EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Food Safety and Quality Unit
B-2440 GEEL (Belgium)

Validation of a Method for the Detection of Cocoa Butter Equivalents in Cocoa Butter and Plain Chocolate

Report on the Validation Study

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2003 EUR 20685 EN

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Luxembourg: Office for Official Publications of the European Communities, 2003
ISBN 92-894-5510-1
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Abstract

A European collaborative study has been conducted to validate an analytical procedure for the detection of cocoa butter equivalents in cocoa butter and plain chocolate. In principle the fat is separated by high-resolution gas chromatography into triglyceride fractions according to their acyl-C-numbers, and within a given number, also according to unsaturation. The presence of CBE is detected by linear regression analysis applied to individual triglyceride fractions of the fat analysed. 15 laboratories participated in the validation study. The results of the ring test clearly demonstrated that the applied method performs well with a detection limit of at least 2 % cocoa butter equivalent admixture to cocoa butter corresponding to 0.4 % in chocolate (assumed fat content of chocolate 20 %).

Keywords: Cocoa butter, Cocoa butter equivalents, HR-GC, triglyceride profile, regression analysis, validation

CONTENTS

ABSTRACT 2
CONTENTS 3
LIST OF ABBREVIATIONS 4
1 RATIONALE - BACKGROUND 5
2 OBJECTIVE 6
3 METHOD DESCRIPTION 6
4 PARTICIPANTS 8
4.1 Co-ordination of the validation study 8
4.2 Preparation and distributing of the test samples 8
4.3 Homogeneity tests 8
4.4 Measurements 8
4.5 Collation and statistical evaluation of results 9
5 TEST SAMPLES 9
5.1 Homogeneity study 10
6 THE VALIDATION STUDY 11
6.1 Design of the validation study 11
6.2 Analysis of test samples 12
6.3 Technical evaluation of the results submitted 12
6.4 Methods employed in the validation study 15
6.5 Statistical evaluation of the results submitted 16
7 RESULTS 17
8 CONCLUSIONS 18
9 LITERATURE 18
ANNEX A 19
ANNEX B 22
ANNEX C 31

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
C	correct classification
CB	cocoa butter
CBE	cocoa butter equivalent
CRM	certified reference material
FN	false negative classification
FP	false positive classification
HR-GC	high-resolution gas chromatography
H_2	Hydrogen
He	Helium
IRMM	Institute for Reference Materials and Measurements
JRC	Joint Research Centre
N_2	Nitrogen
OCI	on-column injection
PMF	palm mid fraction
POO	1,2-dioleoyl-3-palmitoyl-rac-glycerol
POP	1,3-dipalmitoyl-2-oleoylglycerol
POS	1-palmitoyl-2-oleoyl-3-stearoylglycerol
PTV	programmed temperature vaporizer
r	repeatability
R	reproducibility
Rs	resolution
RSD_r	repeatability relative standard deviation
RSD_R	reproducibility relative standard deviation
SOO	1,2-dioleoyl-3-stearoyl-rac-glycerol
SOP	standard operation procedure
SOS	1,3-distearoyl-2-oleoylglycerol
s_r	repeatability standard deviation
s_R	reproducibility standard deviation
TG	triglyceride

1 RATIONALE - BACKGROUND

According to the Directive 2000/36/EC of the European Parliament and the Council [1] vegetable fats other than cocoa butter (CB) may be added to chocolate products up to a level of 5 % of the finished product, without reducing the minimum content of CB or total dry cocoa solids. If such fats are added, consumers have to be guaranteed correct, neutral and objective information by indicating in a conspicuous and clearly legible way that vegetable fats other than CB are present in the product. Member States' laws, regulations and administrative provisions need to comply with the new Chocolate Directive before August 2003.

Only vegetable fats fulfilling the technical and scientific criteria as specified in Annex II of the Directive, so called cocoa butter equivalents (CBE), may be used besides CB for the manufacture of chocolate products. In conformity with the above criteria, the following vegetable fats, obtained from the plants listed below, may be used singly or in blends:

- Illipé, Borneo tallow or Tengkawang (*Shorea spp.*)
- Palm oil (*Elaeis guineensis, Elaeis olifera*)
- Sal (*Shorea robusta*)
- Shea (*Butyrospermum parkii*)
- Kokum gurgi (*Garcinia indica*)
- Mango kernel (*Mangifera indica*)

CBEs resemble the chemical composition and physical properties of CB very closely, making them therefore extremely difficult to quantify and even in some cases to detect (especially at very low levels).
As the statement on the package label indicating that vegetable fats other than CB have not been added to chocolate products is not precluded in the new Chocolate Directive, very sensitive methods for detection are needed to assess compliance with labelling. A specific and reliable analytical method for the detection and quantification of these CBEs is not prescribed, however, the Statement of the Council's Reasons refers to the final report of the European Commission's Joint Research Centre (JRC) devising analytical methods for the determination of cocoa butter and other vegetable fats in chocolate [2].

Separation of triglycerides (TGs) by high-resolution gas chromatography (HR-GC) in combination with statistical evaluation of the results proved to be the most accurate

technique for the detection and quantification of CBEs in genuine CB. Furthermore, the necessity for a certified cocoa butter reference material (CB-CRM) in order to facilitate the work of the analytical chemists was recognised. The latter has been prepared and will be available to the public by the JRC's Institute for Reference Materials and Measurements (IRMM).

The CB-CRM (IRMM 801) was used as a calibrant for TG profiling by HR-GC to create a standardised database containing data from more than 74 different CBs and 94 CBEs. This data base was established by the JRC. An algorithm, based on a modified method proposed by Padley and Timms [3], for the interpretation of TG data obtained by HR-GC was also elaborated by the JRC to be used for the detection of CBEs in cocoa butter and plain chocolate [4].

