosapentaenoic acid (C22:5n-3, DP3)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DP3**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	47.4	50.9			0	16.1	15.9			102.3	5.6
	2		47.0	50.8	48.9			16.0	16.2	16.2			
	3		44.8	52.6				16.7	16.3				
Sample + Spike 1	1	76.2	127	133			114	129	118				
	2		121	136	131	107.8		126	130	129	98.4		
	3		130	139				137	132				
Sample + Spike 2	1	152	209	214			152	173	169				
	2		210	245	215	108.7		162	162	165	97.7		
	3		207	204				160	165				
Sample + Spike 3	1	254	308	339			191	204	204				
	2		288	330	316	105.3		196	203	199	95.9		
	3		320	314				187	199				

8. Docosapentaenoic acid (C22:5n-6, DP6)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DP6**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1	0	24.6	29.7			0	8.19	7.99			102.3	6.7
	2		24.7	26.9	26.1			8.32	8.32	8.2			
	3		23.5	27.1				8.43	8.12				
Sample + Spike 1	1	45.0	75.9	75.8			67.5	75.0	67.5				
	2		73.8	84.3	76.3	111.5		73.5	74.1	74.1	97.7		
	3		69.5	78.3				79.0	75.8				
Sample + Spike 2	1	90	122	116			90.0	101	97.2				
	2		125	131	123	107.3		93.7	92.9	95.6	97.1		
	3		117	124				93.3	95.5				
Sample + Spike 3	1	150	209	201			113	119	117				
	2		145	183	184	105.3		114	117	115	94.9		
	3		187	179				109	114				

9. Docosatetraenoic acid (C22:4n-6, DTA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DTA**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		39.5	41.2				12.7	13.0				
	2	0	37.0	36.4	37.5		0	12.4	13.6	13.0		107.2	12.0
	3		33.1	37.7				13.0	13.5				
Sample + Spike 1	1		87.7	90.9				79.7	73.7				
	2	46.5	90.3	94.7	92.0	117.3	69.75	78.1	80.5	80.9	97.3		
	3		89.1	99.4				87.9	85.4				
Sample + Spike 2	1		135	145				110	104				
	2	93	142	161	146	116.6	93	101	103	104	97.5		
	3		139	154				99.6	104				
Sample + Spike 3	1		235	269				125	127				
	2	155	158	239	224	120.3	116.25	123	126	123	94.3		
	3		214	229				115	120				

10. Eicosadienoic acid (C20:2n-6, ED1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ED1**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		12.3	14.4				8.18	8.24				
	2	0	13.4	12.1	12.9		0	8.28	8.66	8.4		96.3	4.7
	3		11.8	13.3				8.78	8.26				
Sample + Spike 1	1		35.2	38.7				54.4	50.3				
	2	29.04	38.2	38.2	40.6	95.6	43.56	55.1	56.7	53.7	103.9		
	3		38.1	55.4				51.5	54.0				
Sample + Spike 2	1		62.9	66.4				65.0	67.1				
	2	58.08	67.3	71.7	66.3	92.0	58.08	64.9	65.7	65.3	98.0		
	3		64.8	64.9				63.5	65.7				
Sample + Spike 3	1		93.7	105.6				80.9	80.7				
	2	96.8	95.5	109.6	100.9	90.9	72.6	79.3	79.2	79.2	97.5		
	3		104.4	96.6				74.2	80.7				

11. Eicosenoic acid (C20:1n-9, EN1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**EN1**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		7.61	7.89				3.91	3.93				
	2	0	7.94	8.01	7.77		0	3.91	4.08	4.0		95.3	4.2
	3		6.81	8.34				4.25	4.01				
Sample + Spike 1	1		33.2	34.4				51.8	48.7				
	2	30.6	35.5	32.8	35.6	90.9	45.9	51.7	53.5	50.9	102.2		
	3		35.9	41.5				48.7	51.2				
Sample + Spike 2	1		63.5	66.5				62.6	65.4				
	2	61.2	66.9	63.0	64.6	92.8	61.2	63.0	64.8	63.6	97.4		
	3		63.8	63.7				61.6	64.3				
Sample + Spike 3	1		98.2	106				78.5	79.0				
	2	102	99.1	105	102	92.5	76.5	77.6	77.2	77.4	95.9		
	3		108	96.1				72.6	79.6				

12. Eicosapentaenoic acid (C20:5n-3, EPA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**EPA**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		31.1	36.6				19.4	18.5				
	2	0	34.3	34.2	35.2		0	19.7	19.0	18.9		98.2	2.8
	3		37.0	38.3				18.9	18.1				
Sample + Spike 1	1		159	154				197	187				
	2	119	149	155	155	100.3	179	195	199	195	98.5		
	3		154	157				194	198				
Sample + Spike 2	1		268	266				254	251				
	2	238	279	273	270	98.7	238	242	248	247	95.9		
	3		276	260				238	252				
Sample + Spike 3	1		471	432				298	309				
	2	397	446	435	440	101.8	298	289	303	299	94.2		
	3		447	406				288	310				

13. Eicosatrienoic acid (C20:3n-9, ET1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ET1**

		Sample 1: Aalto stripped serum					Sample 2: Low QC					
			Measured concentration					Measured concentration				
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%) / SD (%)
Sample	1		8.22	9.99				3.07	3.02			
	2	0	9.23	8.01	8.69		0	3.19	3.26	3.1		89.6 / 4.6
	3		7.55	9.10				3.38	2.91			
Sample + Spike 1	1		22.0	25.8				34.8	31.8			
	2	20.8	24.1	25.9	26.2	84.3	32.0	35.2	35.9	33.9	96.3	
	3		24.1	35.4				32.5	33.4			
Sample + Spike 2	1		40.2	45.6				41.3	42.8			
	2	42.7	44.2	51.4	44.9	84.8	42.7	41.7	41.6	41.7	90.3	
	3		42.6	45.3				40.9	41.7			
Sample + Spike 3	1		61.1	88.3				52.4	51.5			
	2	71.8	64.4	91.6	75.3	92.8	53.8	50.6	50.6	51.0	88.8	
	3		70.9	75.7				48.3	52.3			

14. *gamma*-Linolenic acid (C18:3n-6, GLA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**GLA**

		Sample 1: Aalto stripped serum					Sample 2: Low QC					
			Measured concentration					Measured concentration				
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%) / SD (%)
Sample	1		36.4	43.1				23.7	22.4			
	2	0	51.3	37.1	42.7		0	24.9	23.1	23.4		101.9 / 4.8
	3		43.8	44.4				25.4	21.1			
Sample + Spike 1	1		142	138				161	147			
	2	89.7	123	138	139	107.9	135	157	162	157	99.6	
	3		161	135				159	158			
Sample + Spike 2	1		228	207				205	204			
	2	179	228	224	219	98.2	179	200	199	201	99.1	
	3		225	201				196	203			
Sample + Spike 3	1		374	358				251	247			
	2	299	396	348	366	108.2	224	238	247	245	98.6	
	3		374	348				233	251			

15. *homo-gamma*-Linolenic acid (C20:3n-6, HGL)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**HGL**

		Sample 1: Aalto stripped serum					Sample 2: Low QC						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		137	132				55.7	55.0				
	2	0	172	108	132		0	56.1	55.9	55.7		95.0	4.7
	3		125	120				55.7	55.9				
Sample + Spike 1	1		272	283				255	239				
	2	139	295	289	277	104.3	208	248	258	253	94.6		
	3		269	255				256	261				
Sample + Spike 2	1		378	373				324	322				
	2	278	330	446	388	92.1	278	311	320	318	94.3		
	3		419	383				305	324				
Sample + Spike 3	1		603	584				378	387				
	2	463	499	611	558	92.0	347	369	390	377	92.4		
	3		514	539				353	384				

16. Lauric acid (C12:0, LAR)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LAR**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		10.8	14.0				5.62	5.00				
	2	0	13.4	12.1	12.8		0	5.24	4.62	5.30		97.6	9.0
	3		11.9	14.7				6.42	4.88				
Sample + Spike 1	1		34.4	39.7				40.7	36.8				
	2	24.8	34.6	40.9	37.6	99.6	37.7	39.7	41.5	39.8	91.7		
	3		37.1	38.8				39.9	40.3				
Sample + Spike 2	1		64.1	59.9				51.6	50.8				
	2	50.2	80.1	67.0	66.0	106.0	50.2	50.3	50.0	50.3	89.6		
	3		60.2	64.9				48.4	50.6				
Sample + Spike 3	1		114	100				63.1	61.8				
	2	84.0	99.7	110	105	109.9	63.0	60.7	62.0	61.2	88.7		
	3		105	103				58.1	61.3				

17. Lignoceric acid (C24:0, LG1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LG1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		44.3	53.7				23.2	22.7				
	2	0	57.8	49.6	52.1		0	23.1	23.2	22.9		97.2	2.0
	3		52.7	54.7				23.4	22.1				
Sample + Spike 1	1		97.8	95.0				83.9	76.4				
	2	42.0	83.1	103	93.7	99.1	63.0	82.9	84.6	82.7	94.9		
	3		88.0	95.4				84.3	84.2				
Sample + Spike 2	1		118	132				104	104				
	2	84.0	146	137	133	96.9	84.0	104	102	103	95.4		
	3		127	141				101	103				
Sample + Spike 3	1		196	179				133	124				
	2	140	203	222	192	100.0	105	126	124	125	97.2		
	3		189	163				117	126				

18. Linoleic acid (C18:2n-6, LNA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LNA**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		1827	2264				1047	991				
	2	0	2132	2001	2102		0	1078	1028	1031		98.2	1.2
	3		2085	2303				1096	946				
Sample + Spike 1	1		4847	4643				4934	4571				
	2	2591	4139	4765	4680	99.5	3886	4831	5083	4861	98.6		
	3		4635	5053				4838	4910				
Sample + Spike 2	1		7225	7145				6155	6246				
	2	5181	7295	7269	7254	99.4	5181	6074	6126	6117	98.2		
	3		7103	7488				5924	6177				
Sample + Spike 3	1		10779	10555				7401	7403				
	2	8635	10598	10344	10456	96.8	6476	7180	7349	7312	97.0		
	3		10334	10130				7026	7516				

19. Myristoleic acid (C14:1n-5, ML1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ML1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measured concentration				Mean	SD
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	(%)
Sample	1		3.76	5.46				3.52	3.39				
	2	0	3.59	4.95	4.68		0	3.66	3.46	3.5		93.3	3.8
	3		4.53	5.80				3.63	3.20				
Sample + Spike 1	1		44.7	47.4				71.8	64.6				
	2	44.7	43.7	43.3	45.3	90.9	67.1	70.0	72.1	69.5	98.5		
	3		42.8	49.9				69.4	69.0				
Sample + Spike 2	1		87.2	82.0				91.2	91.4				
	2	89.4	90.7	77.3	83.9	88.6	89.4	90.3	88.4	89.8	96.6		
	3		74.0	92.0				87.9	89.8				
Sample + Spike 3	1		133	138				113	107				
	2	149	145	148	140	90.9	112	107	109	109	94.5		
	3		123	154				106	111				

20. Myristic acid (C14:0, MR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**MR1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur				Mean	SD
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	(%)
Sample	1		75.1	87.6				43.9	42.2	43.3			
	2	0	71.3	89.2	83.3		0	44.6	43.6			98.4	1.6
	3		80.1	96.7				44.8	40.6				
Sample + Spike 1	1		409	411				536	501	527	100.1		
	2	323	382	445	409	100.9	484	522	560				
	3		392	414				520	525				
Sample + Spike 2	1		685	696				667	705	673	97.7		
	2	645	779	745	709	97.0	645	667	674				
	3		612	735				650	677				
Sample + Spike 3	1		1173	1028				857	834	833	97.9		
	2	1075	1157	1230	1126	97.0	806	835	815				
	3		1055	1115				795	860				

21. Margaric acid (C17:0, MRG)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**MRG**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		31.2	34.2				9.71	9.54	9.5			
	2	0	33.2	34.5	34.3		0	9.76	9.69			89.4	10.4
	3		35.0	37.6				9.55	9.00				
Sample + Spike 1	1		48.8	46.4				33.8	31.0	33.3	98.2		
	2	17.3	43.5	50.5	47.8	77.9	24.2	33.3	34.8				
	3		47.6	49.6				33.1	33.7				
Sample + Spike 2	1		53.4	56.9				40.9	41.9	40.9	97.4		
	2	32.2	64.2	61.1	59.9	79.5	32.2	40.9	41.0				
	3		61.8	61.8				39.8	41.1				
Sample + Spike 3	1		78.4	75.7				51.2	49.1	48.9	100.7		
	2	52.2	78.8	83.2	77.5	82.9	39.1	49.2	49.5				
	3		79.2	69.9				45.0	49.6				

22. Nervonic acid (C24:1n-9, NR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**NR1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measured concentration					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		70.6	104				34.3	35.4				
	2	0	93.4	90.7	88.7		0	35.8	36.2	35.1		101.0	7.4
	3		85.1	88.6				34.6	34.0				
Sample + Spike 1	1		172	172				124	112				
	2	59.7	124	182	157	114.3	89.6	122	125	122	96.9		
	3		138	154				125	124				
Sample + Spike 2	1		151	223				149	150				
	2	119	229	243	212	103.0	119	150	148	148	94.6		
	3		193	231				143	149				
Sample + Spike 3	1		296	284				190	176				
	2	199	293	337	291	101.5	149	177	178	178	95.5		
	3		268	265				168	178				

23. Oleic acid (C18:1n-9, OL1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**OL1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		1206	1533				554	532	550			
	2	0	1424	1362	1414		0	564	556			97.8	1.2
	3		1404	1554				576	516				
Sample + Spike 1	1		4156	4011				4513	4176	4458	97.1		
	2	2685	3735	4200	4087	99.5	4027	4424	4681				
	3		4026	4393				4424	4529				
Sample + Spike 2	1		6526	6618				5777	5894	5728	96.4		
	2	5369	6770	6918	6722	98.9	5369	5667	5760				
	3		6223	7277				5470	5801				
Sample + Spike 3	1		10331	9825				7389	7183	7107	97.7		
	2	8949	10217	10815	10124	97.3	6712	7090	7137				
	3		9946	9609				6649	7197				

