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ORIGINAL PAPER

Statistical Analysis of the Collaborative Study in Support of the Official Method AOCS Ce 1i-07: Determination of Saturated, *cis*-Monounsaturated and *cis*-Polyunsaturated Fatty Acids in Marine and Other Oils Containing Long Chain Polyunsaturated Fatty Acids by Capillary GLC

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Abstract Analysis was done of the statistical results obtained by following recommended AOCS Collaborative Study Procedure M-86 to evaluate the performance of Official Method AOCS Ce 1i-07, which provides a gas–liquid chromatography (GLC) procedure for the determination of the fatty acid composition of oils containing long chain polyunsaturated fatty acids (PUFAs). The method obtains relative between-lab reproducibility (%RSDR) values on the order of 5% or less for most fatty acids that are present above ~0.5% w/w; however, the reproducibility worsens dramatically for analytes below this threshold. Apparently, several participating labs had problems identifying small peaks in the sample chromatograms. They also had problems correctly identifying certain larger peaks that occurred in a congested area of the sample chromatograms, including the 9c-16:1, 9c-11c-22:1, and 6c,9c,12c,15c-16:4 fatty acids. Finally, several analytes with chain lengths between 16 and 18 and between 21 and 22 carbons that were present at moderate concentrations had worse than expected reproducibilities due to severe overlap of these analytes' peaks. A detailed inspection of the contributed data shows that the relatively poor between-lab reproducibility for analytes in this region is due to differences in the labs' chromatographic efficiencies and perhaps in their methods of quantifying highly overlapped peaks.

Keywords Long chain polyunsaturated fatty acid · Capillary gas–liquid chromatography · Outlier · Reproducibility · AOCS Ce 1i-07 · AOCS M-86

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Introduction

An international collaborative study was undertaken to determine the performance of the Official Method AOCS Ce 1i-07 entitled Determination of Saturated, *cis*-Monounsaturated and *cis*-Polyunsaturated Fatty Acids in Marine and Other Oils Containing Long Chain Polyunsaturated Fatty Acids (PUFAs) by Capillary Gas–Liquid Chromatography (GLC), which is to be used for nutritional labeling of the total fat, saturated, *cis*-monounsaturated, and *cis*-polyunsaturated fatty acid contents of marine oils. This method determines the content and concentrations of saturated fatty acids (SFA), *cis*-monounsaturated fatty acids (MUFA), and *cis*-polyunsaturated fatty acids (PUFA), including arachidonic acid (ArA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), in marine oils and other oils such as single cell oils (SCOs) using capillary GLC. This method, however, is not suitable for separation of *cis*- and *trans*-isomers of unsaturated FAMEs, nor is it suitable for determining the *trans*-fatty acid composition of hydrogenated marine oils. AOCS Ce 1h-05 or Ce 1j-08 are the methods of choice for quantitating and separating oils with *trans*-fatty acids, and short chain fatty acids (≤10 carbons) should be quantitated with AOCS Ce 1j-08.

While the details of the method procedure and the results of the collaborative study were published in the Official Method AOCS Ce 1i-07 document, it is also necessary to publish a statistical analysis of the study results, according to AOCS Procedure M 4-86. Therefore, the statistical analysis presented here will focus on the characterization of outliers and certain analytical figures of merit in the fatty acid concentration data. The discussion will also include recommendations regarding the appropriate use of the method.

Experimental Procedures

As mentioned above, complete details of the procedure are provided in the Official Method AOCS Ce 1i-07 document, but in order to provide a context for the current discussion the procedure will be summarized here.

Summary

A triacylglycerol (23:0 TAG) internal standard (IS) (present as one of the standard fatty acids in the mixture GLC-714 from Nu-Chek-Prep, Inc.) was used to determine the concentration of the individual fatty acid methyl esters (FAMEs) in the oil samples after methylation. GLC-714 was added to the FAMEs of the samples prior to separation on a capillary gas chromatography column having a polar stationary phase. Theoretical Correction Factors (TCFs) were used to quantitate all saturated and monounsaturated fatty acids greater than or equal to 12:0 and polyunsaturated fatty acids (PUFA) of 18 carbons. TCFs were also used for fatty acids which lack standards, such as 6c, 9c, 12c, 15c, 18c-21:5 (21:5n-3). Empirical Correction Factors (ECFs) were used for long chain PUFA of 20 carbons or more and three or more double bonds (for which standards are readily available).

Hardware Setup

1. Gas chromatograph suitable for use with a capillary column equipped with:
   (a) Thermostatic chamber for the capillary column, capable of maintaining the desired temperature to ± 0.1 °C.
   (b) Temperature-controlled split mode injection unit.
   (c) Flame-ionization detector (FID), amplifier and electronic recorder-integrator.
2. Capillary columns: The capillary GC column should be of fused silica capillary 30 m in length, and 0.25 mm i.d., 0.25 μm coating of polyethylene glycol (PEG).
3. Microsyringe for gas chromatography: 10 μL delivery, with a hardened needle.
4. Carrier gas: hydrogen or helium.
5. Flame gases: hydrogen and air.
6. Make-up gas: nitrogen or helium.
7. Operating conditions were as follows:
   (a) Injection port temperature: 235 °C.
   (b) Detector temperature: 325 °C.
   (c) Oven temperature program: Initial temperature 170 °C; ramp 1 °C/min; final temperature 225 °C.