2 OBJECTIVE

- To conduct an international collaborative study in order to validate a gas chromatography-based method combined with an algorithm to detect CBEs in genuine CB and plain chocolate.
- To use a matrix material of genuine CB with a certified TG profile as an aid to ensure high comparability of the results.

3 METHOD DESCRIPTION

Cocoa butter, or the fat obtained by solvent extraction from plain chocolate, has to be separated by HR-GC into TG fractions according to their molecular weight and degree of unsaturation. For the interpretation of TG data obtained by HR-GC an algorithm as originally proposed by Padley and Timms [3], who used TGs of the same carbon number instead of individual TGs, is used [4]. The content of SOS is linearly related to POP when the content of the three major TGs is normalised so that %-POP + %-POS + %-SOS equals 100 % (Figure 1).

The detection is based on the fact that CBs are generally found on a line ("CB-line"), whereas mixtures with CBEs deviate from that line. As a result for genuine CB the relationship is therefore expressed as:

Equation 1: **POP = 44.025 – 0.733 x SOS**

This equation was established by the JRC by using a standardised data base of the TG profile of 74 individual genuine CBs evaluated using the CB-CRM as a calibrant.

- For 99 % of all analyses, pure CB complies with:

Equation 2: **POP < 44.025 – 0.733 x SOS**

- A greater value of POP, as given by Equation 2, means that the sample is not pure CB.

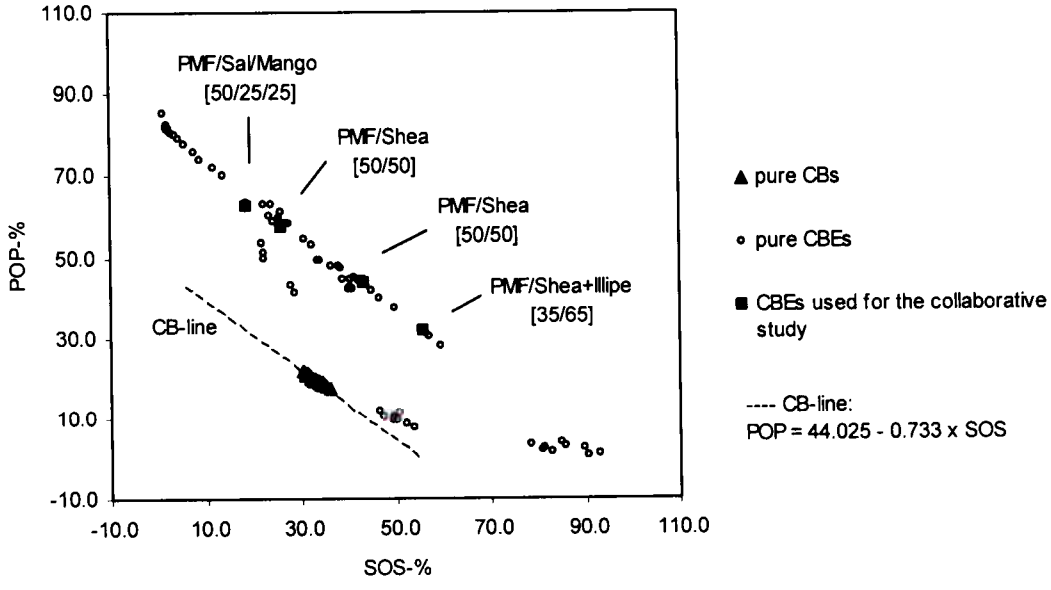

Figure 1: Relationship between the normalised content of POP and SOS of CB and CBE samples

Individual testing laboratories do not need to establish a "CB line". Comparability of results between various laboratories is maintained through calibration of the measurements against the commercially available CB-CRM (IRMM 801).

4 PARTICIPANTS

4.1 Co-ordination of the validation study

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (B)

4.2 Preparation and distributing of the test samples

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (B)

4.3 Homogeneity tests

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (B)

4.4 Measurements

ADM Noble & Thörl GmbH, Hamburg (D)
Barry Callebaut B.V., Bussum (B)
Britannia Food Ingredients Ltd., Goole (UK)
Department of Dairy Research and Bacteriology, University of Agricultural Sciences, Vienna (A)
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Laboratoire Chimie Analytique et Science de l'Aliment, Illkirch-Graffenstaden (F)
Lebensmittelchemisches Institut des Bundesverbandes der Deutschen Süßwarenindustrie, Köln (D)
WEJ GmbH, Hamburg (DE)

4.5 Collation and statistical evaluation of results

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel (B)

5 TEST SAMPLES

Samples of CBs and CBEs (Table 1) were obtained from the cocoa processing industry and other vegetable fat producers and chocolate samples (Table 3) in retail stores. The CBEs used for preparing the CB/CBE blends are indicated in Table 1 and Figure 1. The selected CBEs included in this study consisted of a variety of industrially used fats, including Illipé-containing fats in combination with other CBEs.

Eleven fat samples, representing three pure CBs, four samples of 2 % and four samples of 4 % CBE admixture to CB, dissolved in *iso*-octane, were sent to the participating laboratories (Table 2). The CBE admixtures correspond to 0.4 % and 0.8 % in chocolate (assumed fat content of chocolate 20 %). Furthermore, two chocolate samples, one with the addition of CBE and one without had to be measured (Table 3). The samples were labelled randomly (Table A 1, Annex A). Additionally, one ampoule of the CB-CRM (IRMM 801) was provided for calibration purposes and system suitability check.