24. Pentadecanoic acid (C15:0, PDE)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PDE**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		17.5	20.5			0	6.19	5.90	6.1			
	2	0	20.2	18.1	19.5			6.31	6.09			98.0	7.2
	3		20.3	20.1				6.27	5.70				
Sample + Spike 1	1		37.2	34.5			24.0	28.9	26.6	28.5	93.4		
	2	15.1	33.0	37.7	36.1	110.5		28.3	29.6				
	3		36.9	37.3				28.5	29.3				
Sample + Spike 2	1		48.7	49.9			32.0	35.9	36.6	35.8	92.9		
	2	32.0	55.3	53.2	51.8	100.9		35.6	36.1				
	3		51.3	52.5				34.7	36.3				
Sample + Spike 3	1		75.7	70.3			41.0	45.7	43.6	43.5	91.3		
	2	54.7	74.7	81.4	73.7	99.3		43.8	43.8				
	3		73.3	67.1				40.4	43.8				

25. Palmitoleic acid (C16:1n-7, PL1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PL1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		90.0	139				77.0	71.3	75.1			
	2	0	115	122	118		0	79.7	74.7			102.4	6.1
	3		111	133				79.7	68.3				
Sample + Spike 1	1		932	852				1218	1158	1208	99.5		
	2	759	1098	990	961	111.1	1139	1190	1294				
	3		924	972				1184	1204				
Sample + Spike 2	1		1714	1541				1517	1626	1541	96.5		
	2	1518	1770	1833	1723	105.7	1518	1513	1548				
	3		1757	1722				1469	1573				
Sample + Spike 3	1		3001	2417				1879	1919	1892	95.8		
	2	2530	3236	2456	2790	105.6	1898	1841	1876				
	3		3137	2495				1825	2012				

26. Palmitic acid (C16:0, PM1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PM1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		1927	2259			0	885	872	883			
	2	0	2071	1997	2110			897	901			101.7	6.8
	3		2252	2153				893	853				
Sample + Spike 1	1		5078	4784			4316	5330	4716	5171	99.4		
	2	2877	4870	5438	5074	103.0		5216	5082				
	3		4564	5708				5320	5365				
Sample + Spike 2	1		7230	8192			5755	6631	6144	6400	95.9		
	2	5755	8556	9379	8505	111.1		6416	6412				
	3		7647	10026				6339	6459				
Sample + Spike 3	1		12438	11708			7193	8368	7652	7604	93.4		
	2	9591	12542	13258	12434	107.6		7849	7841				
	3		13301	11356				6900	7017				

27. Stearidonic acid (C18:4n-3, SD1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**SD1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur				Mean	SD
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	(%)
Sample	1		0.95	1.82				1.53	1.40	1.5			
	2	0	1.19	1.45	1.44		0	1.61	1.44			86.2	11.4
	3		1.30	1.93				1.56	1.28				
Sample + Spike 1	1		11.1	11.9				25.2	22.7	24.0	101.8		
	2	14.0	9.8	12.8	12.1	76.1	22.2	25.3	26.7				
	3		9.2	17.8				22.1	22.2				
Sample + Spike 2	1		22.7	25.7				28.8	31.1	29.5	94.8		
	2	29.6	21.6	26.3	23.3	73.9	29.6	30.0	28.2				
	3		17.7	25.9				29.7	29.1				
Sample + Spike 3	1		36.4	51.9				37.9	35.5	36.1	91.9		
	2	50.3	22.9	48.7	41.1	78.9	37.7	35.1	34.5				
	3		41.2	45.6				35.8	38.1				

28. Stearic acid (C18:0, ST1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ST1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur				Mean	SD
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	recovery (%)	(%)
Sample	1		619	849				241	243	238			
	2	0	624	790	703		0	239	245			102.3	8.7
	3		641	697				231	231				
Sample + Spike 1	1		1611	1764				1427	1347	1415	96.1		
	2	816	1597	1710	1598	109.5	1224	1394	1520				
	3		1507	1397				1375	1426				
Sample + Spike 2	1		2298	2329				1739	1870	1775	94.1		
	2	1633	2769	2385	2429	105.7	1633	1748	1792				
	3		2331	2461				1705	1797				
Sample + Spike 3	1		4278	3106				2214	2173	2164	94.4		
	2	2721	4353	3866	3813	114.3	2041	2169	2122				
	3		4184	3094				2045	2260				

29. Tricosanoic acid (C23:0, TSA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**TSA**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		27.7	26.9				10.5	10.4	10.5			
	2	0	35.3	31.5	31.7		0	10.6	10.6			96.4	4.5
	3		33.6	35.5				10.4	10.4				
Sample + Spike 1	1		41.6	40.6				30.6	28.1	30.1	92.1		
	2	14.4	40.4	53.2	45.5	95.9	21.3	29.6	31.1				
	3		47.0	50.4				30.3	31.1				
Sample + Spike 2	1		53.2	57.1				37.4	37.4	36.8	92.4		
	2	28.5	66.4	50.4	60.3	100.3	28.5	36.2	36.6				
	3		59.0	75.5				36.0	37.1				
Sample + Spike 3	1		77.2	70.4				46.0	44.3	44.0	94.7		
	2	47.2	87.2	76.2	80.4	103.2	35.4	44.5	44.4				
	3		82.1	89.5				40.6	44.1				

30. *cis*-Vaccenic acid (C18:1n-7, VC1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**VC1**

		Sample 1: Aalto stripped serum					Sample 2						
			Measured concentration					Measur					
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Mean recovery (%)	SD (%)
Sample	1		69.9	92.2			0	41.7	41.0	43.0			
	2	0	86.1	85.7	85.0			45.5	44.1			85.5	5.3
	3		79.0	97.3				43.6	42.3				
Sample + Spike 1	1		255	241			329	343	308	338	89.7		
	2	220	241	266	252	76.2		333	335				
	3		222	288				348	364				
Sample + Spike 2	1		455	458			439	458	431	434	89.1		
	2	439	437	477	447	82.3		428	436				
	3		394	458				423	429				
Sample + Spike 3	1		710	770			549	544	544	531	88.8		
	2	732	690	773	722	87.0		510	565				
	3		662	724				492	530				

Precision

The method precision of the 30 analytes in the low QC and high QC is shown below.

1. *alpha*-Linolenic acid (C18:3n-3, ALN)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ALN**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	45.3	44.2	44.75	0.3025	0.3025	4005.125
2	44.3	44.8	44.55	0.0625	0.0625	3969.405
3	41.2	43.7	42.45	1.5625	1.5625	3604.005
4	44.4	44.7	44.55	0.0225	0.0225	3969.405
5	43.2	44.1	43.65	0.2025	0.2025	3810.645
6	40.6	44.5	42.55	3.8025	3.8025	3621.005
7	41.1	44.6	42.85	3.0625	3.0625	3672.245
8	43.3	44.8	44.05	0.5625	0.5625	3880.805
9	39.8	39.9	39.85	0.0025	0.0025	3176.045
10	39.9	39.6	39.75	0.0225	0.0225	3160.125
Grand sum	858	**Grand mean**	42.9			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	19.2	1.92	1.39	3.23		
Between Run	60.6	6.73	1.55	3.62		
Total	79.8		2.08	**4.85**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	82.4	82.8	82.60	0.04	0.04	13645.52
2	78.9	82.3	80.60	2.89	2.89	12992.72
3	79.1	79.0	79.05	0.0025	0.0025	12497.805
4	79.2	82.3	80.75	2.4025	2.4025	13041.125
5	80.4	80.6	80.50	0.01	0.01	12960.5
6	75.2	82.8	79.00	14.44	14.44	12482
7	75.0	82.2	78.60	12.96	12.96	12355.92
8	81.1	81.1	81.10	0	0	13154.42
9	80.7	81.3	81.00	0.09	0.09	13122
10	79.9	80.8	80.35	0.2025	0.2025	12912.245
Grand sum	1607.1	**Grand mean**	80.355			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	66.1	6.61	2.57	3.20		
Between Run	25.7	2.86	0.00	0.00		
Total	91.8		2.57	**3.20**		

2. Arachidic acid (C20:0, AR1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**AR1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	17.4	16.3	16.9	0.302	0.303	568
2	17.4	17.5	17.5	0.003	0.003	609
3	17.5	17.2	17.4	0.022	0.023	602
4	17.7	18.4	18.1	0.122	0.123	652
5	18.3	18.2	18.3	0.003	0.003	666
6	18.0	18.2	18.1	0.010	0.010	655
7	17.3	18.1	17.7	0.160	0.160	627
8	17.4	17.1	17.3	0.022	0.022	595
9	17.7	17.4	17.6	0.023	0.022	616
10	17.1	16.6	16.9	0.063	0.063	568
Grand sum	351	**Grand mean**	17.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.46	0.146	0.382	2.18		
Between Run	4.37	0.485	0.412	2.35		
Total	5.83		0.562	**3.20**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	23.3	21.7	22.5	0.64	0.64	1013
2	22.1	21.7	21.9	0.04	0.04	959
3	22.5	21.7	22.1	0.16	0.16	977
4	22.9	22.5	22.7	0.04	0.04	1031
5	22.2	22.1	22.2	0.00	0.00	981
6	22.0	22.4	22.2	0.04	0.04	986
7	22.6	23.1	22.9	0.06	0.06	1044
8	22.7	22.1	22.4	0.09	0.09	1004
9	21.8	22.5	22.2	0.12	0.12	981
10	23.2	21.9	22.6	0.42	0.42	1017
Grand sum	447	**Grand mean**	22.35			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	3.24	0.324	0.569	2.55		
Between Run	1.61	0.179	0.000	0.00		
Total	4.85		0.569	**2.55**		

3. Arachidonic acid (C20:4n-6, ARA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ARA**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	650	639	645	30.3	30.3	830760.5
2	664	662	663	1.00	1.00	879138
3	613	665	639	676	676	816642
4	642	663	653	110	110	851512.5
5	658	644	651	49	49	847602
6	663	641	652	121	121	850208
7	677	687	682	25	25	930248
8	679	671	675	16	16	911250
9	598	616	607	81	81	736898
10	622	652	637	225	225	811538
Grand sum	13006	**Grand mean**	650.3			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	2669	267	16.3	2.51		
Between Run	7995	888	17.6	2.71		
Total	10664		24.0	**3.70**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	1250	1210	1230	400	400	3025800
2	1260	1100	1180	6400	6400	2784800
3	1260	1080	1170	8100	8100	2737800
4	1250	1190	1220	900	900	2976800
5	1120	1130	1125	25	25	2531250
6	1120	1130	1125	25	25	2531250
7	1260	1300	1280	400	400	3276800
8	1280	1150	1215	4225	4225	2952450
9	1060	1170	1115	3025	3025	2486450
10	1270	1080	1175	9025	9025	2761250
Grand sum	23670	**Grand mean**	1183.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	65050	6505	80.7	6.81		
Between Run	51205	5689	0.0	0.00		
Total	116255		80.7	**6.81**		

4. Capric acid (C10:0, CAP)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**CAP**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	2.94	2.92	2.93	0.0001	1E-04	17.1698
2	3.13	3.51	3.32	0.0361	0.0361	22.0448
3	2.98	3.27	3.13	0.021025	0.021025	19.53125
4	3.09	3.02	3.06	0.001225	0.001225	18.66605
5	3.70	3.59	3.65	0.003025	0.003025	26.57205
6	3.70	3.51	3.61	0.009025	0.009025	25.99205
7	3.20	2.77	2.99	0.046225	0.046225	17.82045
8	3.10	2.92	3.01	0.0081	0.0081	18.1202
9	3.05	3.04	3.05	2.5E-05	2.5E-05	18.54405
10	3.13	3.07	3.10	0.0009	0.0009	19.22
Grand sum	63.64	**Grand mean**	3.182			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	0.252	0.025	0.159	4.98		
Between Run	1.178	0.131	0.230	7.23		
Total	1.430		0.279	**8.78**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	4.77	4.82	4.80	0.000625	0.000625	45.98405
2	4.96	4.88	4.92	0.0016	0.0016	48.4128
3	4.99	4.70	4.85	0.021025	0.021025	46.94805
4	4.78	4.52	4.65	0.0169	0.0169	43.245
5	4.84	4.96	4.90	0.0036	0.0036	48.02
6	4.89	4.98	4.94	0.002025	0.002025	48.70845
7	4.92	4.99	4.96	0.001225	0.001225	49.10405
8	4.88	4.64	4.76	0.0144	0.0144	45.3152
9	4.45	4.67	4.56	0.0121	0.0121	41.5872
10	5.14	4.57	4.86	0.081225	0.081225	47.14205
Grand sum	96.35	**Grand mean**	4.82			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	0.309	0.031	0.176	3.65		
Between Run	0.301	0.033	0.035	0.73		
Total	0.610		0.179	**3.72**		

5. Docosanoic acid (C22:0, DA1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**DA1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	52.2	51.4	51.8	0.160	0.160	5366
2	52.8	51.8	52.3	0.250	0.250	5471
3	52.0	52.6	52.3	0.090	0.090	5471
4	53.5	54.1	53.8	0.090	0.090	5789
5	53.3	53.2	53.3	0.002	0.002	5671
6	53.5	53.3	53.4	0.010	0.010	5703
7	53.9	54.0	54.0	0.003	0.002	5821
8	54.0	53.4	53.7	0.090	0.090	5767
9	52.3	53.1	52.7	0.160	0.160	5555
10	53.0	52.9	53.0	0.002	0.003	5607
Grand sum	1060	**Grand mean**	53.0			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.715	0.172	0.414	0.78		
Between Run	9.531	1.059	0.666	1.26		
Total	11.25		0.784	**1.48**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	72.3	70.4	71.4	0.9025	0.9025	10182
2	70.5	69.3	69.9	0.36	0.36	9772
3	71.1	69.3	70.2	0.81	0.81	9856
4	73.2	72.7	73.0	0.0625	0.0625	10643
5	70.9	73.2	72.1	1.3225	1.3225	10382
6	71.4	73.0	72.2	0.64	0.64	10426
7	73.0	72.4	72.7	0.09	0.09	10571
8	72.7	73.0	72.9	0.0225	0.0225	10614
9	69.3	74.0	71.7	5.5225	5.5225	10267
10	71.8	71.6	71.7	0.01	0.01	10282
Grand sum	1435.1	**Grand mean**	71.8			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	19.49	1.95	1.40	1.95		
Between Run	19.68	2.19	0.35	0.48		
Total	39.17		1.44	**2.00**		