Calculations

1. The amount (in g) of individual fatty acids was calculated, expressed as FAME (W_{FAMEx}) using Eq. 1:

$$W_{FAME_x} = \frac{A_x \times W_{TAG\,IS} \times 1.0037 \times R_x}{A_{is}} \quad (1)$$

where A_x = area counts for fatty acid x; $W_{TAG\ Is}$ = weight of the GLC-714 IS (in g) added to the oils; A_{is} = peak area counts of the 23:0 IS peak; 1.0037, conversion of IS in test portion and R_x = theoretical flame ionization detector correction factor (TCF) for FAMEs relative to the 23:0 FAME of the GLC-714 IS, respectively or the Empirical Correction Factor (ECF) as determined via the GLC-714 IS. TCF should be applied to the analytical data for optimum accuracy and to minimize variation between laboratories because of differences in calculating response factors. TCFs are also used for fatty acids where standards are not available. ECFs were needed due to the large deviation from TCFs for long chain PUFA of 20 carbons or more and three or more double bonds. The TCFs were calculated using Eq. 2:

$$TCF_X = \frac{MW_X}{(N_x - 1) \times (AWC) \times (1.3344)} \quad (2)$$

where TCF = theoretical flame ionization detector response factor for fatty acid x (as methyl ester) with respect to the 23:0 FAME of the GLC-714 IS; MW_x = molecular weight of component x; N_x = number of carbon atoms in the FAME of component x; AWC = atomic weight of carbon (12.011); 1.3344 = TCF for the 23:0 FAME of the GLC-714 IS.

The ECFs were calculated using the Certificate of Analysis (COA) supplied with each lot of the GLC-714 IS. The COA listed both the purity (P) and amount (Amt_{FAME_X}) of each FAME used to make up the GLC-714 standard. The actual amount ($AAmt_{FAME_X}$)was calculated by Eq. 3:

$$AAmt_{FAME_X} = P \times Amt_{FAME_X} \quad (3)$$

The response factor (RF) for each peak was determined by Eq. 4:

$$RF_{FAME_X} = Area_{FAME}/AAmt_{FAME_X} \quad (4)$$

Each RF was then made relative to the GLC-714 IS by Eq. 5:

$$RRF_{FAME_X} = RF_{FAME_X}/RF_{IS} \quad (5)$$

The Empirical Correction Factor (ECF) for each FAME was then calculated by taking the inverse of the RRF:

$$ECF_{FAME_X} = 1/RRF_{FAME_X} \quad (6)$$

Data Collection

Fifteen international laboratories agreed to participate in the collaborative study in support of Official Method AOCS Ce 1i-07, but only 13 returned data. Their coded identities with respect to the study protocol and their corresponding lab number index for the purpose of this discussion are listed in Table 1. Each lab was given a spreadsheet on which to enter GLC peak areas (A_x from Eq. 1) for 85 fatty acid analytes for each of seven oil samples. The identities of the seven samples are listed in Table 1. Note that samples 3 and 5 were blind replicates. The fatty acids measured for these samples ranged in size from 8:0 to 24:0 and from 0 to 3 degrees of unsaturation; their identities are listed in Table 2. Analytes listed in bold font were included in the GLC-714 mixture of chromatography calibration standards that was supplied to the participating labs. In addition, the labs were supplied with Menhaden Oil from a commercial source (Arista Industries) along with a reference chromatogram to use for identifying each of the requested analytes. The spreadsheet also contained functions for automatically converting peak areas into individual FAME amounts, which were then converted to Fatty Acid weight-percent concentrations (FA wt.%) by Eq 7:

$$\text{FA wt.}\% = W_{\text{FAME}_X} \times F_{\text{FA}_X} / W_{\text{sample}} \quad (7)$$

where W_{sample} = total FA amount per sample; F_{FA_X} = a specific FAME to FA conversion factor for each analyte (these factors are based on molecular weight and are listed in the Official Method AOCS Ce 1i-07 document).

Statistical Analysis

The collected FA wt.% data were analyzed according to the IUPAC-1987 [1] harmonized statistical procedure, which is summarized in AOCS Procedure 4-86 [2]. The statistical procedure consists of applying analysis of variance and outlier treatments in order to estimate analytical figures of merit related to within-lab repeatability (%RSDr) and inter-lab reproducibility (%RSDR) for each of the measured FA analytes. The figures of merit for each of the measured analytes with respect to the seven samples included in the collaborative study have been published in the Ce 1i-07 official method document [3]. For the current discussion, the outliers discovered by the IUPAC-1987 procedure were examined more closely. In particular, the outliers were classified according to whether they were the result of zero or trace-level concentration of a particular analyte, from erroneous assignment or measurement of a chromatographic peak, or simply due to compounding of errors in the analytical procedure. It was also helpful to examine trends in the repeatability and reproducibility figures of merit in order to characterize potential weaknesses of the Ce 1i-07 method [3].