Table 1: Genuine CBs and CBEs used in the study and for the preparation of CB/CBE mixtures

CB No.	*Country Origin*	*CBE No.*	*Type [composition; %]*
I	Grenada	I	PMF/Sal/Mango [50/25/25]
II	Ghana	II	PMF/Shea [50/50]
III	Ivory Coast/Nigeria/Cameroon'	III	PMF/(Shea + Illipe) [35/65]
		IV	PMF/Shea/Illipe [50/25/25]

Table 2: Samples used for the study

Sample No.	*CB used*	*CBE used*	*CB [%]*	*CBE [%]*
1	CB I	-	100.00	0.00
2	CB II	-	100.00	0.00
3	CB III	-	100.00	0.00
4	CB I	CBE III	97.94	2.06
5	CB I	CBE III	95.95	4.05
6	CB II	CBE II	98.02	1.98
7	CB II	CBE II	96.04	3.96
8	CB III	CBE I	97.96	2.04
9	CB III	CBE I	95.96	4.04
10	CB III	CBE IV	97.91	2.09
11	CB III	CBE IV	96.00	4.00

Table 3: Chocolates used for the study

Sample No.	*Brandname and type*	*Sample description*
12	Cadbury's Bournville	The original plain chocolate [ingredients: vegetable fat]
13	Leysieffer	Chocolat Noir Intense 99 % [no vegetable fats added]

5.1 Homogeneity study

The diluted fat samples (Table 2) were considered to be homogeneous. Homogeneity of the chocolate samples (Table 3) was mandatory in order to make data from various laboratories for matrix materials comparable too. Therefore, homogeneity of the two chocolate samples was investigated by determining the three major TGs (POP, POS and SOS) by HR-GC (CP-TAP, 25 m x 0.25 mm x 0.1 µm). From each sample five unit sub-samples were taken randomly. The fat from each unit sub-sample was extracted with ether according to the AOAC Official Method 920.75 [5]. Two independent sub-samples from each unit were analysed in randomised order by HR-GC. The within- and between-units standard deviation for the content of POP, POS and SOS was calculated by using one-way analysis of variance (ANOVA). The between-units standard deviation was used as an estimate of the inhomogeneity between-units and the within-units standard deviation as an estimate of the combined effects of the repeatability of the method and the possible within-unit inhomogeneity. In order to separate these two effects, five sub-samples were taken from one unit, and each analysed once by HR-GC. The ratios of the variances of the between- and within-unit series were compared by means of a Snedecor F-test to determine whether the between-unit variances differed significantly from zero.

Table 4: Between- and within-units standard deviation of three main TGs in randomly chosen chocolate samples (duplicate determinations carried out on 5 units)

Sample 12	*POP*	*POS*	*SOS*
Average	25.63	43.95	30.42
SD between-units	(2)	(2)	0.041
SD within-units	0.083	0.124	0.065
F-Ratio	0.49	0.85	1.76
P	0.74	0.55	0.27
SD for repeated measurements (1)	0.075	0.071	0.036

(1) five sub-samples taken from one unit analysed (single determination)
(2) mean squares between < mean squares within

Table 5: Between- and within-units standard deviation of three main TGs in randomly chosen chocolate samples (duplicate determinations carried out on 5 units)

Sample 13	*POP*	*POS*	*SOS*
Average	19.77	47.71	32.53
SD between-units	0.052	0.063	0.077
SD within-units	0.065	0.066	0.079
F-Ratio	2.26	2.83	2.87
P	0.19	0.14	0.13
SD for repeated measurements (1)	0.029	0.099	0.097

(1) five sub-samples taken from one unit analysed (single determination)

All tests confirmed that the between-units inhomogeneity was insignificant (P>0.05). In addition, the figures for standard deviations determined on sub-samples of a single unit were of the same magnitude as those determined by ANOVA (Tables 4 to 5). Therefore, the homogeneity of the chocolate samples was considered sufficient to be used as test material for the validation study.

6 THE VALIDATION STUDY

6.1 Design of the validation study

15 laboratories with experience in TG analysis were asked to participate in the study. Each participating laboratory received a code for identification. For the validation study of the method the participants were provided with a provisional standard operation procedure (SOP; Annex B), instruction guidelines and the samples. The participants were requested to follow the SOP exactly. However, the SOP gave some freedom to choose certain equipment, parameters etc. within certain limits. Hence, in order to demonstrate that the

HR-GC method applied was fit-for-purpose participants had to meet predefined performance criteria:

- Separation of critical pairs of components POS/POO and SOS/SOO with a chromatographic resolution of at least 1.0.
- Detector response factors determined for the five main TGs (POP, POS, POO, SOS, SOO) should vary within a range of 0.80 – 1.20.

Both criteria had to be demonstrated by analysing the provided CB-CRM (IRMM 801).

6.2 Analysis of test samples

Before analysing the test samples the participants had to check the system suitability. The test samples had to be analysed in random order. Response factors for the five main TGs (POP, POO, POS, SOS, SOO) had to be determined before analysing the first test sample and after every 10th analyses by using the CB-CRM. A flow scheme of the sample handling is given in Annex A in Figure A 1. The sample number, retention time and the area counts of individual TG fractions had to be reported in an electronic spreadsheet provided by the co-ordinator. By using the electronic spreadsheet the raw data were automatically applied to the proposed algorithm (Equation 1 and 2).

6.3 Technical evaluation of the results submitted

The results of the individual laboratories were examined along with the submitted raw data and chromatograms. Two laboratories (14 and 15) out of the 15 participants were rejected in advance from the validation study since they did not follow the SOP and consequently not fulfilled the performance criteria as laid down in the SOP.