6. Docosahexaenoic acid (C22:6n-3, DHA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	DHA					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	77.0	74.4	75.7	1.69	1.69	11461
2	80.0	78.3	79.2	0.7225	0.7225	12529
3	75.1	78.6	76.9	3.0625	3.0625	11812
4	77.6	79.8	78.7	1.21	1.21	12387
5	76.5	76.7	76.6	0.01	0.01	11735
6	77.5	76.6	77.1	0.2025	0.2025	11873
7	79.1	81.5	80.3	1.44	1.44	12896
8	80.1	78.4	79.3	0.7225	0.7225	12561
9	74.1	77.5	75.8	2.89	2.89	11491
10	77.2	77.9	77.6	0.1225	0.1225	12028
Grand sum	1554	**Grand mean**	77.7			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	24.15	2.41	1.55	2.00		
Between Run	44.50	4.94	1.12	1.45		
Total	68.65		1.92	**2.47**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	175	172	174	2.25	2.25	60205
2	176	166	171	25	25	58482
3	174	169	172	6.25	6.25	58825
4	179	176	178	2.25	2.25	63013
5	168	169	169	0.25	0.25	56785
6	167	169	168	1	1	56448
7	181	180	181	0.25	0.25	65161
8	178	170	174	16	16	60552
9	164	172	168	16	16	56448
10	174	168	171	9	9	58482
Grand sum	3447	**Grand mean**	172			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	157	15.7	3.96	2.30		
Between Run	308	34.2	3.05	1.77		
Total	465		4.99	**2.90**		

7. Docosapentaenoic acid (C22:5n-3, DP3)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	DP3					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	33.6	32.9	33.3	0.1225	0.1225	2211.125
2	33.7	33.2	33.5	0.0625	0.0625	2237.805
3	33.1	34.6	33.9	0.5625	0.5625	2291.645
4	31.5	35.1	33.3	3.24	3.24	2217.78
5	32.2	34.3	33.3	1.1025	1.1025	2211.125
6	32.4	34.5	33.5	1.1025	1.1025	2237.805
7	33.2	32.6	32.9	0.09	0.09	2164.82
8	34.0	33.0	33.5	0.25	0.25	2244.5
9	33.8	34.1	34.0	0.0225	0.0225	2305.205
10	34.7	32.1	33.4	1.69	1.69	2231.12
Grand sum	669	**Grand mean**	33.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	16.49	1.65	1.28	3.84		
Between Run	1.63	0.18	0.00	0.00		
Total	18.12		1.28	**3.84**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	74.5	73.0	73.8	0.5625	0.5625	10878.125
2	72.7	72.1	72.4	0.09	0.09	10483.52
3	71.8	71.7	71.8	0.0025	0.0025	10296.125
4	70.9	73.5	72.2	1.69	1.69	10425.68
5	71.0	74.2	72.6	2.56	2.56	10541.52
6	70.6	74.2	72.4	3.24	3.24	10483.52
7	73.8	71.7	72.8	1.1025	1.1025	10585.125
8	71.1	72.2	71.7	0.3025	0.3025	10267.445
9	71.0	72.9	72.0	0.9025	0.9025	10353.605
10	70.3	74.4	72.4	4.2025	4.2025	10469.045
Grand sum	1447.6	**Grand mean**	72.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	29.31	2.93	1.71	2.37		
Between Run	6.42	0.71	0.00	0.00		
Total	35.73		1.71	**2.37**		

8. Docosapentaenoic acid (C22:5n-6, DP6)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**DP6**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	17.4	16.9	17.2	0.063	0.063	588
2	17.4	17.3	17.4	0.002	0.003	602
3	16.8	18.1	17.5	0.423	0.422	609
4	15.9	17.9	16.9	1.000	1.000	571
5	16.4	17.4	16.9	0.250	0.250	571
6	17.0	17.8	17.4	0.160	0.160	606
7	17.1	16.8	17.0	0.022	0.023	575
8	17.5	17.4	17.5	0.003	0.003	609
9	17.0	17.2	17.1	0.010	0.010	585
10	17.6	16.6	17.1	0.250	0.250	585
Grand sum	344	**Grand mean**	17.2			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	4.37	0.44	0.66	3.85		
Between Run	0.89	0.10	0.00	0.00		
Total	5.26		0.66	**3.85**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	38.7	38.9	38.8	0.01	0.01	3011
2	38.8	38.3	38.6	0.06	0.06	2972
3	37.6	36.8	37.2	0.16	0.16	2768
4	37.1	38.4	37.8	0.42	0.42	2850
5	36.6	39.6	38.1	2.25	2.25	2903
6	36.5	39.4	38.0	2.10	2.10	2880
7	39.2	37.5	38.4	0.72	0.72	2941
8	37.5	37.2	37.4	0.02	0.02	2790
9	35.5	37.1	36.3	0.64	0.64	2635
10	36.4	38.0	37.2	0.64	0.64	2768
Grand sum	755	**Grand mean**	37.8			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	14.1	1.41	1.19	3.14		
Between Run	10.3	1.14	0.00	0.00		
Total	24.3		1.19	**3.14**		

9. Docosatetraenoic acid (C22:4n-6, DTA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**DTA**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	27.4	26.8	27.1	0.09	0.09	1469
2	26.9	26.7	26.8	0.01	0.01	1436
3	25.7	28.0	26.9	1.32	1.32	1442
4	23.0	28.0	25.5	6.25	6.25	1301
5	25.1	28.2	26.7	2.40	2.40	1420
6	26.2	28.7	27.5	1.56	1.56	1507
7	25.7	24.6	25.2	0.30	0.30	1265
8	26.6	26.8	26.7	0.01	0.01	1426
9	26.9	26.3	26.6	0.09	0.09	1415
10	28.1	24.7	26.4	2.89	2.89	1394
Grand sum	530.4	**Grand mean**	26.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	29.9	2.99	1.73	6.52		
Between Run	8.8	0.97	0.00	0.00		
Total	38.6		1.73	**6.52**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	53.9	54.3	54.1	0.04	0.04	5854
2	53.3	54.8	54.1	0.56	0.56	5843
3	52.0	51.8	51.9	0.01	0.01	5387
4	49.6	53.0	51.3	2.89	2.89	5263
5	50.5	56.3	53.4	8.41	8.41	5703
6	51.2	57.0	54.1	8.41	8.41	5854
7	53.6	50.4	52.0	2.56	2.56	5408
8	51.3	52.3	51.8	0.25	0.25	5366
9	50.1	50.8	50.5	0.12	0.12	5090
10	50.2	54.0	52.1	3.61	3.61	5429
Grand sum	1050.4	**Grand mean**	52.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	53.7	5.37	2.32	4.41		
Between Run	30.5	3.38	0.00	0.00		
Total	84.2		2.32	**4.41**		

10. Eicosadienoic acid (C20:2n-6, ED1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ED1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	16.3	15.5	15.9	0.16	0.16	506
2	16.9	17.5	17.2	0.09	0.09	592
3	15.9	16.1	16.0	0.01	0.01	512
4	17.3	17.6	17.5	0.02	0.02	609
5	17.0	16.4	16.7	0.09	0.09	558
6	16.8	16.5	16.7	0.02	0.02	554
7	16.8	17.8	17.3	0.25	0.25	599
8	16.9	17.6	17.3	0.12	0.12	595
9	18.2	16.3	17.3	0.90	0.90	595
10	15.8	17.8	16.8	1.00	1.00	564
Grand sum	337	**Grand mean**	16.9			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	5.34	0.53	0.73	4.34		
Between Run	5.39	0.60	0.18	1.07		
Total	10.7		0.75	**4.47**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	31.6	29.8	30.7	0.81	0.81	1885
2	31.8	29.8	30.8	1.00	1.00	1897
3	31.0	27.7	29.4	2.72	2.72	1723
4	31.8	31.1	31.5	0.12	0.12	1978
5	29.2	28.4	28.8	0.16	0.16	1659
6	28.9	28.9	28.9	0.00	0.00	1670
7	32.4	34.0	33.2	0.64	0.64	2204
8	31.6	29.5	30.6	1.10	1.10	1867
9	26.9	29.0	28.0	1.10	1.10	1562
10	33.2	30.0	31.6	2.56	2.56	1997
Grand sum	606.6	**Grand mean**	30.3			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	20.4	2.04	1.43	4.71		
Between Run	45.0	5.00	1.22	4.01		
Total	65.5		1.88	**6.19**		

11. Eicosenoic acid (C20:1n-9, EN1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**EN1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	8.20	8.11	8.16	0.002	0.002	133
2	8.78	8.93	8.86	0.006	0.006	157
3	7.77	8.37	8.07	0.090	0.090	130
4	8.07	8.63	8.35	0.078	0.078	139
5	8.75	8.33	8.54	0.044	0.044	146
6	8.59	8.56	8.58	0.000	0.000	147
7	8.45	8.97	8.71	0.068	0.068	152
8	8.61	8.91	8.76	0.023	0.023	153
9	8.73	7.50	8.12	0.378	0.378	132
10	7.70	8.46	8.08	0.144	0.144	131
Grand sum	168	**Grand mean**	8.42			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.67	0.17	0.41	4.85		
Between Run	1.67	0.19	0.10	1.15		
Total	3.33		0.42	**4.98**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	17.1	16.1	16.6	0.25	0.25	551
2	17.0	15.1	16.1	0.90	0.90	515
3	16.5	13.3	14.9	2.56	2.56	444
4	16.9	15.3	16.1	0.64	0.64	518
5	14.5	14.1	14.3	0.04	0.04	409
6	14.7	14.7	14.7	0.00	0.00	432
7	16.9	17.8	17.4	0.20	0.20	602
8	16.7	14.4	15.6	1.32	1.32	484
9	12.9	14.4	13.7	0.56	0.56	373
10	17.0	14.2	15.6	1.96	1.96	487
Grand sum	309.6	**Grand mean**	15.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	16.9	1.69	1.30	8.39		
Between Run	22.3	2.48	0.63	4.07		
Total	39.2		1.44	**9.33**		

12. Eicosapentaenoic acid (C20:5n-3, EPA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**EPA**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	40.0	38.2	39.1	0.81	0.81	3058
2	41.0	40.0	40.5	0.25	0.25	3281
3	37.3	40.2	38.8	2.10	2.10	3003
4	39.4	41.0	40.2	0.64	0.64	3232
5	40.8	39.6	40.2	0.36	0.36	3232
6	41.1	40.3	40.7	0.16	0.16	3313
7	40.7	41.0	40.9	0.02	0.02	3337
8	41.7	41.0	41.4	0.12	0.12	3420
9	38.4	40.0	39.2	0.64	0.64	3073
10	39.6	39.8	39.7	0.01	0.01	3152
Grand sum	801.1	**Grand mean**	40.1			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	10.235	1.0235	1.011681768	2.53		
Between Run	12.8745	1.4305	0.451109743	1.13		
Total	23.1095		1.10770032	**2.77**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	78.2	75.9	77.1	1.32	1.32	11873
2	79.8	70.8	75.3	20.3	20.3	11340
3	78.8	67.7	73.3	30.8	30.8	10731
4	77.8	75.1	76.5	1.82	1.82	11689
5	71.9	71.3	71.6	0.09	0.09	10253
6	72.9	72.5	72.7	0.04	0.04	10571
7	79.5	81.8	80.7	1.32	1.32	13009
8	81.1	71.9	76.5	21.2	21.2	11705
9	66.8	73.6	70.2	11.6	11.6	9856
10	80.8	70.8	75.8	25.0	25.0	11491
Grand sum	1499	**Grand mean**	75.0			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	226.74	22.674	4.76	6.35		
Between Run	168.27	18.70	0	0.00		
Total	395.01		4.76	**6.35**		

13. Eicosatrienoic acid (C20:3n-9, ET1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ET1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	6.31	6.10	6.21	0.011025	0.011025	77.00405
2	6.85	7.07	6.96	0.0121	0.0121	96.8832
3	5.97	6.16	6.07	0.009025	0.009025	73.56845
4	6.96	6.88	6.92	0.0016	0.0016	95.7728
5	6.69	6.14	6.42	0.075625	0.075625	82.30445
6	6.65	6.16	6.41	0.060025	0.060025	82.04805
7	6.7	7.15	6.93	0.050625	0.050625	95.91125
8	6.55	6.85	6.70	0.0225	0.0225	89.78
9	6.61	5.95	6.28	0.1089	0.1089	78.8768
10	5.87	7.03	6.45	0.3364	0.3364	83.205
Grand sum	130.65	**Grand mean**	6.53			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.37565	0.138	0.371	5.68		
Between Run	1.882925	0.209	0.189	2.90		
Total	3.258575		0.416	**6.37**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	14.8	14.1	14.5	0.1225	0.1225	417.605
2	15.2	13.8	14.5	0.49	0.49	420.5
3	14.5	12.4	13.5	1.1025	1.1025	361.805
4	15.4	14.7	15.1	0.1225	0.1225	453.005
5	13.6	12.6	13.1	0.25	0.25	343.22
6	13.3	12.8	13.1	0.0625	0.0625	340.605
7	15.6	16.5	16.1	0.2025	0.2025	515.205
8	15.0	13.2	14.1	0.81	0.81	397.62
9	11.8	12.8	12.3	0.25	0.25	302.58
10	15.7	13.4	14.6	1.3225	1.3225	423.405
Grand sum	281.2	**Grand mean**	14.1			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	9.47	0.95	0.97	6.92		
Between Run	21.878	2.43	0.86	6.13		
Total	31.348		1.30	**9.24**		

14. *gamma*-Linolenic acid (C18:3n-6, GLA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	GLA					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	58.7	56.8	57.8	0.9025	0.9025	6670.125
2	56.8	57.7	57.3	0.2025	0.2025	6555.125
3	51.2	56.1	53.7	6.0025	6.0025	5756.645
4	57.1	58.0	57.6	0.2025	0.2025	6624.005
5	55.6	57.1	56.4	0.5625	0.5625	6350.645
6	49.7	56.7	53.2	12.25	12.25	5660.48
7	49.8	56.6	53.2	11.56	11.56	5660.48
8	55.5	57.3	56.4	0.81	0.81	6361.92
9	48.0	48.0	48.0	0	0	4608
10	47.9	47.7	47.8	0.01	0.01	4569.68
Grand sum	1082.3	**Grand mean**	54.1			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	65.005	6.5005	2.549607813	4.71		
Between Run	248.4405	27.6045	3.248384214	6.00		
Total	313.4455		4.129467278	**7.63**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	93.3	93.7	93.5	0.04	0.04	17484.5
2	87.2	93.7	90.5	10.5625	10.5625	16362.405
3	86.5	86.6	86.6	0.0025	0.0025	14981.805
4	87.1	93.7	90.4	10.89	10.89	16344.32
5	90.5	92.2	91.4	0.7225	0.7225	16689.645
6	79.6	91.8	85.7	37.21	37.21	14688.98
7	79.6	91.8	85.7	37.21	37.21	14688.98
8	90.9	91.9	91.4	0.25	0.25	16707.92
9	90.1	91.4	90.8	0.4225	0.4225	16471.125
10	90.5	91.4	91.0	0.2025	0.2025	16543.805
Grand sum	1793.5	**Grand mean**	89.7			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	195.025	19.5025	4.416163493	4.92		
Between Run	131.3725	14.59694444	0	0.00		
Total	326.3975		4.416163493	**4.92**		