Table 1 Laboratory IDs and samples used in the collaborative study

Lab ID	Lab #	Sample ID	Sample #
802	1	18/12 fish oil	1
803	2	30/20 EE	2
804	3	Tuna oil	3
805	4	ArA SCO	4
806	5	Tuna oil	5
807	6	DHA SCO	6
808	7	48/25 fish oil	7
809	8		
810	9		
811	10		
813	11		
814	12		
815	13		

Results and Discussion

Outlier Trends

Trends in the appearance of outliers will be presented as a function of analyte, laboratory, and sample. Table 2 lists the tabulation of outliers for each analyte across all 13 laboratories and all 7 samples. Outliers were classified as (1) "Not Present" or zero concentration, (2) "Trace-level" or due to a majority of labs not reporting a value for the particular analyte, and (3) "Stat" or supposedly due to normal compounding of random error. Note that a given analyte had (13 labs) × (7 samples) = 91 total measured values.

Analytes that exhibited very few "Not present" or trace-level outliers and a small amount of normal statistical outliers were usually present at mean FA wt.% above 0.5%, see for example the 16:0 and 18:0 analytes. The mean FA wt.% for these two analytes was above 0.5% for every sample, and was below 1.0% for only one sample each. Thus, it is expected that most of the labs would correctly identify these and similar analyte peaks. Inspection of the other analytes that exhibited a similar trend in outliers revealed that, if the analyte was present at greater than ~0.5% in every sample, then the vast majority of outliers for that analyte were of the usual statistical type.

Several analytes had many zero-level outliers, a few trace-level outliers, and no normal outliers; see for example the 11:0, 10c-17:1, and iso 21:0 analytes (among others). In this case, either a few labs were wrongfully attributing very small features in the sample chromatograms to these analytes, or several labs were incapable of seeing small but

Table 2 Number of zero entries, trace-level outliers, and statistical outliers per analyte

Analyte	Not present	Trace outliers	Stat outliers	Analyte	Not present	Trace outliers	Stat outliers	Analyte	Not present	Trace outliers	Stat outliers
8:0	**70**	**10**	**1**	Iso 17:0	39	9	5	8c,11c,14c,17c-18:4	54	6	4
9:0	91	0	0	7c,10c-16:2 (anteiso 17:0)	52	6	8	**20:0 (arachidic)**	**8**	**0**	**21**
Iso 10:0	91	0	0	9c,12c-16:2	43	7	7	9c-20:1	40	6	5
10:0	**70**	**6**	**4**	3,7,11,15-tetra Me 16:0 (phytanic)	41	5	9	**11c-20:1 (gondoic)**	**8**	**1**	**18**
11:0	81	10	0	**17:0**	**18**	**5**	**16**	13c-20:1	32	1	12
Iso 12:0	91	0	0	6c,9c,12c-16:3	32	9	9	3c,6c,9c,12c,15c-18:5	50	1	5
10c-11:1	89	2	0	10c-17:1	65	18	0	Iso 21:0	80	11	0
Anteiso 12:0	90	1	0	7c,10c,13c-16:3	49	6	11	11c,14c-20:2	26	0	19
12:0 (lauric)	**38**	**5**	**8**	Iso 18:0	73	17	0	8c,11c,14c-20:3 (homogamma)	21	0	19
Iso 13:0	79	9	0	4c,7c,10c,13c-16:4	45	9	6	**21:0**	**51**	**13**	**2**
11c-12:1	86	5	0	Anteiso 18:0	87	4	0	**5c,8c,11c,14c-20:4 (ArA)**	**12**	**4**	**20**
Anteiso 13:0	89	2	0	6c,9c,12c,15c-16:4	43	15	1	**11c,14c,17c-20:3**	**34**	**2**	**18**
13:0	**53**	**13**	**0**	**18:0 (stearic)**	**0**	**0**	**23**	8c,11c,14c,17c-20:4	33	2	15
Iso 14:0	63	5	1	**9c-18:1 (oleic)**	**0**	**0**	**23**	**5c,8c,11c,14c,17c-20:5 (EPA)**	**13**	**4**	**20**
12c-13:1	88	3	0	11c-18:1	14	0	20	**22:0 (behenic)**	**14**	**0**	**18**
Anteiso 14:0	85	6	0	13c-18:1	51	13	2	9c-11c-22:1	34	2	12
14:0 (myristic)	**6**	**2**	**22**	Iso 19:0	80	12	0	**13c-22:1 (erucic)**	**32**	**3**	**16**
9c-14:1	42	8	7	5c,11c-18:2	42	7	5	13c,16c-22:2	64	16	8
Iso 15:0	51	7	6	8c,11c-18:2	53	13	0	6c,9c,12c,15c,18c-21:5	35	3	15
Anteiso 15:0	55	10	3	**9c,12c-18:2 (linoleic)**	**1**	**0**	**24**	13c,16c,19c-22:3	61	15	0
15:0	24	5	14	Anteiso 19:0	82	9	0	7c,10c,13c,16c-22:4	63	19	0
10c-15:1	82	9	0	11c,14c-18:2	31	1	15	4c,7c,10c,13c,16c-22:5 (n-6 DPA)	36	0	15
Iso 16:0	58	5	4	6c,9c,12c-18:3 (gamma linolenic)	18	2	11	**7c,10c,13c,16c,19c-22:5 (n-3 DPA)**	**13**	**1**	**17**
Anteiso 16:0	83	8	0	19:0	53	3	9	**24:0 (lignoceric)**	**37**	**9**	**7**
2,6,10,14 tetra Me 15:0 (pristanic)	72	15	0	10c-19:1	81	10	0	**4c,7c,10c,13c,16c,19c-22:6 (DHA)**	**12**	**1**	**16**
16:0 (palmitic)	**5**	**0**	**25**	8c,11c,14c-18:3	32	4	4	**24:1 isomers**	**28**	**4**	**8**
7c-16:1	31	8	14	**9c,12c,15c-18:3 (alpha linolenic)**	**20**	**6**	**17**				
9c-16:1	**11**	**0**	**21**	Iso 20:0	80	11	0				
11c-16:1	48	8	6	6c,9c,12c,15c-18:4	22	1	17				

Analytes included in the GLC-714 calibration standard are shown in bold font

valid peaks caused by these analytes. Inspection of the outliers as a function of laboratory suggests that these analytes' outliers were usually due to certain labs' inability to correctly identify minor peaks in a chromatogram, especially considering that the analytes listed above are not normally observed in marine oils.