Laboratory 14 applied a capillary column of 10 m length with an apolar stationary phase (DB1; J&W Scientific) instead of a fused silica column of 25 to 30 m length, coated with medium-polarity stationary phase. Thus, the samples were only separated according to their molecular weight and not as requested in the SOP according to their molecular weight and degree of unsaturation. Laboratory 15 employed a fused silica column of the type CP-SIL 24 CB/MS. In addition N_2 instead of He or H_2 was used as carrier gas. Though the resolution of the critical pairs was almost fulfilled (Table 6) the figures of the determined

response factors (Table 7) of the five main TGs clearly demonstrated that the chromatographic system was not working properly. The response factors spread from 0.66 to 11.47, having a relative standard deviation (RSD) of repeatability up to 33.5 %. In comparison the response factors of laboratories 1 to 13 varied within a range of 0.82 to 1.35. The RSD of repeatability of the response factors for the individual TGs were less than 5 % for most of the laboratories.

Laboratory 4 was not able to achieve a resolution of 1.0 for the critical pair POS and POO. However, inspection of the chromatograms submitted revealed that the separation of the critical pair was still sufficient in order to be used for the study.

Table 6: Separation power (expressed as Rs) of the chromatographic systems used by the participants

Lab code	*Resolution (POS/POO)*	*Resolution (SOS/SOO)*
1	0.99	1.21
2	1.11	1.59
3	1.61	1.94
4	0.87	1.38
5	1.26	1.66
6	1.34	1.59
7	1.36	1.85
8	1.48	1.74
9	1.09	1.13
10	0.99	0.98
11	1.01	1.31
12	1.05	1.22
13	1.00	1.30
14	n.a	n.a
15	0.97	1.07

n.a. = not applicable

Although the determined response factors for SOO of laboratory 5 and 13 were greater than the suggested limit of 1.20 in the SOP the data were not rejected since the final results were in close agreement with the results of the remaining laboratories.

In conclusion, based on the technical evaluation of the results 13 data sets out of the 15 submitted were accepted for the validation; laboratory 14 did not comply with the general instructions of the SOP and laboratory 15 failed to determine proper and repeatable response factors demonstrating a sound functioning chromatographic system.

Table 7: Determined response factors for the five main triglycerides by using the CB-CRM (n = 7 replicates)

	mean	*min*	*max*	*SD*	*RSD*	*mean*	*min*	*max*	*SD*	*RSD*
Lab code			*POP*					*POS*		
1	0.88	0.87	0.88	0.004	0.42	0.97	0.97	0.97	0.001	0.13
2	0.90	0.90	0.90	0.001	0.12	0.97	0.97	0.97	0.001	0.14
3	0.87	0.87	0.88	0.003	0.35	0.97	0.97	0.97	0.001	0.14
4	0.86	0.85	0.87	0.005	0.58	0.97	0.97	0.97	0.002	0.21
5	0.84	0.82	0.85	0.007	0.80	0.96	0.95	0.97	0.004	0.39
6	0.93	0.92	0.94	0.008	0.85	0.98	0.97	0.99	0.005	0.53
7	0.84	0.82	0.85	0.009	1.13	0.97	0.97	0.98	0.004	0.44
8	0.86	0.85	0.87	0.009	1.04	0.96	0.96	0.96	0.001	0.13
9	0.85	0.85	0.85	0.003	0.30	0.96	0.96	0.96	0.001	0.07
10	0.92	0.91	0.92	0.007	0.80	0.97	0.96	0.97	0.006	0.60
11	0.95	0.95	0.95	0.004	0.37	0.98	0.98	0.98	0.001	0.10
12	0.96	0.95	0.96	0.004	0.41	0.99	0.99	0.99	0.002	0.22
13	0.84	0.84	0.85	0.005	0.65	0.96	0.96	0.97	0.003	0.34
14	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	0.69	0.66	0.71	0.018	2.64	0.93	0.92	0.94	0.004	0.45
Lab code			POO					SOS		
1	1.05	1.04	1.06	0.008	0.78	1.12	1.11	1.13	0.005	0.48
2	0.94	0.90	0.97	0.023	2.46	1.12	1.12	1.13	0.004	0.40
3	1.03	1.02	1.05	0.011	1.07	1.13	1.12	1.13	0.004	0.37
4	0.92	0.87	0.95	0.030	3.22	1.15	1.13	1.15	0.006	0.57
5	1.12	1.08	1.18	0.039	3.45	1.17	1.16	1.18	0.006	0.55
6	0.95	0.89	1.02	0.052	5.48	1.07	1.06	1.09	0.009	0.81
7	0.88	0.83	0.97	0.052	5.83	1.18	1.15	1.20	0.015	1.27
8	1.07	1.06	1.10	0.013	1.21	1.14	1.13	1.16	0.011	0.94
9	0.95	0.93	0.97	0.014	1.45	1.16	1.16	1.17	0.004	0.30
10	1.15	1.03	1.29	0.104	9.06	1.09	1.08	1.10	0.007	0.60
11	1.06	0.95	1.12	0.065	6.13	1.05	1.04	1.06	0.006	0.55
12	0.95	0.92	0.97	0.020	2.08	1.04	1.04	1.05	0.004	0.38
13	1.04	1.00	1.06	0.021	2.01	1.16	1.15	1.17	0.007	0.63
14	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15	7.44	4.31	11.47	2.496	33.55	1.29	1.24	1.35	0.043	3.35
Lab code			SOO							
1	1.18	1.17	1.20	0.013	1.06					
2	1.04	1.01	1.07	0.023	2.19					
3	1.20	1.19	1.23	0.016	1.32					
4	1.18	1.16	1.22	0.026	2.22					
5	1.30	1.26	1.35	0.043	3.31					
6	1.09	0.97	1.17	0.071	6.51					
7	1.07	1.01	1.15	0.055	5.17					
8	1.22	1.21	1.23	0.012	0.97					
9	1.22	1.21	1.23	0.012	0.96					
10	1.13	1.11	1.20	0.032	2.79					
11	1.01	0.93	1.06	0.044	4.35					
12	1.05	1.02	1.07	0.017	1.63					
13	1.28	1.26	1.32	0.020	1.60					
14	n.a.	n.a.	n.a.	n.a.	n.a.					
15	4.68	2.24	6.21	1.410	30.13					

n.a. = not applicable

6.4 Methods employed in the validation study

13 laboratories performed the analysis according to the preliminary SOP distributed. A brief outline of the HR-GC methods used by the participants accepted on technical grounds is given in Table 8.