15. *homo-gamma*-Linolenic acid (C20:3n-6, HGL)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	HGL					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	117	117	117	0	0	27378
2	115	115	115	0	0	26450
3	112	121	117	20.25	20.25	27144.5
4	109	121	115	36	36	26450
5	116	121	119	6.25	6.25	28084.5
6	115	121	118	9	9	27848
7	116	115	116	0.25	0.25	26680.5
8	120	122	121	1	1	29282
9	117	120	119	2.25	2.25	28084.5
10	116	117	117	0.25	0.25	27144.5
Grand sum	2343	**Grand mean**	117			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	150.5	15.05	3.88	3.31		
Between Run	64.05	7.12	0.00	0.00		
Total	214.55		3.88	**3.31**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	227	227	227	0	0	103058
2	222	214	218	16	16	95048
3	223	215	219	16	16	95922
4	218	225	222	12.25	12.25	98124.5
5	214	232	223	81	81	99458
6	212	231	222	90.25	90.25	98124.5
7	225	225	225	0	0	101250
8	226	222	224	4	4	100352
9	210	229	220	90.25	90.25	96360.5
10	222	217	220	6.25	6.25	96360.5
Grand sum	4436	**Grand mean**	222			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	632	63.20	7.95	3.58		
Between Run	153.2	17.02	0.00	0.00		
Total	785.2		7.95	**3.58**		

16. Lauric acid (C12:0, LAR)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**LAR**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	9.86	9.47	9.67	0.038025	0.038025	186.82445
2	10.6	10.9	10.8	0.0225	0.0225	231.125
3	9.86	10.1	10.0	0.0144	0.0144	199.2008
4	10.2	10.3	10.3	0.0025	0.0025	210.125
5	11.7	10.5	11.1	0.36	0.36	246.42
6	11.7	10.5	11.1	0.36	0.36	246.42
7	10.7	10.3	10.5	0.04	0.04	220.5
8	10.7	10.3	10.5	0.04	0.04	220.5
9	9.90	10.2	10.1	0.0225	0.0225	202.005
10	10.4	10.3	10.4	0.0025	0.0025	214.245
Grand sum	208.49	**Grand mean**	10.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.80485	0.180485	0.424835262	4.08		
Between Run	3.961245	0.440138333	0.360314677	3.46		
Total	5.766095		0.557056251	**5.34**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	17.6	17.1	17.4	0.0625	0.0625	602.045
2	18.3	16.7	17.5	0.64	0.64	612.5
3	17.7	15.5	16.6	1.21	1.21	551.12
4	18.2	16.8	17.5	0.49	0.49	612.5
5	16.5	16.3	16.4	0.01	0.01	537.92
6	16.6	16.5	16.6	0.0025	0.0025	547.805
7	18.2	18.1	18.2	0.0025	0.0025	658.845
8	18.2	16.6	17.4	0.64	0.64	605.52
9	15.1	16.4	15.8	0.4225	0.4225	496.125
10	18.3	15.9	17.1	1.44	1.44	584.82
Grand sum	340.6	**Grand mean**	17.0			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	9.84	0.984	0.991967741	5.82		
Between Run	8.782	0.975777778	0	0.00		
Total	18.622		0.991967741	**5.82**		

17. Lignoceric acid (C24:0, LG1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**LG1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	46.9	45.7	46.3	0.36	0.36	4287.38
2	47.1	46.4	46.8	0.1225	0.1225	4371.125
3	46.2	46.8	46.5	0.09	0.09	4324.5
4	47.3	48.1	47.7	0.16	0.16	4550.58
5	48.2	47.6	47.9	0.09	0.09	4588.82
6	47.6	47.4	47.5	0.01	0.01	4512.5
7	48.4	48.7	48.6	0.0225	0.0225	4714.205
8	48.7	48.1	48.4	0.09	0.09	4685.12
9	46.9	47.2	47.1	0.0225	0.0225	4427.405
10	47.7	47.2	47.5	0.0625	0.0625	4503.005
Grand sum	948.2	**Grand mean**	47.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	2.06	0.21	0.45	0.96		
Between Run	10.478	1.16	0.69	1.46		
Total	12.538		0.83	**1.75**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	64.7	63.2	64.0	0.5625	0.5625	8179.205
2	62.4	61.5	62.0	0.2025	0.2025	7675.605
3	63.6	61.3	62.5	1.3225	1.3225	7800.005
4	65.0	64.5	64.8	0.0625	0.0625	8385.125
5	63.0	64.7	63.9	0.7225	0.7225	8153.645
6	63.4	64.3	63.9	0.2025	0.2025	8153.645
7	64.8	64.7	64.8	0.0025	0.0025	8385.125
8	64.8	65.0	64.9	0.01	0.01	8424.02
9	60.9	65.0	63.0	4.2025	4.2025	7925.405
10	64.1	63.0	63.6	0.3025	0.3025	8077.205
Grand sum	1273.9	**Grand mean**	63.7			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	15.185	1.52	1.23	1.93		
Between Run	17.9245	1.99	0.49	0.76		
Total	33.1095		1.32	**2.08**		

18. Linoleic acid (C18:2n-6, LNA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**LNA**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	2680	2600	2640	1600	1600	13939200
2	2630	2630	2630	0	0	13833800
3	2300	2570	2435	18225	18225	11858450
4	2590	2610	2600	100	100	13520000
5	2500	2560	2530	900	900	12801800
6	2260	2580	2420	25600	25600	11712800
7	2270	2570	2420	22500	22500	11712800
8	2500	2560	2530	900	900	12801800
9	2160	2160	2160	0	0	9331200
10	2160	2150	2155	25	25	9288050
Grand sum	49040	**Grand mean**	2452			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	139700	13970	118	4.82		
Between Run	553820	61535.55556	154	6.29		
Total	693520		194	**7.92**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	4730	4640	4685	2025	2025	43898450
2	4410	4700	4555	21025	21025	41496050
3	4320	4340	4330	100	100	37497800
4	4360	4670	4515	24025	24025	40770450
5	4500	4520	4510	100	100	40680200
6	3990	4550	4270	78400	78400	36465800
7	4000	4560	4280	78400	78400	36636800
8	4490	4510	4500	100	100	40500000
9	4480	4520	4500	400	400	40500000
10	4440	4510	4475	1225	1225	40051250
Grand sum	89240	**Grand mean**	4462			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	411600	41160	203	4.55		
Between Run	307920	34213	0.0	0.00		
Total	719520		203	**4.55**		

19. Myristoleic acid (C14:1n-5, ML1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ML1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	7.17	6.89	7.03	0.0196	0.0196	98.8418
2	7.90	7.98	7.94	0.0016	0.0016	126.0872
3	6.89	7.15	7.02	0.0169	0.0169	98.5608
4	7.87	7.50	7.69	0.034225	0.034225	118.11845
5	7.51	7.14	7.33	0.034225	0.034225	107.31125
6	7.63	7.14	7.39	0.060025	0.060025	109.07645
7	7.54	7.95	7.75	0.042025	0.042025	119.97005
8	7.71	7.36	7.54	0.030625	0.030625	113.55245
9	7.12	7.16	7.14	0.0004	0.0004	101.9592
10	7.27	7.73	7.50	0.0529	0.0529	112.5
Grand sum	148.61	**Grand mean**	7.43			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	0.58505	0.059	0.242	3.26		
Between Run	1.731045	0.192	0.259	3.48		
Total	2.316095		0.354	**4.77**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	9.52	9.31	9.42	0.011025	0.011025	177.28445
2	10.3	8.95	9.63	0.455625	0.455625	185.28125
3	9.71	7.78	8.75	0.931225	0.931225	152.95005
4	10.5	9.22	9.86	0.4096	0.4096	194.4392
5	8.71	8.07	8.39	0.1024	0.1024	140.7842
6	8.84	8.16	8.50	0.1156	0.1156	144.5
7	10.3	10.3	10.3	0	0	212.18
8	10.1	8.66	9.38	0.5184	0.5184	175.9688
9	7.72	8.78	8.25	0.2809	0.2809	136.125
10	10.2	8.06	9.13	1.1449	1.1449	166.7138
Grand sum	183.19	**Grand mean**	9.16			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	7.93935	0.794	0.891	9.73		
Between Run	8.297945	0.922	0.253	2.76		
Total	16.237295		0.926	**10.11**		

20. Myristic acid (C14:0, MR1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**MR1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	94.3	90.1	92.2	4.41	4.41	17001.68
2	99.0	99.3	99.2	0.0225	0.0225	19661.445
3	87.5	93.4	90.5	8.7025	8.7025	16362.405
4	95.3	96.7	96.0	0.49	0.49	18432
5	101	96.4	98.7	5.29	5.29	19483.38
6	101	96.9	99.0	4.2025	4.2025	19582.205
7	97.5	97.0	97.3	0.0625	0.0625	18915.125
8	98.6	95.4	97.0	2.56	2.56	18818
9	90.2	91.2	90.7	0.25	0.25	16452.98
10	92.7	94.2	93.5	0.5625	0.5625	17465.805
Grand sum	1907.7	**Grand mean**	95.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	53.105	5.31	2.30	2.42		
Between Run	209.0605	23.23	2.99	3.14		
Total	262.1655		3.78	**3.96**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	192	183	188	20.25	20.25	70312.5
2	193	162	178	240.25	240.25	63012.5
3	188	149	169	380.25	380.25	56784.5
4	194	177	186	72.25	72.25	68820.5
5	164	161	163	2.25	2.25	52812.5
6	165	162	164	2.25	2.25	53464.5
7	194	194	194	0	0	75272
8	194	163	179	240.25	240.25	63724.5
9	146	166	156	100	100	48672
10	194	150	172	484	484	59168
Grand sum	3491	**Grand mean**	175			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	3083.5	308	17.6	10.06		
Between Run	2689.45	299	0.0	0.00		
Total	5772.95		17.6	**10.06**		

21. Margaric acid (C17:0, MRG)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	MRG					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	19.6	18.9	19.3	0.1225	0.1225	741.125
2	21.3	20.9	21.1	0.04	0.04	890.42
3	19.4	19.8	19.6	0.04	0.04	768.32
4	20.6	20.2	20.4	0.04	0.04	832.32
5	21.2	20.4	20.8	0.16	0.16	865.28
6	21.4	20.5	21.0	0.2025	0.2025	877.805
7	20.4	20.0	20.2	0.04	0.04	816.08
8	20.8	20.2	20.5	0.09	0.09	840.5
9	19.6	19.1	19.4	0.0625	0.0625	748.845
10	20.3	19.5	19.9	0.16	0.16	792.02
Grand sum	404.1	**Grand mean**	20.2			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.915	0.192	0.438	2.17		
Between Run	7.8745	0.875	0.585	2.89		
Total	9.7895		0.730	**3.61**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	33.9	33.4	33.7	0.0625	0.0625	2264.645
2	34.6	30.7	32.7	3.8025	3.8025	2132.045
3	34.2	29.0	31.6	6.76	6.76	1997.12
4	34.8	32.2	33.5	1.69	1.69	2244.5
5	30.6	30.1	30.4	0.0625	0.0625	1842.245
6	30.5	30.0	30.3	0.0625	0.0625	1830.125
7	34.6	34.0	34.3	0.09	0.09	2352.98
8	34.9	30.4	32.7	5.0625	5.0625	2132.045
9	28.1	29.8	29.0	0.7225	0.7225	1676.205
10	34.9	28.1	31.5	11.56	11.56	1984.5
Grand sum	638.8	**Grand mean**	31.9			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	59.75	5.98	2.44	7.65		
Between Run	53.138	5.90	0.00	0.00		
Total	112.888		2.44	**7.65**		

22. Nervonic acid (C24:1n-9, NR1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**NR1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	68.8	65.8	67.3	2.25	2.25	9058.58
2	73.0	73.4	73.2	0.04	0.04	10716.48
3	68.5	69.6	69.1	0.3025	0.3025	9535.805
4	70.9	73.2	72.1	1.3225	1.3225	10382.405
5	72.1	69.5	70.8	1.69	1.69	10025.28
6	70.9	69.4	70.2	0.5625	0.5625	9842.045
7	73.7	71.4	72.6	1.3225	1.3225	10527.005
8	73.1	75.6	74.4	1.5625	1.5625	11055.845
9	76.5	63.8	70.2	40.3225	40.3225	9842.045
10	69.7	69.8	69.8	0.0025	0.0025	9730.125
Grand sum	1418.7	**Grand mean**	70.9			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	98.755	9.88	3.14	4.43		
Between Run	80.1305	8.90	0.00	0.00		
Total	178.8855		3.14	**4.43**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	90.0	88.4	89.2	0.64	0.64	15913.28
2	91.0	93.6	92.3	1.69	1.69	17038.58
3	87.8	89.6	88.7	0.81	0.81	15735.38
4	89.9	94.2	92.1	4.6225	4.6225	16946.405
5	90.0	95.7	92.9	8.1225	8.1225	17242.245
6	90.2	93.9	92.1	3.4225	3.4225	16946.405
7	92.6	91.4	92.0	0.36	0.36	16928
8	89.6	92.8	91.2	2.56	2.56	16634.88
9	85.1	83.1	84.1	1	1	14145.62
10	90.3	91.3	90.8	0.25	0.25	16489.28
Grand sum	1810.5	**Grand mean**	90.5			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	46.955	4.70	2.17	2.39		
Between Run	124.5625	13.84	2.14	2.36		
Total	171.5175		3.04	**3.36**		

23. Oleic acid (C18:1n-9, OL1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**OL1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	1400	1360	1380	400	400	3808800
2	1380	1370	1375	25	25	3781250
3	1210	1350	1280	4900	4900	3276800
4	1370	1380	1375	25	25	3781250
5	1320	1360	1340	400	400	3591200
6	1180	1330	1255	5625	5625	3150050
7	1190	1340	1265	5625	5625	3200450
8	1300	1340	1320	400	400	3484800
9	1140	1140	1140	0	0	2599200
10	1160	1150	1155	25	25	2668050
Grand sum	25770	**Grand mean**	1289			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	34850	3485	59.0	4.58		
Between Run	137205	15245	76.7	5.95		
Total	172055		96.8	**7.51**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	3150	3080	3115	1225	1225	19406450
2	2920	3110	3015	9025	9025	18180450
3	2930	2950	2940	100	100	17287200
4	2920	3120	3020	10000	10000	18240800
5	3010	3020	3015	25	25	18180450
6	2600	2970	2785	34225	34225	15512450
7	2600	2990	2795	38025	38025	15624050
8	2940	2960	2950	100	100	17405000
9	2970	2970	2970	0	0	17641800
10	2960	2980	2970	100	100	17641800
Grand sum	59150	**Grand mean**	2958			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	185650	18565	136	4.61		
Between Run	184325	20481	30.9	1.05		
Total	369975		140	**4.72**		