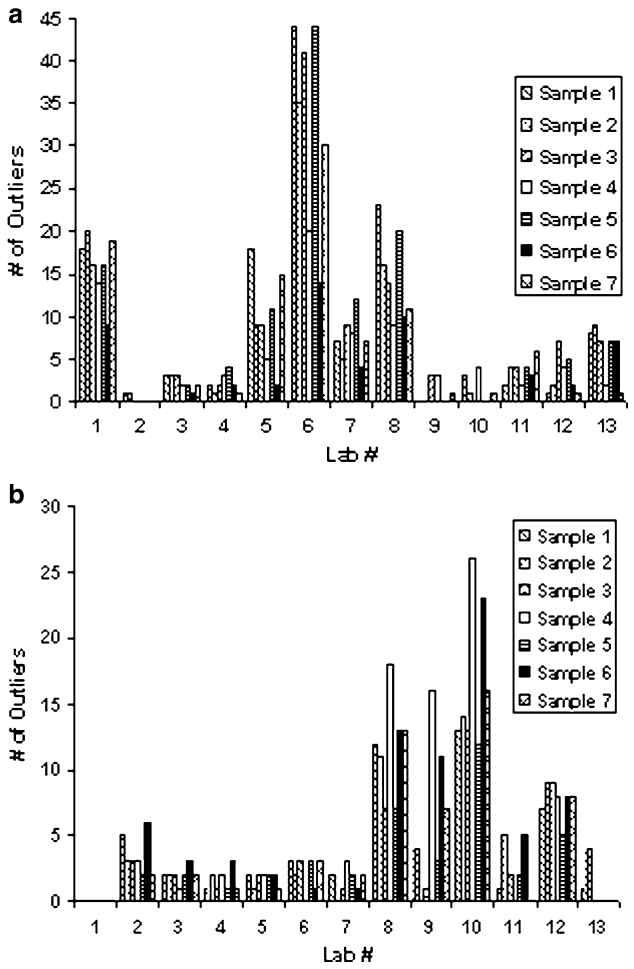

Fig. 1 **a** Number of statistical outliers per sample for every laboratory, **b** number of trace-level outliers per sample for every laboratory

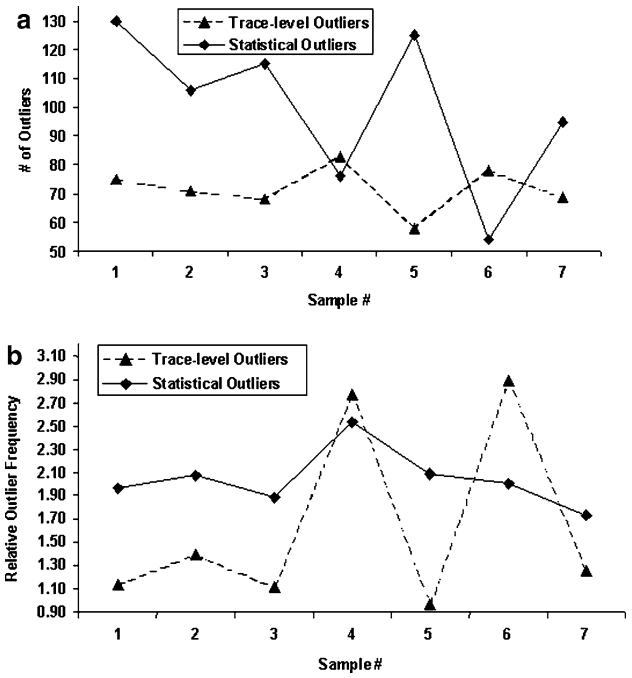

Fig. 2 **a** Number of statistical outliers and trace-level outliers per sample, summed over all laboratories, **b** statistical and trace-level outlier frequency per sample relative to the number of analytes present in each sample

The two bar graphs in Fig. 1 display the number of normal statistical outliers and trace-level outliers, respectively, as a function of lab number. Lab #1, lab #6 and lab #8 each had relatively large amounts of statistical outliers, indicating either that these labs either had poorly calibrated chromatographs or incorrectly identified certain elution peaks. Lab #9, lab #10, and lab #12 had large amounts of trace outliers but very few statistical outliers, implying that these labs were more thorough in identifying small peaks in the sample chromatograms. Many of the recorded trace-level analyte FA wt.%s that were identified as outliers by the IUPAC-1987 procedure may very well have been valid results that were counted as outliers simply because the majority of labs did not identify a small but valid analyte peak in a given sample.