In order to obtain the fat from the chocolate samples the AOAC Official Method 920.75 [5] was recommended by the SOP. However, participants were allowed to use alternative extraction procedures provided that the same results were obtained. Five laboratories applied the AOAC method, four laboratories extracted the fat by the Soxhlet extraction procedure and the rest of the laboratories obtained the fat by using solvents such as hexane or a mixture of chloroform/hexane.

Table 8: HR-GC conditions employed for the validation study

	Participant					
	1	2	3	4	5	6
Column characteristics						
phase	CB-TAP ultimetal	CB-TAP ultimetal	CB-TAP	RTx-65TG	RTx-65TG	CB-TAP
length [m]	25	25	25	30	30	25
i.d. [mm]	0.25	0.25	0.25	0.25	0.25	0.25
film thickness [µm]	0.1	0.1	0.1	0.1	0.1	0.1
Temperature mode						
- oven						
injection temperature [°C] / hold time [min]	100 / 0.1	100 / 0.1	340 / 0	340 / 1	100 / 0.5	200 / 2
programme rate 1 [°C/min]	50	35	1	1	50	12
temperature [°C] / hold time [min]	330 / 0	-			330 / 1	272 / 0
programme rate 2 [°C/min]	1	-			5	4
temperature [°C] / hold time [min]	-	-	-	-	-	-
programme rate 3 [°C/min]	-	-	-	-	-	-
final temperature [°C] / hold time [min]	350 / 10	350 / 15	360 / 10	360 / 3	350 / 30	340 / 15
- injector temperature [°C]	oven track	103	360	399	80-355	oven track
- detector temperature [°C]	360	360	360	370	360	360
Injection mode	OCI automatic	OCI	split manual	split manual	OCI "SPI" automatic	OCI manual
Carrier gas						
type	He	H_2	He	H_2	He	H_2
constant pressure [kPa]	-		250	120	190	120
constant flow [ml/min]	2	1.5	-		-	-
Sample						
concentration [mg/ml]	0.5	2.5	4.5	50	0.5	0.25
volume injected [µl]	0.5	0.5	1	0.18	0.5	1

	Participant						
	7	8	9	10	11	12	13
Column characteristics							
phase	CB-TAP	RTx-65TG	DB-17HT	RTx-65TG	RTx-65TG	CB-TAP	CB-TAP
length [m]	25	30	30	30	25	25	25
i.d. [mm]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
film thickness [µm]	0.1	0.1	0.15	0.1	0.1	0.1	0.1
Temperature mode							
- oven							
injection temperature [°C] / hold time [min]	100 / 2	200 / 1	80 / 2	60 / 0	100 / 0.5	100 / 1	200 / 2
programme rate 1 [°C/min]	30	15	50	60	50	30	20
temperature [°C] / hold time [min]	280 / 0	360 / 2	300 / 0	300 / 0	300 / 2		320 / 0
programme rate 2 [°C/min]	5	1	30	10	30		1
temperature [°C] / hold time [min]	-	-			-	-	-
programme rate 3 [°C/min]	-	-			-	-	-
final temperature [°C] / hold time [min]	340 / 23	369 / 0	350 / 30	360 / 15	340 / 13.5	340 / 35	360 / 10
- injector temperature [°C]	60	390	oven track	63	oven track	100	65 - 370
- detector temperature [°C]	350	390	360	365	360	360	370
Injection mode	PTV automatic	split automatic	OCI automatic	OCI automatic	OCI automatic	OCI manual	OCI - "spy" manual
Carrier gas							
type	H_2	H_2	H_2	H_2	H_2	H_2	He
constant pressure [kPa]	70	180				150	90
constant flow [ml/min]	-	-	0.8	1.4	3.5	-	-
Sample							
concentration [mg/ml]	0.1	12.5	0.5	1	0.2	0.5	1 - 2
volume injected [µl]	3	0.5	0.5	0.2	1	0.4	0.1

6.5 Statistical evaluation of the results submitted

The individual results accepted on technical basis as submitted by the participants are listed in Annex C (Tables C 1 to C 13). For each data set, identified by a laboratory code, the three major peaks POP, POS and SOS, normalised to 100 %, are given. Furthermore, the resulting POP values of Equation 1 and the decision if the sample consists of pure CB or a mixture of CB/CBE, made by comparing the calculated POP values with the measured POP values (Equation 2), are listed in Tables C1 to C13 (Annex C).

After checking the data for plausibility laboratory means of POP, POS and SOS were calculated and plotted in increasing order with the corresponding range (Figures C 1 to C 13, Annex C).

The data sets accepted on technical grounds were subjected to statistical tests as described in ISO 5725:

- Cochran test to identify outlying variances
- Single Grubbs and double Grubbs test to detect outlying data set averages.

In Annex C, Table C 14 all data accepted for technical reasons were included in the computation of precision figures, while Table C 15 contains the results of the statistical evaluation performed after removal of the detected outliers.

The RSD_R of POP, POS and SOS without removing the outliers was < 2.6 %. Removal of statistical outliers improved the precision figures to RSD_R < 1.1 %. Table 9 gives a brief outline of the resulting range of laboratory mean values and the corresponding standard deviation of reproducibility after removal of statistical outliers.