24. Pentadecanoic acid (C15:0, PDE)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	PDE					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	11.2	10.7	11.0	0.0625	0.0625	239.805
2	11.3	11.4	11.4	0.0025	0.0025	257.645
3	10.6	11.4	11.0	0.16	0.16	242
4	11.0	11.2	11.1	0.01	0.01	246.42
5	11.6	11.0	11.3	0.09	0.09	255.38
6	11.8	11.0	11.4	0.16	0.16	259.92
7	11.4	11.3	11.4	0.0025	0.0025	257.645
8	11.4	11.1	11.3	0.0225	0.0225	253.125
9	10.6	10.6	10.6	0	0	224.72
10	10.8	11.0	10.9	0.01	0.01	237.62
Grand sum	222.4	**Grand mean**	11.1			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.04	0.10	0.32	2.90		
Between Run	1.192	0.13	0.12	1.07		
Total	2.232		0.34	**3.09**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	18.3	17.6	18.0	0.1225	0.1225	644.405
2	18.0	15.6	16.8	1.44	1.44	564.48
3	18.2	14.9	16.6	2.7225	2.7225	547.805
4	18.0	16.8	17.4	0.36	0.36	605.52
5	15.6	15.4	15.5	0.01	0.01	480.5
6	15.7	15.5	15.6	0.01	0.01	486.72
7	18.4	18.4	18.4	0	0	677.12
8	18.7	15.7	17.2	2.25	2.25	591.68
9	14.1	15.7	14.9	0.64	0.64	444.02
10	18.4	14.9	16.7	3.0625	3.0625	554.445
Grand sum	333.9	**Grand mean**	16.7			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	21.235	2.12	1.46	8.73		
Between Run	22.2345	2.47	0.42	2.49		
Total	43.4695		1.52	**9.08**		

25. Palmitoleic acid (C16:1n-7, PL1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**PL1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	167	162	165	6.25	6.25	54120.5
2	168	169	169	0.25	0.25	56784.5
3	149	162	156	42.25	42.25	48360.5
4	167	168	168	0.25	0.25	56112.5
5	161	166	164	6.25	6.25	53464.5
6	151	167	159	64	64	50562
7	151	169	160	81	81	51200
8	167	172	170	6.25	6.25	57460.5
9	140	140	140	0	0	39200
10	140	140	140	0	0	39200
Grand sum	3176	**Grand mean**	159			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	413	41.3	6.426507605	4.05		
Between Run	2116.2	235.1333333	9.844626284	6.20		
Total	2529.2		11.75655845	**7.40**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	475	468	472	12.25	12.25	444624.5
2	442	476	459	289	289	421362
3	439	442	441	2.25	2.25	388080.5
4	440	469	455	210.25	210.25	413140.5
5	463	460	462	2.25	2.25	425964.5
6	408	468	438	900	900	383688
7	413	469	441	784	784	388962
8	467	476	472	20.25	20.25	444624.5
9	454	454	454	0	0	412232
10	442	455	449	42.25	42.25	402304.5
Grand sum	9080	**Grand mean**	454			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	4525	452.5	21.27204739	4.69		
Between Run	2663	295.8888889	0	0.00		
Total	7188		21.27204739	**4.69**		

26. Palmitic acid (C16:0, PM1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**PM1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	1890	1840	1865	625	625	6956450
2	1970	1960	1965	25	25	7722450
3	1800	1900	1850	2500	2500	6845000
4	1910	1940	1925	225	225	7411250
5	1980	1910	1945	1225	1225	7566050
6	1970	1900	1935	1225	1225	7488450
7	1960	1970	1965	25	25	7722450
8	1980	1930	1955	625	625	7644050
9	1820	1840	1830	100	100	6697800
10	1850	1890	1870	400	400	6993800
Grand sum	38210	**Grand mean**	1911			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	13950	1395	37.34969879	1.95		
Between Run	47545	5282.777778	44.08955533	2.31		
Total	61495		57.78311941	**3.02**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	4200	3980	4090	12100	12100	33456200
2	4130	3570	3850	78400	78400	29645000
3	4170	3520	3845	105625	105625	29568050
4	4200	3930	4065	18225	18225	33048450
5	3720	3720	3720	0	0	27676800
6	3710	3710	3710	0	0	27528200
7	4260	4250	4255	25	25	36210050
8	4310	3810	4060	62500	62500	32967200
9	3600	3970	3785	34225	34225	28652450
10	4270	3510	3890	144400	144400	30264200
Grand sum	78540	**Grand mean**	3927			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	911000	91100	301.8277655	7.69		
Between Run	590020	65557.77778	0	0.00		
Total	1501020		301.8277655	**7.69**		

27. Stearidonic acid (C18:4n-3, SD1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**SD1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	3.38	2.83	3.11	0.075625	0.075625	19.28205
2	2.62	3.13	2.88	0.065025	0.065025	16.53125
3	2.47	2.92	2.70	0.050625	0.050625	14.52605
4	2.63	3.02	2.83	0.038025	0.038025	15.96125
5	2.35	3.11	2.73	0.1444	0.1444	14.9058
6	2.52	2.94	2.73	0.0441	0.0441	14.9058
7	2.47	2.77	2.62	0.0225	0.0225	13.7288
8	2.46	3.10	2.78	0.1024	0.1024	15.4568
9	2.97	2.60	2.79	0.034225	0.034225	15.51245
10	2.75	2.30	2.53	0.050625	0.050625	12.75125
Grand sum	55.34	**Grand mean**	2.77			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.2551	0.126	0.354	12.80		
Between Run	0.43572	0.048	0.000	0.00		
Total	1.69082		0.354	**12.80**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	3.65	4.13	3.89	0.0576	0.0576	30.2642
2	3.61	3.99	3.80	0.0361	0.0361	28.88
3	3.54	3.64	3.59	0.0025	0.0025	25.7762
4	3.54	3.91	3.73	0.034225	0.034225	27.75125
5	3.50	4.38	3.94	0.1936	0.1936	31.0472
6	3.22	3.63	3.43	0.042025	0.042025	23.46125
7	3.28	3.62	3.45	0.0289	0.0289	23.805
8	3.64	4.25	3.95	0.093025	0.093025	31.12605
9	3.72	4.17	3.95	0.050625	0.050625	31.12605
10	3.44	4.14	3.79	0.1225	0.1225	28.7282
Grand sum	75	**Grand mean**	3.75			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	1.3222	0.132	0.364	9.70		
Between Run	0.7154	0.079	0.000	0.00		
Total	2.0376		0.364	**9.70**		

28. Stearic acid (C18:0, ST1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**ST1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	477	445	461	256	256	425042
2	512	516	514	4	4	528392
3	479	479	479	0	0	458882
4	508	491	500	72.25	72.25	499000.5
5	525	504	515	110.25	110.25	529420.5
6	522	506	514	64	64	528392
7	488	478	483	25	25	466578
8	505	472	489	272.25	272.25	477264.5
9	486	457	472	210.25	210.25	444624.5
10	491	438	465	702.25	702.25	431520.5
Grand sum	9779	**Grand mean**	489			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	3432.5	343	18.5	3.79		
Between Run	7674.45	853	16.0	3.26		
Total	11106.95		24.5	**5.00**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	996	934	965	961	961	1862450
2	976	911	944	1056.25	1056.25	1780384.5
3	979	903	941	1444	1444	1770962
4	1010	950	980	900	900	1920800
5	937	922	930	56.25	56.25	1727940.5
6	934	927	931	12.25	12.25	1731660.5
7	1000	990	995	25	25	1980050
8	1020	944	982	1444	1444	1928648
9	911	913	912	1	1	1663488
10	979	834	907	5256.25	5256.25	1643484.5
Grand sum	18970	**Grand mean**	949			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	22312	2231	47.2	4.98		
Between Run	16823	1869	0	0.00		
Total	39135		47.2	**4.98**		

29. Tricosanoic acid (C23:0, TSA)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	TSA					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	21.2	21.2	21.2	0	0	898.88
2	21.6	20.8	21.2	0.16	0.16	898.88
3	21.4	21.7	21.6	0.0225	0.0225	928.805
4	21.9	22.0	22.0	0.0025	0.0025	963.605
5	22.0	22.1	22.1	0.0025	0.0025	972.405
6	21.8	22.2	22.0	0.04	0.04	968
7	22.1	22.1	22.1	0	0	976.82
8	22.2	21.5	21.9	0.1225	0.1225	954.845
9	21.2	22.3	21.8	0.3025	0.3025	946.125
10	21.8	21.7	21.8	0.0025	0.0025	946.125
Grand sum	434.8	**Grand mean**	21.7			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	1.31	0.131	0.362	1.66		
Between Run	1.938	0.215	0.205	0.94		
Total	3.248		0.416	**1.91**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	29.5	29.2	29.4	0.0225	0.0225	1722.845
2	29.0	27.8	28.4	0.36	0.36	1613.12
3	29.3	28.5	28.9	0.16	0.16	1670.42
4	30.2	29.3	29.8	0.2025	0.2025	1770.125
5	29.4	29.7	29.6	0.0225	0.0225	1746.405
6	29.5	29.9	29.7	0.04	0.04	1764.18
7	29.7	29.6	29.7	0.0025	0.0025	1758.245
8	30.0	29.8	29.9	0.01	0.01	1788.02
9	28.5	31.0	29.8	1.5625	1.5625	1770.125
10	29.3	29.3	29.3	0	0	1716.98
Grand sum	588.5	**Grand mean**	29.4			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	4.765	0.477	0.690	2.35		
Between Run	3.8525	0.428	0.000	0.00		
Total	8.6175		0.690	**2.35**		

30. cis-Vaccenic acid (C18:1n-7, VC1)

Method name:	Fatty acids in serum					
Method #:	4028					
Matrix:	Serum					
Units:	uM					
Analyte:	**VC1**					
Quality material 1: Low Pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	95.5	95.0	95.3	0.0625	0.0625	18145.125
2	96.9	96.2	96.6	0.1225	0.1225	18643.805
3	84.1	93.6	88.9	22.5625	22.5625	15788.645
4	91.1	93.7	92.4	1.69	1.69	17075.52
5	96.6	93.8	95.2	1.96	1.96	18126.08
6	95.4	89.1	92.3	9.9225	9.9225	17020.125
7	95.0	94.1	94.6	0.2025	0.2025	17879.405
8	98.9	98.2	98.6	0.1225	0.1225	19424.205
9	87.7	93.9	90.8	9.61	9.61	16489.28
10	92.1	99.7	95.9	14.44	14.44	18393.62
Grand sum	1880.6	**Grand mean**	94.0			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev (%)**		
Within Run	121.39	12.1	3.48	3.71		
Between Run	152.992	17.0	1.56	1.66		
Total	274.382		3.82	**4.06**		
Quality material 2: High pool						
Run	**Result 1**	**Result 2**	**Mean**	**SS 1**	**SS 2**	**2*mean^2**
1	218	217	218	0.25	0.25	94612.5
2	217	184	201	272.25	272.25	80400.5
3	211	173	192	361	361	73728
4	212	198	205	49	49	84050
5	186	184	185	1	1	68450
6	182	186	184	4	4	67712
7	216	207	212	20.25	20.25	89464.5
8	216	182	199	289	289	79202
9	157	191	174	289	289	60552
10	231	197	214	289	289	91592
Grand sum	3965	**Grand mean**	198			
	Sum squares	**Mean Sq Error**	**Std Dev**	**Rel Std Dev**		
Within Run	3149.5	315	17.75	8.95		
Between Run	3702.25	411	6.94	3.50		
Total	6851.75		19.06	**9.61**		

Stability

The freeze/thaw, bench-top, processed sample, and long-term stability of the 30 analytes in the low QC and high QC is shown below.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions	
Describe condition:	QC vials thawed three times and re-frozen at -80°C (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)	
Describe condition:	QC vials stored on the bench top for 3 hours after removing from -80°C freezer
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler	
Describe condition:	Processed samples stored at room temperature for 3 days
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis	
Describe condition:	QC pools prepared stored continuously at -80°C compared to data obtained 1/24/2012

1. *alpha*-Linolenic acid (C18:3n-3, ALN)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ALN**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	36.4	37.0	41.1	40.1	41.1	41.2	35.4	41.1
Replicate 2	37.7	38.0	38.2	40.0	38.2	37.7	42.2	38.2
Replicate 3	36.8	33.5	39.1	36.9	39.1	39.3	40.8	39.1
Mean	37.0	36.2	39.5	39.0	39.5	39.4	39.5	39.5
% difference from initial measurement	--	**-2.2**	--	**-1.2**	--	**-0.2**	--	**0.0**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	53.7	56.8	56.1	62.0	56.1	55.7	63.8	56.1
Replicate 2	56.4	58.2	55.2	58.0	55.2	54.4	64.5	55.2
Replicate 3		55.0	61.8	60.0	61.8	63.0	64.0	61.8
Mean	55.1	56.7	57.7	60.0	57.7	57.7	64.1	57.7
% difference from initial measurement	--	**2.9**	--	**4.1**	--	**0.0**	--	**-10.0**

2. Arachidic acid (C20:0, AR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**AR1**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	17.1	17.6	17.2	17.9	17.2	16.8	17.0	17.2
Replicate 2	17.3	17.9	17.0	19.0	17.0	15.7	19.1	17.0
Replicate 3	18.9	16.0	18.7	18.3	18.7	18.4	18.4	18.7
Mean	17.8	17.2	17.7	18.4	17.7	17.0	18.1	17.7
% difference from initial measurement	--	**-3.5**	--	**4.2**	--	**-3.9**	--	**-2.6**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	19.9	22.7	20.5	20.6	20.5	20.0	23.1	20.5
Replicate 2	21.1	23.8	20.0	21.6	20.0	18.9	22.7	20.0
Replicate 3		21.8	23.1	23.2	23.1	21.8	22.8	23.1
Mean	20.5	22.8	21.2	21.8	21.2	20.2	22.9	21.2
% difference from initial measurement	--	**10.9**	--	**2.8**	--	**-4.7**	--	**-7.3**

3. Arachidonic acid (C20:4n-6, ARA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ARA**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	549	562	614	592	614	613	546	614
Replicate 2	572	573	571	573	571	579	633	571
Replicate 3	554	503	598	554	598	603	615	598
Mean	558	546	594	573	594	598	598	594
% difference from initial measurement	--	**-2.2**	--	**-3.6**	--	**0.6**	--	**-0.6**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	981	1026	1017	1017	1017	1012	1096	1017
Replicate 2	1015	1048	966	995	966	964	1079	966
Replicate 3		1024	1108	1020	1108	1103	1059	1108
Mean	998	1032	1030	1010	1030	1026	1078	1030
% difference from initial measurement	--	**3.4**	--	**-1.9**	--	**-0.4**	--	**-4.4**