The line plot in Fig. 2a displays the number of trace-level outliers and statistical outliers as a function of sample. The natural fish oil samples 1, 3, 5, and 7 have more statistical outliers than the two single-cell oil samples 4 and 6, but this is expected since the fish oils contain many more detected analytes per sample than the single-cell oils. The fish oils had on average over 60 detected analytes per sample, whereas the two SCO oils (samples 4 and 6) had only 30 and 27 detected analytes, respectively. Here a detected analyte is one for which a majority of the labs reported a mean FA wt.% value greater than zero. The single-cell oils actually contain slightly more trace-level outliers than statistical outliers, as well as containing more trace-level outliers than the fish oil samples. When the relative complexity of the samples is taken into account by dividing the number of outliers by the number of detected analytes, as is shown in Fig. 2b, it can be seen that the frequency of statistical outliers per sample is roughly the same; however, the frequency of trace-level outliers is much higher for the single-cell oil samples. Figure 1b shows that the labs reporting more trace-level outliers (labs #8, #9, and #10) tended to report the most trace-level outliers for the SCO samples; also, 95% of the trace-level outliers for samples 4 and 6 were values less than 0.2 FA wt.%. This combination of circumstances suggests that nearly all of the SCO trace-level outliers were due to several labs ignoring very small peaks their chromatograms.

Figure 3 reveals that 95% of trace-level outliers for all 7 samples were values less than 0.5 FA wt.%. Of the 22 values greater than 0.5 FA wt., 7 were reported by lab #6, which also had by far the greatest number of statistical

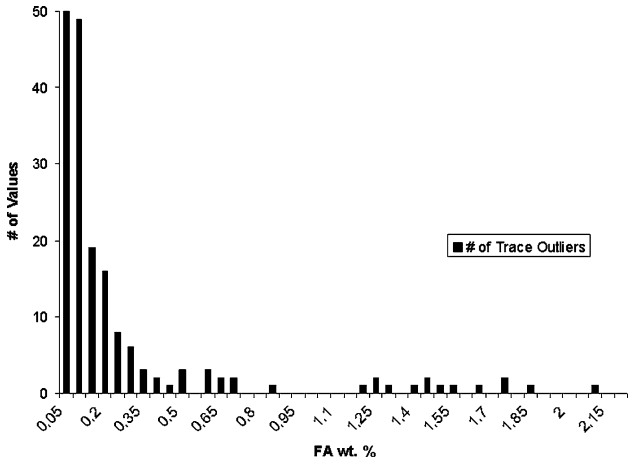

Fig. 3 Number of trace-level outliers having values within the labeled FA wt.% ranges. Note: there were 280 values in the 0-0.1 FA wt.% range, the histogram is scaled to ≤50 in order to clearly display values >0.5 FA wt %

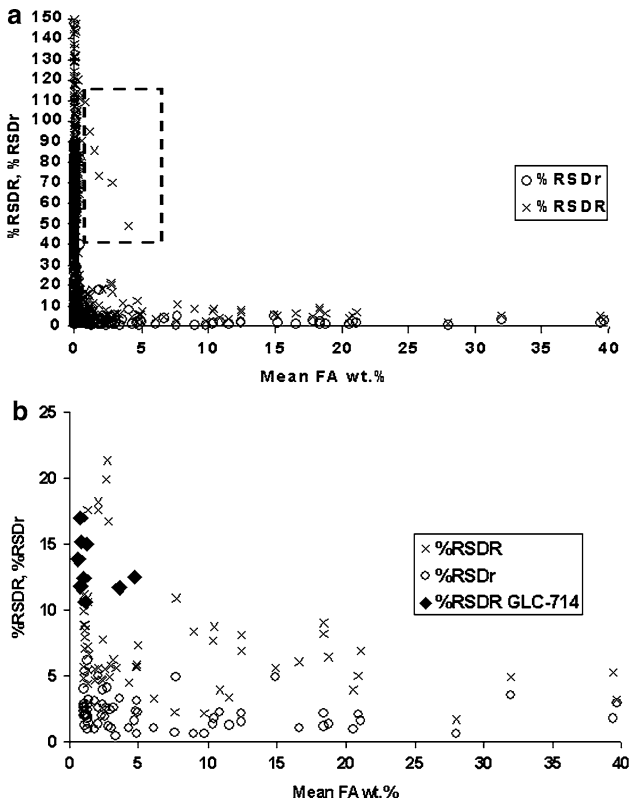

Fig. 4 **a** Repeatability (%RSDr) and reproducibility (%RSDR) values for every analyte versus its mean fatty acid weight percent concentration (Mean FA wt.%), analytes with unusual %RSDR are enclosed in the dashed box, **b** close-up of the data in part a after removing analytes in the dashed box and analytes with Mean FA wt.% below 0.5%. In addition, %RSDR values above 10% are highlighted for analytes contained in the GLC-714 calibration standard

outliers, suggesting that these are erroneously large values due to calibration errors. 13 of the values above 0.5 FA wt.% occurred within sample 2: some labs had particular trouble correctly identifying certain peaks in the 30/20 EE (ethyl ester) sample.