Table 9: Range of accepted laboratory mean values on technical and statistical grounds and corresponding standard deviation of reproducibility (results reported in g TG / 100 g total TGs (= POP+POS+SOS=100 %))

Sample. Number	*POP range of means*	s_R	*POS range of means*	s_R	*SOS range of means*	s_R
1	20.11-20.67	0.142	47.57-47.95	0.108	31.64-32.08	0.169
2	19.39-19.62	0.081	47.13-47.57	0.148	32.81-33.33	0.166
3	18.88-19.19	0.120	47.11-47.33	0.090	33.54-33.96	0.168
4	20.38-20.86	0.193	46.91-47.31	0.132	31.94-32.62	0.250
5	20.50-20.99	0.156	46.05-46.53	0.182	32.50-33.42	0.279
6	19.78-20.11	0.118	46.64-46.90	0.091	33.00-33.48	0.181
7	19.88-20.54	0.214	46.08-46.26	0.066	33.21-33.87	0.213
8	19.47-19.78	0.113	46.36-46.85	0.142	33.53-33.87	0.149
9	20.24-20.72	0.157	45.85-46.30	0.148	33.16-33.81	0.196
10	19.70-20.02	0.094	46.46-46.85	0.118	33.13-33.84	0.185
11	20.52-21.01	0.164	45.91-46.30	0.125	32.68-33.46	0.226
12	24.92-25.77	0.265	43.48-44.21	0.210	30.59-32.13	0.198
13	19.52-19.91	0.120	47.23-47.57	0.106	32.69-32.99	0.111

7 RESULTS

The outcome of the study was summarised as a number of "correct", "false positive" and "false negative" results. By using the CB-CRM for calibration purpose, the efficiency of the regression method (percentage of correctly classified samples) was 100 %. Pure CBs, CB/CBE blends as well as the plain chocolate samples were classified correctly. This

suggests a detection limit of 2 % CBE in CB, resulting in 0.4 % CBE in chocolate (assumed fat content of chocolate 20 %).

8 CONCLUSIONS

The results of the collaborative study showed that the analytical approach (= TG profiling by HR-GC + simple regression) can be applied for the detection of 2 % CBE in CB covering a broad range of commercially available CBEs. However, a thorough in-house investigation by the JRC of arithmetically prepared CBE/CB mixtures by using the standardised data base covering the full range of available CBEs suggested for some CBEs a detection limit of 3 %. Illipé was included in the study only in combination with other CBEs since pure Illipé is according to chocolate manufacturers of no practical importance as an admixture to chocolate. Taking in account the results of the in-house investigation and validation study the analytical procedure can detect at least 0.4 to 0.6 % CBEs in the final product (assumed fat content of chocolate 20 %).

The main advantage of the tested methodological approach is that individual testing laboratories do not need to establish a "CB line". The end user of the described approach has just to calibrate the gas chromatographic separation system using the certified reference material, separate the sample in question and use the mathematical equations for subsequent data treatment in order to detect CBEs. The method offers an important measure to assess compliance with labelling provisions and is suitable for a rapid screening of large numbers of samples to detect foreign fats in CB and plain chocolate.

9 LITERATURE

[1] Directive 2000/36/EC of the European Parliament and the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. OJ L197, 19-25.

[2] M. Lipp, C. Simoneau, F. Ulberth, E. Anklam. (1999) EUR 18992 EN.

[3] F.B. Padley & R.E. Timms (1980) J. Am. Oil Chem. Soc. 57, 286-293.

[4] M. Buchgraber & E. Anklam (2003) publication in preparation.

[5] Official Methods of Analysis of AOAC International (1995) 16th Edition. Volume II. Chapter 31. AOAC Official Method 920.75.

ANNEX A

Table A 1: Sample codes for individual laboratories

Figure A 1: Sample handling

Table A 1: Sample codes for individual laboratories

Lab code	Sample number 1	2	3	4	5	6	7	8	9	10	11	12	13
	Labelled Sample Codes												
1	324	172	439	34	118	18	305	349	285	420	382	137	373
2	338	168	431	24	111	5	313	348	293	414	398	134	363
3	336	179	422	36	103	1	309	359	298	418	391	122	378
4	321	161	436	32	110	9	303	344	297	403	385	140	366
5	337	166	433	28	102	12	315	343	292	404	392	135	370
6	322	162	424	38	101	19	317	341	299	409	397	124	365
7	334	169	438	26	107	2	302	360	296	408	390	132	368
8	339	180	429	37	120	7	316	352	300	416	388	139	375
9	325	177	434	27	117	14	318	346	282	415	394	133	362
10	335	165	437	22	112	10	311	347	290	410	396	125	364
11	331	178	430	23	115	13	320	351	281	412	399	136	379
12	328	164	440	40	109	6	314	350	283	413	393	138	371
13	333	174	435	39	113	3	312	345	291	405	381	129	361
14	340	167	421	30	119	15	301	358	284	407	389	131	372
15	326	170	426	21	104	8	304	354	289	406	384	121	380