4. Capric acid (C10:0, CAP)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**CAP**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	3.33	3.66	3.04	3.38	3.04	3.01	2.67	3.04
Replicate 2	3.64	3.63	3.39	2.97	3.39	3.45	2.66	3.39
Replicate 3	3.95	2.93	2.85	3.43	2.85	2.78	2.67	2.85
Mean	3.64	3.41	3.09	3.26	3.09	3.08	2.67	3.09
% difference from initial measurement	--	**-6.5**	--	**5.5**	--	**-0.4**	--	**16.0**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	4.63	5.24	4.00	4.55	4.00	4.21	4.03	4.00
Replicate 2	5.19	4.55	3.81	4.56	3.81	3.81	4.29	3.81
Replicate 3		4.58	4.22	4.70	4.22	4.46	4.05	4.22
Mean	4.91	4.79	4.01	4.61	4.01	4.16	4.12	4.01
% difference from initial measurement	--	**-2.4**	--	**14.9**	--	**3.7**	--	**-2.7**

5. Docosanoic acid (C22:0, DA1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DA1**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	50.1	50.2	50.8	51.4	50.8	50.6	50.1	50.8
Replicate 2	51.9	51.4	50.9	50.9	50.9	50.6	53.5	50.9
Replicate 3	50.7	45.4	51.4	50.0	51.4	51.6	52.0	51.4
Mean	50.9	49.0	51.1	50.7	51.1	50.9	51.9	51.1
% difference from initial measurement	--	**-3.7**	--	**-0.6**	--	**-0.2**	--	**-1.6**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	66.3	67.5	67.4	67.0	67.4	67.7	72.3	67.4
Replicate 2	66.7	68.2	63.5	67.2	63.5	63.8	71.6	63.5
Replicate 3		67.3	69.1	67.0	69.1	69.7	70.6	69.1
Mean	66.5	67.7	66.6	67.1	66.6	67.1	71.5	66.6
% difference from initial measurement	--	**1.8**	--	**0.6**	--	**0.6**	--	**-6.8**

6. Docosahexaenoic acid (C22:6n-3, DHA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DHA**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	68.9	71.3	74.1	71.9	74.1	74.8	65.9	74.1
Replicate 2	72.2	72.3	73.4	70.1	73.4	73.8	73.9	73.4
Replicate 3	69.7	64.1	76.5	68.7	76.5	78.1	71.2	76.5
Mean	70.3	69.2	74.7	70.2	74.7	75.6	70.3	74.7
% difference from initial measurement	--	**-1.5**	--	**-6.0**	--	**1.2**	--	**6.2**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	151	156	160	153	160	160	164	160
Replicate 2	162	156	144	155	144	151	156	144
Replicate 3		162	169	152	169	166	157	169
Mean	156	158	158	153	158	159	159	158
% difference from initial measurement	--	**1.0**	--	**-3.1**	--	**0.6**	--	**-0.4**

7. Docosapentaenoic acid (C22:5n-3, DP3)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DP3**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	30.8	29.8	32.1	34.0	32.1	31.7	28.3	32.1
Replicate 2	31.4	29.3	32.0	34.8	32.0	32.5	30.6	32.0
Replicate 3	30.2	27.4	32.4	33.3	32.4	33.4	29.6	32.4
Mean	30.8	28.8	32.2	34.0	32.2	32.5	29.5	32.2
% difference from initial measurement	--	**-6.4**	--	**5.7**	--	**1.2**	--	**9.1**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	62.8	62.3	66.0	69.5	66.0	66.4	65.8	66.0
Replicate 2	66.4	63.7	60.7	72.4	60.7	62.0	63.8	60.7
Replicate 3		64.4	69.9	69.5	69.9	68.8	63.3	69.9
Mean	64.6	63.5	65.5	70.4	65.5	65.7	64.3	65.5
% difference from initial measurement	--	**-1.8**	--	**7.5**	--	**0.3**	--	**2.0**

8. Docosapentaenoic acid (C22:5n-6, DP6)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DP6**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	15.5	15.1	16.0	17.0	16.0	15.9	14.6	16.0
Replicate 2	15.6	14.6	15.6	17.5	15.6	16.3	15.9	15.6
Replicate 3	15.4	13.9	16.3	16.0	16.3	16.8	15.3	16.3
Mean	15.5	14.6	16.0	16.8	16.0	16.3	15.3	16.0
% difference from initial measurement	--	**-6.2**	--	**5.5**	--	**2.2**	--	**4.7**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	31.6	31.7	33.8	35.1	33.8	33.9	34.3	33.8
Replicate 2	34.5	31.8	31.1	36.8	31.1	32.0	33.9	31.1
Replicate 3		32.6	35.7	34.4	35.7	35.2	33.8	35.7
Mean	33.0	32.0	33.5	35.5	33.5	33.7	34.0	33.5
% difference from initial measurement	--	**-3.1**	--	**5.8**	--	**0.5**	--	**-1.4**

9. Docosatetraenoic acid (C22:4n-6, DTA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**DTA**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	24.6	23.6	24.2	27.0	24.2	24.4	23.6	24.2
Replicate 2	25.4	22.8	24.7	27.7	24.7	25.5	24.9	24.7
Replicate 3	23.7	21.0	24.5	25.2	24.5	25.5	24.4	24.5
Mean	24.6	22.4	24.5	26.6	24.5	25.2	24.3	24.5
% difference from initial measurement	--	**-8.6**	--	**8.9**	--	**2.8**	--	**0.7**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	43.7	42.8	46.7	49.3	46.7	46.7	48.1	46.7
Replicate 2	47.4	43.8	42.7	53.2	42.7	43.6	47.9	42.7
Replicate 3		44.9	49.0	48.8	49.0	47.6	47.0	49.0
Mean	45.5	43.8	46.1	50.5	46.1	45.9	47.7	46.1
% difference from initial measurement	--	**-3.7**	--	**9.3**	--	**-0.5**	--	**-3.2**

10. Eicosadienoic acid (C20:2n-6, ED1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ED1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	15.5	16.9	17.1	16.0	17.1	16.8	14.5	17.1
Replicate 2	15.8	17.0	17.6	17.4	17.6	16.8	16.5	17.6
Replicate 3	17.3	15.1	18.1	17.0	18.1	18.1	15.9	18.1
Mean	16.2	16.3	17.6	16.8	17.6	17.2	15.6	17.6
% difference from initial measurement	--	**0.8**	--	**-4.7**	--	**-2.0**	--	**12.5**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	26.0	30.3	26.7	27.1	26.7	26.0	28.8	26.7
Replicate 2	26.8	30.5	26.6	27.1	26.6	25.5	27.7	26.6
Replicate 3		28.3	31.3	29.5	31.3	30.0	27.6	31.3
Mean	26.4	29.7	28.2	27.9	28.2	27.2	28.0	28.2
% difference from initial measurement	--	**12.7**	--	**-1.1**	--	**-3.6**	--	**0.7**

11. Eicosenoic acid (C20:1n-9, EN1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**EN1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	7.19	7.93	8.14	7.89	8.14	7.95	7.08	8.14
Replicate 2	7.51	7.95	8.26	8.45	8.26	7.69	8.46	8.26
Replicate 3	8.16	7.15	8.52	8.30	8.52	8.33	8.17	8.52
Mean	7.62	7.68	8.31	8.21	8.31	7.99	7.90	8.31
% difference from initial measurement	--	**0.7**	--	**-1.1**	--	**-3.8**	--	**5.1**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	12.2	13.8	12.5	13.1	12.5	12.2	14.0	12.5
Replicate 2	12.8	14.5	12.4	12.8	12.4	11.8	14.0	12.4
Replicate 3		13.2	14.7	14.7	14.7	14.4	13.6	14.7
Mean	12.5	13.8	13.2	13.5	13.2	12.8	13.9	13.2
% difference from initial measurement	--	**10.4**	--	**2.6**	--	**-2.8**	--	**-4.8**

12. Eicosapentaenoic acid (C20:5n-3, EPA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**EPA**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	36.4	35.2	38.6	39.0	38.6	39.8	32.1	38.6
Replicate 2	36.7	37.3	36.4	37.2	36.4	37.3	36.8	36.4
Replicate 3	36.4	32.9	37.2	36.3	37.2	38.4	35.5	37.2
Mean	36.5	35.1	37.4	37.5	37.4	38.5	34.8	37.4
% difference from initial measurement	--	**-3.8**	--	**0.2**	--	**2.9**	--	**7.5**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	62.2	64.5	61.7	68.4	61.7	64.6	66.8	61.7
Replicate 2	65.2	66.3	60.9	66.3	60.9	64.1	65.3	60.9
Replicate 3		65.4	67.7	65.3	67.7	70.7	64.8	67.7
Mean	63.7	65.4	63.4	66.7	63.4	66.4	65.6	63.4
% difference from initial measurement	--	**2.7**	--	**5.1**	--	**4.7**	--	**-3.3**

13. Eicosatrienoic acid (C20:3n-9, ET1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ET1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	5.72	6.34	6.54	6.06	6.54	6.39	5.26	6.54
Replicate 2	5.84	6.44	6.76	6.45	6.76	6.21	6.37	6.76
Replicate 3	6.37	5.66	6.71	6.15	6.71	6.69	6.08	6.71
Mean	5.97	6.15	6.67	6.22	6.67	6.43	5.90	6.67
% difference from initial measurement	--	**2.9**	--	**-6.7**	--	**-3.6**	--	**13.0**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	11.8	14.0	11.9	12.4	11.9	11.8	12.9	11.9
Replicate 2	12.2	14.3	12.2	12.0	12.2	11.5	12.5	12.2
Replicate 3		12.9	14.3	13.4	14.3	13.6	12.5	14.3
Mean	12.0	13.7	12.8	12.6	12.8	12.3	12.6	12.8
% difference from initial measurement	--	**14.4**	--	**-1.6**	--	**-3.9**	--	**1.4**

14. *gamma*-Linolenic acid (C18:3n-6, GLA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**GLA**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	42.2	44.1	51.0	51.2	51.0	51.1	40.1	51.0
Replicate 2	44.4	44.1	45.6	48.4	45.6	45.4	50.9	45.6
Replicate 3	41.5	39.5	47.6	44.6	47.6	47.5	49.6	47.6
Mean	42.7	42.6	48.1	48.1	48.1	48.0	46.9	48.1
% difference from initial measurement	--	**-0.3**	--	**0.0**	--	**-0.2**	--	**2.5**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	53.3	56.0	55.0	62.1	55.0	54.7	62.1	55.0
Replicate 2	55.2	57.2	55.5	57.1	55.5	55.2	63.3	55.5
Replicate 3		54.4	63.9	59.6	63.9	64.2	62.0	63.9
Mean	54.2	55.9	58.1	59.6	58.1	58.0	62.5	58.1
% difference from initial measurement	--	**3.0**	--	**2.6**	--	**-0.2**	--	**-7.0**

15. *homo-gamma*-Linolenic acid (C20:3n-6, HGL)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**HGL**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	107	106	113	116	113	112	101	113
Replicate 2	109	105	112	115	112	110	110	112
Replicate 3	106	95	111	111	111	110	108	111
Mean	107	102	112	114	112	111	106	112
% difference from initial measurement	--	**-4.8**	--	**1.7**	--	**-1.1**	--	**5.3**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	193	193	202	207	202	200	202	202
Replicate 2	203	201	193	208	193	190	205	193
Replicate 3		199	212	207	212	211	199	212
Mean	198	198	202	207	202	200	202	202
% difference from initial measurement	--	**-0.1**	--	**2.4**	--	**-1.0**	--	**0.0**

16. Lauric acid (C12:0, LAR)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LAR**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	9.67	10.1	10.5	12.0	10.5	10.3	8.74	10.5
Replicate 2	9.43	9.49	10.4	11.0	10.4	10.5	10.1	10.4
Replicate 3	11.0	8.69	9.92	11.4	9.92	9.78	9.67	9.92
Mean	10.0	9.43	10.3	11.5	10.27	10.20	9.52	10.27
% difference from initial measurement	--	**-6.1**	--	**11.8**	--	**-0.8**	--	**8.0**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	13.2	14.8	14.1	15.0	14.1	14.3	16.9	14.1
Replicate 2	14.2	14.4	13.9	15.5	13.9	13.8	15.6	13.9
Replicate 3		13.8	15.7	16.5	15.7	15.8	15.7	15.7
Mean	13.7	14.3	14.5	15.7	14.5	14.6	16.1	14.5
% difference from initial measurement	--	**4.4**	--	**7.6**	--	**0.4**	--	**-9.6**

17. Lignoceric acid (C24:0, LG1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LG1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	44.7	45.2	45.8	45.2	45.8	45.0	43.8	45.8
Replicate 2	45.7	46.5	45.0	44.3	45.0	45.8	47.1	45.0
Replicate 3	45.5	40.8	46.3	43.6	46.3	46.7	46.3	46.3
Mean	45.3	44.2	45.7	44.4	45.7	45.8	45.7	45.7
% difference from initial measurement	--	**-2.5**	--	**-2.9**	--	**0.3**	--	**-0.1**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	58.1	60.3	59.0	58.4	59.0	59.3	62.7	59.0
Replicate 2	60.9	61.1	55.9	59.4	55.9	57.2	62.2	55.9
Replicate 3		59.3	61.6	57.4	61.6	62.1	61.7	61.6
Mean	59.5	60.2	58.8	58.4	58.8	59.5	62.2	58.8
% difference from initial measurement	--	**1.3**	--	**-0.7**	--	**1.2**	--	**-5.4**

18. Linoleic acid (C18:2n-6, LNA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**LNA**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	1917	1965	2222	2213	2222	2213	1859	2222
Replicate 2	1961	2008	2013	2049	2013	2022	2284	2013
Replicate 3	1920	1787	2124	1922	2124	2134	2242	2124
Mean	1933	1920	2120	2062	2120	2123	2128	2120
% difference from initial measurement	--	**-0.7**	--	**-2.7**	--	**0.2**	--	**-0.4**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	2839	2988	2908	3087	2908	2911	3293	2908
Replicate 2	2917	3059	2842	2902	2842	2845	3336	2842
Replicate 3		2933	3309	3015	3309	3313	3248	3309
Mean	2878	2993	3020	3001	3020	3023	3292	3020
% difference from initial measurement	--	**4.0**	--	**-0.6**	--	**0.1**	--	**-8.3**