Table 3 Identities and statistics of anomalous analytes from Fig. 4a

Analyte	Sample	Mean	%RSDr	%RSDR
6c,9c,12c,15c 16:4	1	1.51	1.81	86.00
9c,11c, 22:1	2	0.84	3.50	109.36
9c 16:1	3	2.84	1.23	69.61
9c 16:1	5	4.06	5.41	49.41
9c 16:1	6	1.87	17.50	73.18
9c,11c, 22:1	7	1.11	6.30	94.94

With Mean FA wt.% greater than 0.5

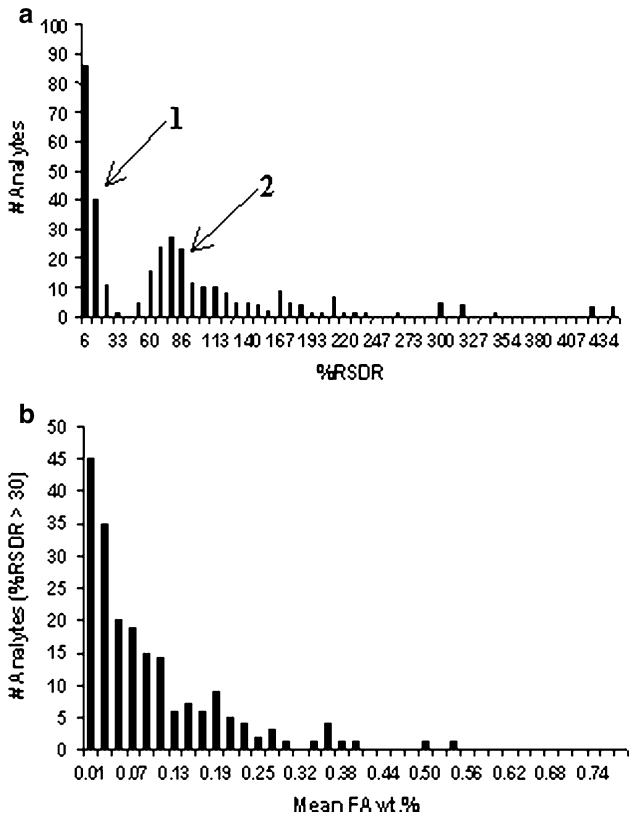

Fig. 5 **a** Number of analytes having reproducibility (%RSDR) values in the stated range bins. There were %RSDR values >300%, but these were omitted for the sake of clarity. The numbers 1 and 2 refer to two distinct sub-populations of analyte mean fatty acid weight percent concentrations (Mean FA wt.%), **b** number of analytes in sub-population 2 from part **a** having Mean FA wt.% values in the stated ranges

Repeatability and Reproducibility Trends

Trends in the repeatability and reproducibility percent standard deviations (%RSDr and %RSDR, respectively) were examined in order to obtain an indication of the overall precision and reliable concentration threshold of the

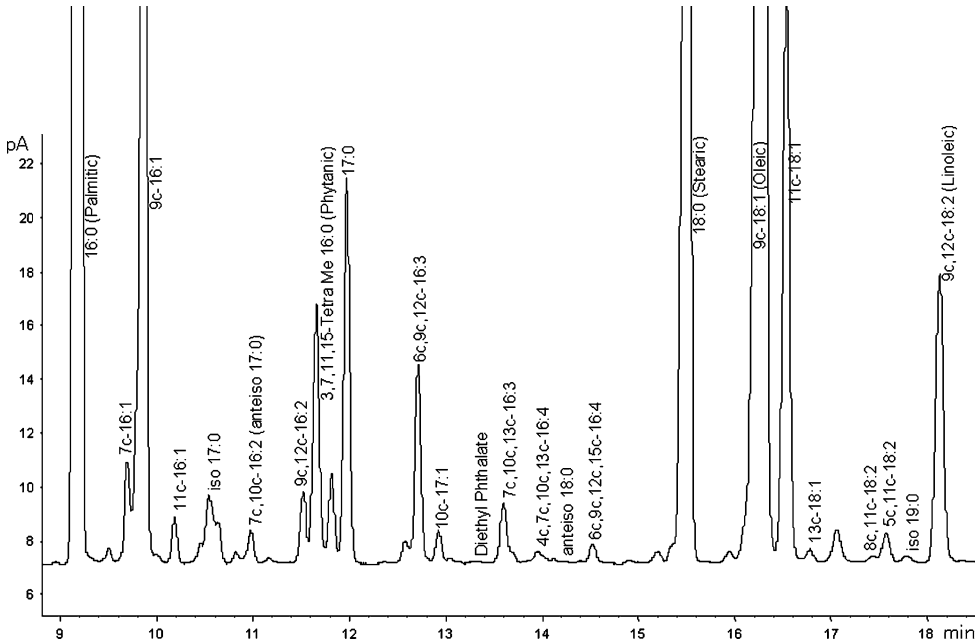

Fig. 6 Example chromatogram for sample 3 detailing the elution time region that includes analytes between 16:0 (palmitic) and 9c,12c-18:2 (linoleic)