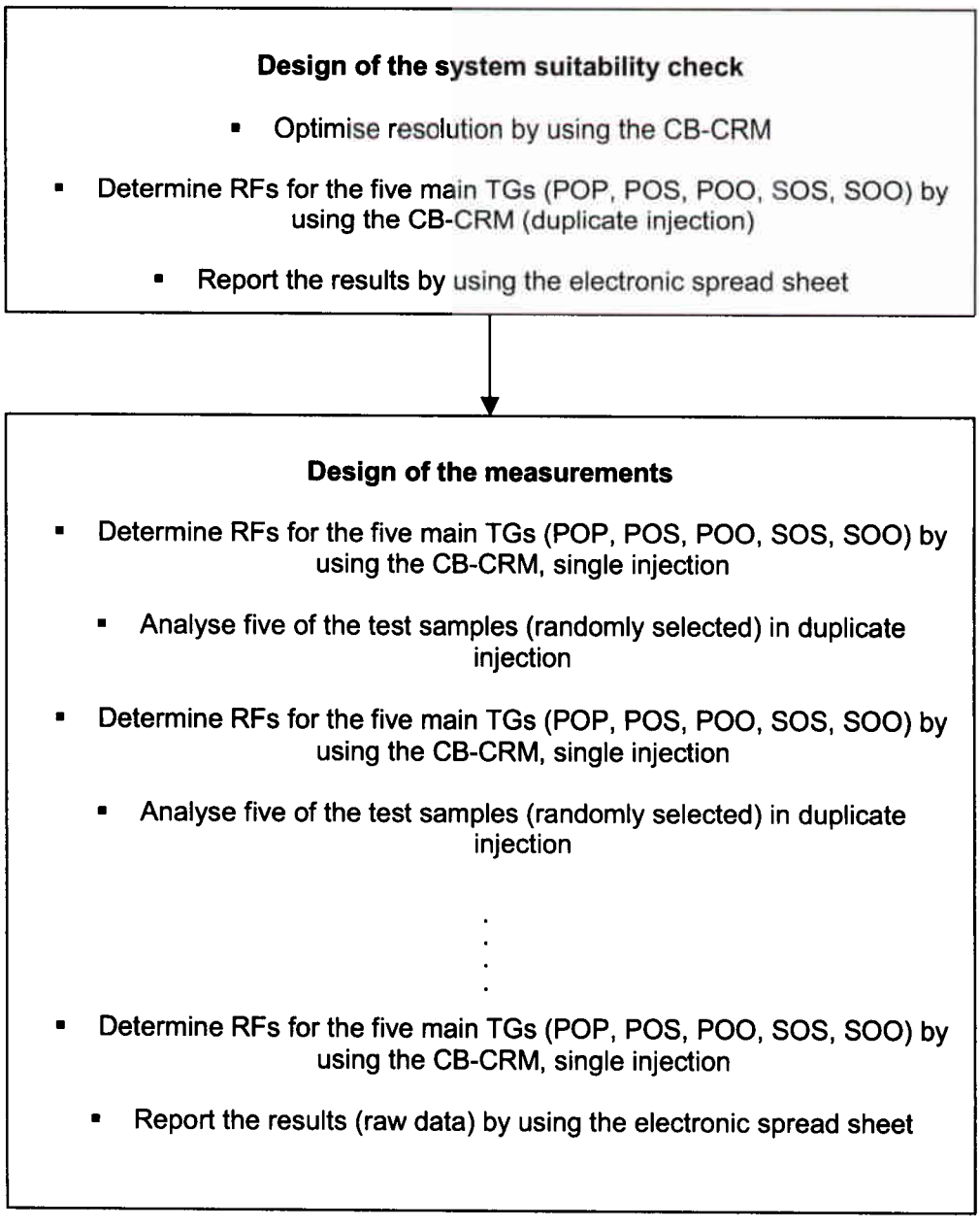


Figure A 1: Flow-scheme detailing the handling of the samples

ANNEX B

Standard operation procedure used for the study

Standard operation procedure for the detection of cocoa butter equivalents in cocoa butter and plain chocolate

1 Scope and Field of Application

This draft standard specifies a procedure for the detection of cocoa butter equivalents (CBE) in cocoa butter and plain chocolate by high resolution capillary gas chromatography (HR-GC) of triglycerides and subsequent data evaluation by regression analysis.

2 Reference

AOAC Official Method 970.20 - Cacao Products. Preparation of Sample
AOAC Official Method 920.75 - Separation of Fat in Cacao Products
Report EUR: Certification of the triglyceride profile of cocoa butter (in press)

3 Principle

Cocoa butter, or the fat obtained by solvent extraction from plain chocolate, is separated by HR-GC into triglyceride fractions according to their molecular weight and degree of unsaturation. The presence of CBE is detected by linear regression analysis applied to individual triglyceride fractions of the fat analysed.

4 Reagents and Materials

All reagents shall be of recognized analytical grade, unless otherwise stated.

4.1 Fat solvent (non-chlorinated solvents e.g. *n*-heptane, *iso*-octane)

4.2 Cocoa butter, Certified Reference Material (CRM) IRMM-801, for calibration purposes and system suitability check

5 Apparatus

5.1 Balance, sensitivity ±1 mg
5.2 Volumetric flasks, of capacity 20 mL
5.3 Pipettes, of capacity 1 mL
5.4 Drying oven, maintained at 55 °C (dry heater block may be used)
5.5 Gas chromatograph (GC): a chromatograph fitted with a cold on-column injection system and a flame ionisation detector (FID). (*Note*: alternative injection system, e.g. a split injector, a programmed-temperature vaporizer (PTV) or a moving-needle injector, may be used provided the same results are obtained as indicated in 9.1).

The separation and quantification have proven to be satisfactory if the following experimental conditions are followed:

- GC column: 25 - 30 m x 0.25 i.d. fused silica coated with thermo stable 50 % phenylmethylpolysiloxane to a film thickness of 0.1 – 0.15 μm (*Note*: Suitable columns are listed in Annex 1).
- temperature programme: 100 °C (initial temperature), programme rate 30 °C/min to 340 °C (final temperature). (*Note*: Operating conditions may be changed to obtain optimum separation of cocoa butter triglycerides)
- carrier gas: helium or hydrogen (purity ≥ 99.999 %).

Alternative experimental conditions, used in an international collaborative study, are listed in Annex 1.