19. Myristoleic acid (C14:1n-5, ML1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ML1**

Quality material 1: Low pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	6.26	6.69	7.25	6.90	7.25	7.37	5.96	7.25
Replicate 2	6.71	6.90	7.01	6.99	7.01	6.67	6.88	7.01
Replicate 3	6.52	6.01	7.18	6.47	7.18	7.27	6.74	7.18
Mean	6.49	6.54	7.15	6.79	7.15	7.10	6.53	7.15
% difference from initial measurement	--	**0.6**	--	**-5.1**	--	**-0.6**	--	**9.5**

Quality material 2: High pool								
	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	7.10	7.80	7.21	8.09	7.21	7.14	7.89	7.21
Replicate 2	7.49	7.55	7.33	7.55	7.33	7.30	7.76	7.33
Replicate 3		7.39	8.12	8.12	8.12	8.21	7.77	8.12
Mean	7.30	7.58	7.55	7.92	7.55	7.55	7.81	7.55
% difference from initial measurement	--	**3.9**	--	**4.9**	--	**-0.1**	--	**-3.2**

20. Myristic acid (C14:0, MR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**MR1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	81.2	83.2	90.3	93.0	90.3	90.6	77.3	90.3
Replicate 2	83.2	84.9	84.8	88.9	84.8	85.0	89.5	84.8
Replicate 3	83.7	74.2	88.8	81.1	88.8	88.8	89.6	88.8
Mean	82.7	80.8	87.9	87.7	87.9	88.1	85.5	87.9
% difference from initial measurement	--	**-2.3**	--	**-0.3**	--	**0.2**	--	**2.9**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	129	135	133	145	133	132	146	133
Replicate 2	134	140	130	141	130	131	149	130
Replicate 3		135	150	145	150	149	149	150
Mean	132	137	138	144	138	137	148	138
% difference from initial measurement	--	**3.8**	--	**4.5**	--	**-0.1**	--	**-7.2**

21. Margaric acid (C17:0, MRG)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**MRG**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	18.3	18.1	20.0	20.5	20.0	20.4	18.7	20.0
Replicate 2	18.7	18.6	19.6	19.6	19.6	19.3	20.4	19.6
Replicate 3	18.8	16.4	20.0	18.5	20.0	20.4	20.4	20.0
Mean	18.6	17.7	19.9	19.5	19.9	20.0	19.8	19.9
% difference from initial measurement	--	**-5.0**	--	**-1.7**	--	**0.8**	--	**0.2**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	25.6	26.7	27.3	28.2	27.3	27.7	28.7	27.3
Replicate 2	26.6	27.3	26.3	28.2	26.3	26.5	28.6	26.3
Replicate 3		27.0	29.8	28.4	29.8	29.9	28.8	29.8
Mean	26.1	27.0	27.8	28.3	27.8	28.0	28.7	27.8
% difference from initial measurement	--	**3.5**	--	**1.8**	--	**0.8**	--	**-3.2**

22. Nervonic acid (C24:1n-9, NR1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**NR1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	66.1	69.2	71.3	67.3	71.3	71.3	65.2	71.3
Replicate 2	66.6	69.0	74.1	69.8	74.1	76.1	70.0	74.1
Replicate 3	68.7	59.9	71.1	66.0	71.1	70.3	68.4	71.1
Mean	67.1	66.1	72.1	67.7	72.1	72.5	67.9	72.1
% difference from initial measurement	--	**-1.6**	--	**-6.2**	--	**0.6**	--	**6.3**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	83.0	86.5	86.0	85.2	86.0	87.5	91.6	86.0
Replicate 2	88.7	88.2	82.5	89.7	82.5	83.4	89.7	82.5
Replicate 3		88.2	91.9	86.9	91.9	92.6	88.1	91.9
Mean	85.8	87.6	86.8	87.3	86.8	87.8	89.8	86.8
% difference from initial measurement	--	**2.0**	--	**0.5**	--	**1.1**	--	**-3.3**

23. Oleic acid (C18:1n-9, OL1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**OL1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1008	1043	1158	1179	1158	1155	1013	1158
Replicate 2	1058	1069	1090	1150	1090	1070	1227	1090
Replicate 3	1049	949	1141	1082	1141	1126	1198	1141
Mean	1039	1020	1129	1137	1129	1117	1146	1129
% difference from initial measurement	--	**-1.8**	--	**0.7**	--	**-1.1**	--	**-1.4**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1854	1961	1902	2062	1902	1892	2203	1902
Replicate 2	1902	2006	1859	1899	1859	1851	2231	1859
Replicate 3		1930	2177	2038	2177	2200	2198	2177
Mean	1878	1966	1979	2000	1979	1981	2211	1979
% difference from initial measurement	--	**4.7**	--	**1.0**	--	**0.1**	--	**-10.5**

24. Pentadecanoic acid (C15:0, PDE)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PDE**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	11.4	11.3	12.4	13.0	12.4	12.6	10.5	12.4
Replicate 2	12.2	11.6	11.8	12.6	11.8	12.0	12.3	11.8
Replicate 3	11.5	10.2	12.6	11.6	12.6	12.3	11.8	12.6
Mean	11.7	11.0	12.3	12.4	12.3	12.3	11.5	12.3
% difference from initial measurement	--	**-5.5**	--	**0.7**	--	**-0.1**	--	**6.7**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	15.0	16.0	15.6	16.8	15.6	15.6	16.6	15.6
Replicate 2	15.8	16.0	15.2	17.0	15.2	15.3	16.9	15.2
Replicate 3		15.8	17.3	16.9	17.3	17.4	16.7	17.3
Mean	15.4	15.9	16.0	16.9	16.0	16.1	16.7	16.0
% difference from initial measurement	--	**3.6**	--	**5.3**	--	**0.5**	--	**-4.3**

25. Palmitoleic acid (C16:1n-7, PL1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PL1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	137	144	159	160	159	159	128	159
Replicate 2	144	143	145	155	145	144	156	145
Replicate 3	136	127	153	144	153	153	153	153
Mean	139	138	152	153	152	152	146	152
% difference from initial measurement	--	**-0.7**	--	**0.7**	--	**-0.2**	--	**4.1**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	303	318	306	337	306	312	340	306
Replicate 2	313	327	307	318	307	308	348	307
Replicate 3		313	355	336	355	352	342	355
Mean	308	319	323	330	323	324	343	323
% difference from initial measurement	--	**3.7**	--	**2.4**	--	**0.4**	--	**-6.0**

26. Palmitic acid (C16:0, PM1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**PM1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1647	1664	1854	1832	1854	1830	1662	1854
Replicate 2	1704	1706	1733	1768	1733	1737	1905	1733
Replicate 3	1683	1493	1815	1670	1815	1820	1857	1815
Mean	1678	1621	1801	1757	1801	1795	1808	1801
% difference from initial measurement	--	**-3.4**	--	**-2.4**	--	**-0.3**	--	**-0.4**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3232	3342	3344	3500	3344	3345	3578	3344
Replicate 2	3348	3407	3185	3438	3185	3189	3572	3185
Replicate 3		3348	3631	3513	3631	3633	3537	3631
Mean	3290	3366	3387	3484	3387	3389	3563	3387
% difference from initial measurement	--	**2.3**	--	**2.9**	--	**0.1**	--	**-4.9**

27. Stearidonic acid (C18:4n-3, SD1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**SD1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.56	2.95	3.09	2.76	3.09	3.07	2.54	2.79
Replicate 2	2.68	3.25	3.10	2.78	3.10	2.94	2.43	2.88
Replicate 3	2.98	2.72	3.16	2.98	3.16	3.23	2.40	2.56
Mean	2.74	2.97	3.12	2.84	3.12	3.08	2.46	2.74
% difference from initial measurement	--	**8.5**	--	**-8.9**	--	**-1.2**	--	**11.6**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3.07	3.67	2.95	3.13	2.95	2.81	2.62	3.00
Replicate 2	2.90	3.60	3.19	2.77	3.19	3.10	2.71	2.95
Replicate 3		3.14	3.68	3.31	3.68	3.50	2.76	2.97
Mean	2.98	3.47	3.27	3.07	3.27	3.14	2.70	2.97
% difference from initial measurement	--	**16.3**	--	**-6.1**	--	**-4.2**	--	**10.3**

28. Stearic acid (C18:0, ST1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**ST1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	476	469	496	513	496	498	485	496
Replicate 2	469	487	483	495	483	476	544	483
Replicate 3	500	417	516	459	516	515	524	516
Mean	482	457	498	489	498	496	518	498
% difference from initial measurement	--	**-5.1**	--	**-1.9**	--	**-0.4**	--	**-3.8**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	864	897	902	865	902	899	983	902
Replicate 2	892	918	841	888	841	838	956	841
Replicate 3		913	953	897	953	949	951	953
Mean	878	909	898	883	898	895	963	898
% difference from initial measurement	--	**3.6**	--	**-1.7**	--	**-0.3**	--	**-6.7**

29. Tricosanoic acid (C23:0, TSA)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**TSA**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	20.5	20.6	21.2	21.5	21.2	21.1	20.7	21.2
Replicate 2	21.5	20.8	20.4	21.2	20.4	20.7	22.0	20.4
Replicate 3	20.4	18.7	21.4	20.9	21.4	21.5	21.5	21.4
Mean	20.8	20.0	21.0	21.2	21.0	21.1	21.4	21.0
% difference from initial measurement	--	**-3.7**	--	**0.9**	--	**0.4**	--	**-1.7**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	27.0	27.5	27.5	28.2	27.5	27.5	29.3	27.5
Replicate 2	27.6	27.7	26.7	27.8	26.7	26.3	29.0	26.7
Replicate 3		27.0	28.5	27.9	28.5	28.6	28.8	28.5
Mean	27.3	27.4	27.5	28.0	27.5	27.5	29.1	27.5
% difference from initial measurement	--	**0.4**	--	**1.5**	--	**-0.3**	--	**-5.2**

30. cis-Vaccenic acid (C18:1n-7, VC1)

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM
Analyte:	**VC1**

Quality material 1: Low pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	74.4	77.2	90.1	82.6	90.1	87.5	71.6	90.1
Replicate 2	84.1	72.9	79.0	86.1	79.0	76.3	85.4	79.0
Replicate 3	72.5	64.5	85.6	79.8	85.6	83.0	83.2	85.6
Mean	77.0	71.5	84.9	82.8	84.9	82.3	80.1	84.9
% difference from initial measurement	--	**-7.1**	--	**-2.5**	--	**-3.1**	--	**6.0**

Quality material 2: High pool								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	140	146	153	170	153	148	166	153
Replicate 2	153	148	157	161	157	152	166	157
Replicate 3		142	170	162	170	166	162	170
Mean	146	146	160	164	160	155	165	160
% difference from initial measurement	--	**-0.5**	--	**2.7**	--	**-2.8**	--	**-2.9**

LOD, specificity and fit for intended use

The LOD, specificity, and fit for intended use of the 30 analytes is summarized here:

Method name:	Fatty acids in serum
Method #:	4028
Matrix:	Serum
Units:	uM

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
alpha-Linolenic acid (C18:3n-3) (ALN)	1.54	yes	yes
Arachidic acid (C20:0) (AR1)	0.82	yes	yes
Arachidonic acid (C20:4n-6) (ARA)	7.34	yes	yes
Capric acid (C10:0) (CAP)	1.59	yes	yes
Docosanoic acid (C22:0) (DA1)	0.68	yes	yes
Docosahexaenoic acid (C22:6n-3) (DHA)	1.84	yes	yes
Docosapentaenoic acid (C22:5n-3) (DP3)	0.55	yes	yes
Docosapentaenoic acid (C22:5n-6) (DP6)	0.24	yes	yes
Docosatetraenoic acid (C22:4n-6) (DTA)	0.31	yes	yes
Eicosadienoic acid (C20:2n-6) (ED1)	0.31	yes	yes
Eicosenoic acid (C20:1n-9) (EN1)	0.87	yes	yes
Eicosapentaenoic acid (C20:5n-3) (EPA)	0.79	yes	yes
Eicosatrienoic acid (C20:3n-9) (ET1)	0.39	yes	yes
gamma-Linolenic acid (C18:3n-6) (GLA)	0.42	yes	yes
homo-gamma-Linolenic acid (C20:3n-6) (HGL)	1.14	yes	yes
Lauric acid (C12:0) (LAR)	2.33	yes	yes
Lignoceric acid (C24:0) (LG1)	1.09	yes	yes
Linoleic acid (C18:2n-6) (LNA)	22.6	yes	yes
Myristoleic acid (C14:1n-5) (ML1)	0.29	yes	yes
Myristic acid (C14:0) (MR1)	4.9	yes	yes
Margaric acid (C17:0) (MRG)	3.36	yes	yes
Nervonic acid (C24:1n-9) (NR1)	0.69	yes	yes
Oleic acid (C18:1n-9) (OL1)	17.7	yes	yes
Pentadecanoic acid (C15:0) (PDE)	0.75	yes	yes
Palmitoleic acid (C16:1n-7) (PL1)	6.56	yes	yes
Palmitic acid (C16:0) (PM1)	78.1	yes	yes
Stearidonic acid (C18:4n-3) (SD1)	0.24	yes	yes
Stearic acid (C18:0) (ST1)	39.1	yes	yes
Tricosanoic acid (C23:0) (TSA)	0.9	yes	yes
Vaccenic acid (C18:1n-7) (VC1)	2.31	yes	yes

Appendix B – Ruggedness Testing

Detailed information can be found at:

\\cdc\project\CCEHIP_NCEH_DLS_NBB_LABS\Fatty Acids Documents\Fatty Acids 2 Validation 2008

1. Heating Block Temperature

a. Principle: Esterified fatty acids are hydrolyzed from triglycerides, phospholipids and cholesteryl esters using sequential treatment with mineral acid and base in the presence of heat. The temperature of the heating blocks can vary day to day which could have an effect on hydrolysis.

b. Proposal: To vary the temperature during the hydrolysis phase to determine its effect on area counts for ARA, DHA, EPA, and LNA.

c. Findings: Method specifies 219°F (104°C), lower temps 183°F (84°C) and 201°F (94°C), and higher temps 237°F (114°C) and 255°F (124°C) were tested

Analyte	QCID	219°F uM	183°F uM	201°F uM	237°F uM	255°F uM
ARA	HP06+03_6209_QC04	1215	1220	1190	1190	1205
	LP_06553_QC04	528	547	543	527	543
	MP06+02_6208_QC04	893	876	882	874	876
DHA	HP06+03_6209_QC04	187	187	186	184	184
	LP_06553_QC04	123	127	127	122	128
	MP06+02_6208_QC04	196	190	190	191	191
EPA	HP06+03_6209_QC04	67	68	67	66.5	67
	LP_06553_QC04	38.5	39.5	39	38.5	39.5
	MP06+02_6208_QC04	107	104.5	104	105	104
LNA	HP06+03_6209_QC04	5520	5600	5490	5465	5505
	LP_06553_QC04	3010	3125	3110	2995	3080
	MP06+02_6208_QC04	3250	3185	3200	3190	3180

d. Heating Block Temperature Summary: Varying the temperature of the heating blocks within 183 – 255°F does not appear to affect ARA, DHA, EPA or LNA concentrations.