method. Figure 4a shows a scatter plot of the %RSDr and %RSDR for every analyte FA wt.% across all 7 samples in terms of plotted versus its mean. As expected from the outlier analysis discussed above, analyte means of less than ~0.5% FA wt.% vary widely in their reproducibility, often having %RSDR greater than 100%. For analyte means above ~0.5% FA wt.% the %RSDr were consistently below 5%; however, a few analyte measurements had %RSDR significantly higher than 20%. These are contained within the dashed box in part a) of Fig. 4. The identities of these analytes are listed in Table 3. The 9c-16:1 FA analyte had a very poor %RSDR for several samples even when its mean was well above 1% FA wt.%. This particular peak significantly overlaps the 7c-16:1 analyte, and an examination of the individual lab values for these two analytes reveals that, whenever the 9c-16:1 analyte was present above 0.5 FA wt.%, at least two of the labs incorrectly identified this as the 7c-16:1 analyte (one lab even attributed it to the 16:0 (palmitic) analyte). The consistent misidentification of 9c-16:1 is puzzling, given that this analyte was included in the GLC-714 standard mixture that the labs were given in order to standardize their chromatography. With respect to the 6c,9c,12c,15c-16:4 and the 9c,11c-22:1 analytes, instead of mis-assigning the peaks, some of the labs recorded them as not present. While the latter case is more prevalent for analytes with high %RSDR values that are present at levels below 0.5 FA wt.%, all of the % RSDR values above 20% occur because at least three of the participating labs either fail to identify or incorrectly assign a given analytes elution peak.

Figure 4b displays the same information as Fig. 4a, except that analytes with mean FA wt.%s of less than 0.5% have been removed, as were the anomalous measurements contained in the shaded box in Fig. 4a. This plot more clearly shows that virtually all analytes present at greater than 0.5% FA wt.% had %RSDr of less than 5%. Also, it shows that %RSDR improves when an analyte is present at greater FA wt.%. In general, %RSDR is at most 10% for analytes present at greater than 1% FA wt.% and is often 5% or less. Figure 4 suggests that there exists a threshold of roughly 0.5% FA wt.% below which the method does not exhibit a reasonable between-lab reproducibility. To clarify this, the histogram in Fig. 5a plots the number of analytes that had a certain range of %RSDR values. The histogram exhibits a bi-modal pattern, suggesting that there is one sub-population of analytes with %RSDR below 30% and another sub-population with %RSDR significantly higher than 30%. These two sub-populations are indicated with arrows in Fig. 5a. Figure 5b replots the second sub-population in terms of number of analytes within a certain range of mean FA wt.%. Notice that none of the samples in this sub-population had a mean FA wt.% greater than 0.56%. This is further evidence that a given laboratory should be careful when applying this method to reliably determine an analyte FA wt.% of less than 0.5%.

Figure 4b also shows that, after discounting the troublesome 9c-16:1 analyte, there were still 19 analytes with FA wt.% between 0.5% and 5% and having %RSDR > 10%. Moreover, 9 of these 19 analytes were compounds present in the GLC-714 calibration standard, as indicated by the diamond data point marker in Fig. 4b. All of these 19 analytes had carbon chain lengths between 16 and 22. The elution time region for these compounds was especially congested for all 7 samples. As an example, Fig. 6 shows a

Table 4 %RSD of the individual FA wt.% mean amounts of each analyte for the blind duplicate samples, and overall compound means for the blind duplicate samples

Analyte	$\%RSD_{1,2}$	Mean	Analyte	$\%RSD_{1,2}$	Mean	Analyte	$\%RSD_{1,2}$	Mean
8:0	**0.00**	**0.00**	7c,10c-16:2 (anteiso 17:0)	12.30	0.06	9c-20:1	3.64	0.20
9:0	0.00	0.00	9c,12c-16:2	55.24	0.26	**11c-20:1 (gondoic)**	**0.12**	**1.04**
Iso 10:0	0.00	0.00	3,7,11,15-tetra Me 16:0 (phytanic)	6.43	0.52	13c-20:1	3.85	0.07
10:0	**0.00**	**0.00**	**17:0**	**2.67**	**1.14**	3c,6c,9c,12c,15c-18:5	14.43	0.03
11:0	0.00	0.00	6c,9c,12c-16:3	21.88	0.31	Iso 21:0	0.00	0.00
Iso 12:0	0.00	0.00	10c-17:1	35.69	0.02	11c,14c-20:2	4.30	0.21
10c-11:1	0.00	0.00	7c,10c,13c-16:3	25.51	0.10	8c,11c,14c-20:3 (homogamma)	0.60	0.07
Anteiso 12:0	0.00	0.00	Iso 18:0	0.00	0.00	**21:0**	**2.32**	**0.09**
12:0 (lauric)	**19.33**	**0.03**	4c,7c,10c,13c-16:4	31.31	0.06	**5c,8c,11c,14c-20:4 (ArA)**	**2.57**	**1.29**
Iso 13:0	0.00	0.00	Anteiso 18:0	0.00	0.00	**11c,14c,17c-20:3**	**3.93**	**0.12**
11c-12:1	0.00	0.00	6c,9c,12c,15c-16:4	4.85	0.04	8c,11c,14c,17c-20:4	1.61	0.30
Anteiso 13:0	0.00	0.00	**18:0 (stearic)**	**1.48**	**4.95**	**5c,8c,11c,14c,17c-20:5 (EPA)**	**1.37**	**4.77**
13:0	8.91	0.04	**9c-18:1 (oleic)**	**0.23**	**12.43**	**22:0 (behenic)**	**1.23**	**0.25**
Iso 14:0	26.54	0.02	11c-18:1	0.24	2.08	9c-11c-22:1	10.97	0.21
12c-13:1	0.00	0.00	13c-18:1	17.70	0.04	**13c-22:1 (erucic)**	**36.14**	**0.08**
Anteiso 14:0	0.00	0.00	Iso 19:0	0.00	0.00	13c,16c-22:2	141.42	0.00
14:0 (myristic)	**0.98**	**2.95**	5c,11c-18:2	0.62	0.09	6c,9c,12c,15c,18c-21:5	2.32	0.11
9c-14:1	2.07	0.06	8c,11c-18:2	44.25	0.01	13c,16c,19c-22:3	0.60	0.01
Iso 15:0	13.80	0.10	**9c,12c-18:2 (linoleic)**	**0.81**	**1.04**	7c,10c,13c,16c-22:4	30.23	0.03
Anteiso 15:0	24.37	0.03	Anteiso 19:0	0.00	0.00	4c,7c,10c,13c,16c-22:5 (n-6 DPA)	0.11	1.31
15:0	1.55	0.92	11c,14c-18:2	0.66	0.19	**7c,10c,13c,16c,19c-22:5 (n-3 DPA)**	**0.00**	**0.90**
10c-15:1	0.00	0.00	6c,9c,12c-18:3 (gamma linolenic)	6.18	0.20	**24:0 (lignoceric)**	**0.42**	**0.17**
Iso 16:0	18.42	0.07	19:0	2.69	0.14	**4c,7c,10c,13c,16c,19c-22:6 (DHA)**	**1.00**	**20.73**
Anteiso 16:0	0.00	0.00	10c-19:1	0.00	0.00	**24:1 isomers**	**1.68**	**0.37**
2,6,10,14 tetra Me 15:0 (pristanic)	0.00	0.00	8c,11c,14c-18:3	5.83	0.19			
16:0 (palmitic)	**0.11**	**18.39**	**9c,12c,15c-18:3 (alpha linolenic)**	**3.72**	**0.39**			
7c-16:1	15.24	0.20	Iso 20:0	0.00	0.00			
9c-16:1	**24.97**	**3.45**	6c,9c,12c,15c-18:4	1.88	0.65			
11c-16:1	7.28	0.10	8c,11c,14c,17c-18:4	0.00	0.00			
Iso 17:0	7.95	0.26	**20:0 (arachidic)**	**1.80**	**0.32**			

Analytes included in the GLC-714 calibration standard are shown in bold font

chromatogram of the elution time region between the 16:0 (palmitic) and 9c,12c-18:2 (linoleic) analytes for sample 3, which contains three of the aforementioned 14 analytes: 17:0, 3,7,11,15-Tetra Me 16:0 (phytanic), and 11c-18:1. None of these three peaks are baseline-resolved; indeed, by looking at other chromatograms in the region between chain lengths of 16–22 carbons, it can be seen that all 14 of the spurious analytes are partially obscured other peaks. Judging from inspection of the labs' data, it is unlikely that any of these analytes has been mis-identified, since the labs' values are all in rough agreement with each other, and since half of these high %RSDR compounds were included in the GLC-714 mixture. Instead, it seems that differences in the labs' chromatographic efficiency and in the way labs determine baselines and/or resolve peak shapes causes an increased %RSDR statistic for analytes that elute in this congested chromatographic region at concentrations below ~5% FA wt.%.

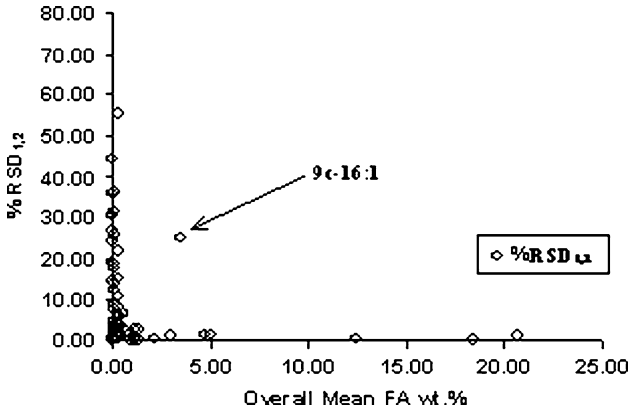

Fig. 7 Percent relative standard deviations (%RSD) for the analyte mean fatty acid weight percent concentrations (Mean FA wt.%) in the blind duplicate samples 3 and 5 versus the overall analyte Mean FA wt.%. The arrow refers to the anomalous %RSD value for the 9c-16:1 analyte

An additional between-lab consistency check involves comparing the results of the two blind duplicates, samples 3 and 5. Table 4 displays the overall FA wt.% means for each analyte in these samples, and also shows the percent relative standard deviations (%RSD) calculation as a measure of the deviation of the individual sample values from the two-sample mean. The mean and %RSD values here were calculated from the combined ($n = 26$) replicates runs from each lab for the two samples after outliers had been removed on a per-analyte basis. For example, DHA had $n = 0$ outliers for both samples, so the statistics were calculated from the full ($n = 26$) set of runs; however, ARA had $n = 3$ outliers from each sample, so the statistics in Table 4 for that analyte were calculated from the valid ($n = 20$) subset of runs. As expected from the analyses above, %RSD for the blind duplicates is much better (less than $\sim$3%) for analytes with mean FA wt.% above the $\sim$0.5% threshold; conversely, %RSD varies widely for those analytes below the threshold, with many values being significantly greater than 10%. Figure 7 shows a scatter plot of the individual %RSD values versus their overall mean FA wt.%. Here the same tendency can be seen for %RSD as was observed for %RSDR in Fig. 4. Also, the anomalous 9c-16:1 analyte appears as the only analyte present above 0.5% FA wt.% that has a %RSD greater than $\sim$3%.

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