2. Heating Block versus Oven for Hydrolysis

a. Principle: The heating block temperature can fluctuate causing high temperature spikes, which can lead to evaporation of the solvent during the hydrolysis step. This loss of solvent can affect the recovery of some of the analytes causing a higher sample repeat rate.

b. Proposal: To compare the high calibrator, high and low QC in triplicate in the oven and heating block and measure the peak area ratio to determine if there are any differences between using an oven or a heating block. Both the heating block and oven were set at 104°C.

c. Findings:

Table1: Effect of using a heating block versus an oven during the hydrolysis step on the mean peak area ratio (PAR)

Analyte Code	cal 40			HP06+03_6209_QC04			LP_06553_QC04			Average
	Heating block	Oven	% diff	Heating block	Oven	% diff	Heating block	Oven	% diff	% diff PAR
ALN	2.51	2.52	0%	0.97	0.95	-2%	0.22	0.22	1%	0%
AR1	1.61	1.69	5%	0.21	0.22	6%	0.13	0.15	11%	7%
ARA	4.76	4.79	1%	2.68	2.65	-1%	1.15	1.18	2%	1%
DA1	1.26	1.35	8%	0.42	0.46	10%	0.25	0.29	15%	11%
DE1	0.37	0.42	15%	0.11	0.15	44%	0.08	0.1	28%	29%
DHA	4.3	4.34	1%	0.76	0.76	-1%	0.5	0.51	3%	1%
DP3	1.23	1.28	4%	0.34	0.35	2%	0.17	0.18	5%	3%
DP6	0.61	0.64	5%	0.15	0.15	1%	0.06	0.06	5%	3%
DTA	0.75	0.8	7%	0.23	0.24	4%	0.11	0.12	12%	7%
ED1	0.13	0.13	4%	0.1	0.1	3%	0.04	0.05	7%	5%
EN1	0.15	0.16	8%	0.09	0.1	5%	0.03	0.03	8%	7%
EPA	3.27	3.3	1%	0.25	0.25	0%	0.14	0.14	1%	0%
GLA	1.38	1.38	0%	0.45	0.45	-1%	0.35	0.35	2%	0%
HGL	1.53	1.57	2%	1.02	1.02	1%	0.79	0.83	5%	2%
LG1	1.21	1.35	12%	0.33	0.38	14%	0.21	0.25	17%	14%
LNA	3.37	3.39	1%	2.39	2.37	-1%	1.3	1.32	2%	0%
ML1	0.51	0.5	-1%	0.05	0.05	-3%	0.06	0.06	-1%	-2%
MR1	4.36	4.39	1%	1.63	1.63	0%	0.95	0.97	2%	1%
NR1	7.66	8.52	11%	0.47	0.52	11%	0.39	0.46	17%	13%
OL1	4.06	4.09	1%	3.32	3.31	0%	1.08	1.09	1%	1%
PL1	6.53	6.55	0%	1.34	1.26	-7%	0.55	0.54	-3%	-3%
PM1	5.13	5.15	0%	3.17	3.14	-1%	1.29	1.31	2%	0%
ST1	6.17	6.2	1%	3.48	3.45	-1%	1.71	1.72	1%	0%
VC1	0.77	0.78	1%	0.38	0.39	1%	0.16	0.16	-1%	0%

d. Using a heating block versus an oven for hydrolysis summary: The overall average percent difference for all analytes was 4%. There appears to be no difference in using the heating blocks verus the oven on any analyte, therefore either can be used. The oven is preferred because the temperature is easier to maintain and there are fewer samples evaporating as compared to when the heating blocks were used.

3. Derivitization

a. Principle: The extract is derivatized with pentaflurobenzyl bromide in the presence of triethylamine (TEA) to form pentaflurobenzyl (PFB) esters. The current method specifies to add 10uL of TEA to the bottom of the tube and then add 100uL of 7% PFBBr in acetonitrile and wait 15 minutes to allow sufficient time for reaction.

b. Proposal: To compare adding TEA and PFBBr solution separately or mixed together prior to addition to extract. To allow reaction to take place for an additional 10 minutes to verify that 15 minutes is sufficient. Only one set of calibrators (set defined as cal 40a, 30a, 20a, 10a, and blank) per factor was used.

c. Findings:

Table1: Effect of mixing TEA with PFBBr solution or adding separately on the concentrations of ARA, DHA, EPA, and LNA

Analyte	Factor	cal 40a (uM)	cal 30a (uM)	cal 20a (uM)	cal 10a (uM)	blank (uM)
ARA	TEA added separate; rxn time 15 min	2120	470	198	80.9	12.1
	TEA mixed prior to addition; rxn time 15 min	2100	462	191	83.5	11.7
	TEA added separate; rxn time 25 min	2105	461	189	79.8	11.7
	TEA mixed prior to addition; rxn time 25 min	2069	472	196	80.3	11.8
DHA	TEA added separate; rxn time 15 min	1042	240	99.2	40.3	6.60
	TEA mixed prior to addition; rxn time 15 min	1032	236	97	41.2	6.30
	TEA added separate; rxn time 25 min	1058	236	95.6	38.8	6.40
	TEA mixed prior to addition; rxn time 25 min	1036	242	98.8	39.4	6.40
EPA	TEA added separate; rxn time 15 min	829	192	79.9	32.2	5.10
	TEA mixed prior to addition; rxn time 15 min	831	189	77.9	33.1	5.00
	TEA added separate; rxn time 25 min	848	191	77.3	31.2	5.00
	TEA mixed prior to addition; rxn time 25 min	828	192	80.2	31.7	5.00
LNA	TEA added separate; rxn time 15 min	8072	1954	814	319	12.2
	TEA mixed prior to addition; rxn time 15 min	7941	1920	794	328	10.0
	TEA added separate; rxn time 25 min	8224	1956	779	310	9.70
	TEA mixed prior to addition; rxn time 25 min	8022	1945	806	314	10.5

d. Adding TEA separately or mixed with PFBBr solution summary: Mixing the TEA with the PFBBr solution prior to adding to the extract does not appear to affect ARA, DHA, EPA, or LNA, however in the past if TEA is not properly added to the bottom of the tube containing the extract, then the derivatization is not complete. So since it yields similar results, TEA will be added to the PFBBr solution just prior to aliquotting to achieve the most consistent derivatization possible.

Table 2: Effect of longer reaction time of TEA and PFBBr has on the peak area of ARA, DHA, EPA, and LNA in calibrators

Analyte	Factor	cal 40a (analyte peak area)	cal 30a (analyte peak area)	cal 20a (analyte peak area)	cal 10a (analyte peak area)	blank (analyte peak area)
ARA	TEA added separate; rxn time 15 min	10,342,800	2,423,610	690,931	272,022	2,418
	TEA mixed prior to addition; rxn time 15 min	11,474,800	1,841,460	799,494	297,310	704
	TEA added separate; rxn time 25 min	7,828,580	871,874	608,026	231,309	488
	TEA mixed prior to addition; rxn time 25 min	10,408,500	1,854,230	763,023	258,501	1,080
DHA	TEA added separate; rxn time 15 min	16,794,800	4,197,870	1,181,700	460,744	5,053
	TEA mixed prior to addition; rxn time 15 min	21,171,200	3,372,700	1,465,720	519,113	1,757
	TEA added separate; rxn time 25 min	13,903,800	846,652	1,033,040	380,262	1,841
	TEA mixed prior to addition; rxn time 25 min	18,192,600	3,370,860	1,389,410	438,293	2,848
EPA	TEA added separate; rxn time 15 min	14,293,900	3,506,330	971,106	375,213	3,419
	TEA mixed prior to addition; rxn time 15 min	17,305,900	2,731,130	1,195,730	423,339	2,067
	TEA added separate; rxn time 25 min	11,268,600	879,146	864,994	311,999	1,149
	TEA mixed prior to addition; rxn time 25 min	15,188,900	2,722,590	1,136,990	356,939	2,323
LNA	TEA added separate; rxn time 15 min	2,192,640	479,788	128,988	50,473	860
	TEA mixed prior to addition; rxn time 15 min	2,225,670	338,265	145,263	53,237	434
	TEA added separate; rxn time 25 min	1,545,730	221,902	111,004	41,484	358
	TEA mixed prior to addition; rxn time 25 min	2,087,520	337,335	134,607	46,523	540

e. **Increasing reaction time summary:** Increasing the reaction time, does not appear to increase the peak area for ARA, DHA, EPA or LNA, therefore either 15 or 25 minutes are equivalent and may be used.

4. Time length before injection

a. Principle: Occasionally it is necessary to store the prepared samples at -20°C until they can be run on an instrument at a later time.

b. Proposal: To vary storage time of prepared samples at -80°C to determine if there are effects on analyte concentrations. Experiment 1: 325 patient samples, quality control materials and calibrators were analyzed in March 2010 and re-injected May 2011. Experiment 2: 266 patient samples, quality control materials and calibrators were analyzed in March- April 2010 and re-injected June 2013.

c. Findings: Tables 1 and 2 show the comparison of linear regressions for each experiment.

Table 1: 14 months at -80°C

Analyte	n	R^2	Intercept	Slope
ALN	325	1.00	0.67	0.99
AR1	325	1.00	-0.68	1.01
ARA	325	1.00	-9.96	1.01
DA1	325	0.99	-0.70	1.00
DE1	325	1.00	0.12	1.00
DHA	325	1.00	-2.14	1.00
DP3	325	1.00	-0.02	1.01
DP6	325	1.00	-0.01	1.00
DTA	325	1.00	0.00	1.01
ED1	325	1.00	0.07	1.00
EN1	325	1.00	-0.13	1.00
EPA	325	1.00	-0.74	1.01
GLA	325	1.00	0.26	0.99
HGL	325	0.99	-0.81	0.99
LG1	325	0.99	-0.07	1.00
LNA	325	1.00	-102	1.02
ML1	323	1.00	0.2	1.00
MR1	325	1.00	-1.22	1.00
NR1	325	1.00	-1.25	1.02
OL1	325	1.00	-29.4	1.01
PL1	325	0.99	4.41	1.02
PM1	325	1.00	45.0	0.98
ST1	325	1.00	-18.1	1.02
VC1	325	0.92	29.2	0.93

Table 2: 3 years and 3 months at -80°C

Analyte	n	R^2	Intercept	Slope
ALN	265	1.00	0.36	1.00
AR1	266	1.00	-0.67	1.02
ARA	264	1.00	-24.0	1.01
DA1	266	0.99	-2.30	1.03
DE1	266	1.00	-0.11	1.01
DHA	264	1.00	-2.34	1.01
DP3	264	1.00	0.69	1.02
DP6	262	1.00	-0.23	1.00
DTA	262	1.00	-0.11	1.01
ED1	262	1.00	0.37	1.02
EN1	265	1.00	0.00	1.00
EPA	263	1.00	-0.25	1.01
GLA	263	1.00	0.73	1.00
HGL	263	1.00	-0.23	1.00
LG1	265	0.99	-1.98	1.03
LNA	266	1.00	10.5	1.01
ML1	262	1.00	0.21	1.01
MR1	266	1.00	-6.55	1.02
NR1	264	1.00	0.48	1.03
OL1	266	1.00	-11.6	1.01
PL1	264	0.99	3.91	1.01
PM1	266	0.99	-117	1.08
ST1	266	0.98	-43.1	1.01
VC1	266	0.95	29.4	0.91

d. Stored extracted samples for varying lengths of time summary: There is essentially no difference in prepared samples tested up to 3 years later.

5. Sonication of calibrators/samples prior to aliquotting

a. Principle: When preparing stock standard solutions (p.6), it is necessary to sonicate some of the individual analytes to go into toluene prior to preparing the working standards. Determine how long the sonication should be to solubilize the material but not to destroy it.

b. Proposal: To vary sonication time (15 – 90 minutes) of the high calibrator to determine if there is an effect on analyte concentration.

c. Findings: The table shows the average of 4 replicates across 4 sonication time points (15, 30, 60, and 90 minutes)

Analyte	Avg (uM)	SD	CV
Fatty acids - Saturated			
AR1	164	15	9%
DA1	236	27	11%
LG1	123	16	13%
MR1	473	9	2%
PM1	5537	100	2%
ST1	1710	33	2%
Fatty acids - Monounsaturated			
EN1	62.9	3	5%
DE1	63.5	5	8%
ML1	50.8	2	3%
NR1	131	11	9%
OL1	6591	129	2%
PL1	380	7	2%
VC1	961	26	3%
Fatty acids - Polyunsaturated			
ALN	361	7	2%
ARA	2542	46	2%
DHA	801	17	2%
DP3	158	4	2%
DP6	79.9	2	2%
DTA	77.9	2	3%
ED1	64.3	2	4%
EPA	1101	23	2%
GLA	181	4	2%
HGL	383	7	2%
LNA	7898	125	2%

d. Sonication time summary: There does not appear to be a difference in concentration for any analyte with increased sonication time. Therefore a minimum of 15 minutes sonication time will be done for all standards, QC and samples prior to aliquotting.

Appendix C: Method Comparison: #4026 vs #4028

A method comparison consisting of 20 patient samples was completed. Excluding DE1, 23 fatty acids (common to both methods) showed good to excellent agreement with average difference < 1% and excellent correlation with average R^2 of 0.98.

Analyte	Average % Difference*
ALN_N	2.4%
AR1_N	0.6%
ARA_N	2.2%
DA1_N	3.7%
DE1_N	102%
DHA_N	5.5%
DP3_N	0.4%
DP6_N	-4.5%
DTA_N	-12.2%
ED1_N	-3.7%
EN1_N	11.5%
EPA_N	3.7%
GLA_N	1.1%
HGL_N	-6.5%
LG1_N	4.2%
LNA_N	-3.3%
ML1_N	4.8%
MR1_N	-3.2%
NR1_N	18%
OL1_N	-2.8%
PL1_N	3.5%
PM1_N	-3.4%
ST1_N	-10.2%
VC1_N	-7.1%
Mean	-0.1%
SD	6.4%

* (#4028 - #4026)/#4026 [(new method – old method)/old method]

DE1 is a low-level MUFA that is not measured with precision (generally close to the LOD) and thus has been deemed non-reportable in assay #4028.