5.6 Chromatographic data system

5.7 Micro syringe: maximum volume 10 µl, graduated to 0.1 µl. (*Note*: an automatic sampler may be used).
5.8 Food grater: a kitchen blender with a design featuring the motor above the receiving container to avoid melting the samples (e.g. Philips HR2833)

6 Preparation of cocoa butter CRM for calibration purposes and system suitability check

Before opening and using the cocoa butter CRM (4.2), the ampoule has to be warmed in a drying oven (5.4) until the contents have melted. When a clear solution is obtained, mix the contents by repeated inversion for not less than 20 sec., open and transfer the contents to a clean vial, which can be tightly sealed and preserved in a cool place for future usage.

7 Preparation of the test sample

Chill ca 200 g chocolate until hard, and grate to fine granular condition using a mechanical device (5.8). Mix thoroughly and preserve in tightly stoppered bottle in a cool place.

8 Procedure

8.1 Fat extraction

Obtain the fat as described in AOAC Official Method 920.75. That is to say, fat is separated from 10 – 40 g grated chocolate by extracting with two or three 100 mL portions of ether. (*Note*: alternative extraction procedures may be used, e.g. by Soxhlet, by supercritical carbon dioxide or by using microwaves, provided that the same results are obtained).

8.2 Separation of individual triglycerides by HR-GC

The test samples (cocoa butter, fat extracted from chocolate, cocoa butter CRM (4.2)) have to be warmed in a drying oven (5.4) until completely melted. If the liquid sample contains sediment, filter the sample inside the oven to obtain a clear filtrate. Pipettes (or similar equipment) used for transferring the sample during weighing operations should be brought to a temperature of ca. 50 °C in a drying oven in order to avoid partial fat fractionation.

Weigh ca 0.2 g test sample in a 20 mL volumetric flask (5.2) and bring to volume with a suitable fat solvent (4.1). Pipette 1 mL of the resulting solution in another 20 mL volumetric flask and bring to volume with the same solvent.
Inject 0.5-1.0 µl of the final test solution (0.5 mg fat/mL) into the HR-GC system using the cold on-column injection system. (*Note*: alternative sample amounts and injectors may be used provided that the detection system employed gives a linear response and the system suitability criteria (9.1) are met).

8.3 Identification

Identification of the five major triglyceride fractions 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS), 1-palmitoyl-2,3-dioleoyl-glycerol (POO), 1,3-distearoyl-2-oleoylglycerol (SOS), and 1-stearoyl-2,3-dioleoyl-glycerol (SOO) is made by comparison of the retention times of the test samples with those of the cocoa butter CRM (4.2). The elution order of the triglycerides of the cocoa butter CRM is given in the example chromatogram (Annex 2).

8.4 Calculations

8.4.1 Determination of response factors

Determine the response factors of the triglycerides POP, POS, POO, SOS and SOO by injection of the cocoa butter CRM solution using experimental conditions identical to those used for the samples. Calculate the area percentage for each of the five triglyceride fractions by:

$$AR_i\,[\%] = \frac{AR_i}{\sum AR_i} \times 100 \qquad \textbf{[Equation 1]}$$

$$RF_i = \frac{MR_i\,[\%]}{AR_i\,[\%]} \qquad \textbf{[Equation 2]}$$

AR_i ..... area under the peak corresponding to triglyceride i in the cocoa butter CRM
ΣAR_i ..... sum of the areas under the peaks attributed to POP, POS, POO, SOS, SOO in the cocoa butter CRM
MR_i [%]..... mass-% of triglyceride i in the cocoa butter CRM as given in the certificate
AR_i [%] ..... area-% of triglyceride i in the cocoa butter CRM
RF_i detector response factor of triglyceride i in the cocoa butter CRM

8.4.2 Calculation of weight percentages of triglycerides

Calculate the weight percentage of the triglycerides POP, POS, POO, SOS, SOO in the test sample by

$$MS_i\,[\%] = \frac{RF_i \times AS_i}{\sum (RF_i \times AS_i)} \times 100 \qquad \textbf{[Equation 3]}$$

AS_i..... area under the peak corresponding to the triglyceride i in the test sample
RF_i..... response factors as determined in 8.4.1
MS_i [%]..... mass-% of triglyceride i in the test samples

8.4.3 Decision if sample is pure cocoa butter

Weight percentage data for the three major fractions POP, POS and SOS of the test sample are normalized so that:

POP + POS +SOS = 100 % **[Equation 4]**

The variability of the triglyceride composition is expressed by an equation of the form:

POP = 43.734 – 0.733 x SOS (residual standard deviation = 0.125) **[Equation 5]**

This equation was established by using a standardised data base of the triglyceride profile of 74 individual genuine cocoa butters evaluated and in-house validated by the authors. The cocoa butter CRM (4.2) was used to standardise the applied analytical methodology for the determination of the triglyceride profile of the cocoa butters.

For 99 % of all analyses, pure cocoa butter complies with:

$$POP < 43.734 - 0.733 \times SOS + 2.326 \times 0.125 \qquad \textbf{[Equation 6]}$$

A greater value of POP, as given by equation 6, means that the sample is not pure cocoa butter.

9 Notes on Procedure

The details of the chromatographic procedure depend, among other factors, on the equipment, the type, age, and supplier of the column, the means of introduction of the test solution, the sample size, and the detector. Different column lengths and brands may be used, and injection volumes may be varied, if the requirements of the system suitability tests (9.1) are met.

9.1 System suitability

The cocoa butter CRM (4.2) has to be used to check the suitability of the separation system.

9.1.1 Resolution

The HR-GC separation system must be capable of separating the critical pairs POS/POO and SOS/SOO with a chromatographic resolution of at least 1.0. In case of failure, the chromatographic conditions (e.g. sample size, column temperature, carrier gas flow, etc) have to be optimised.

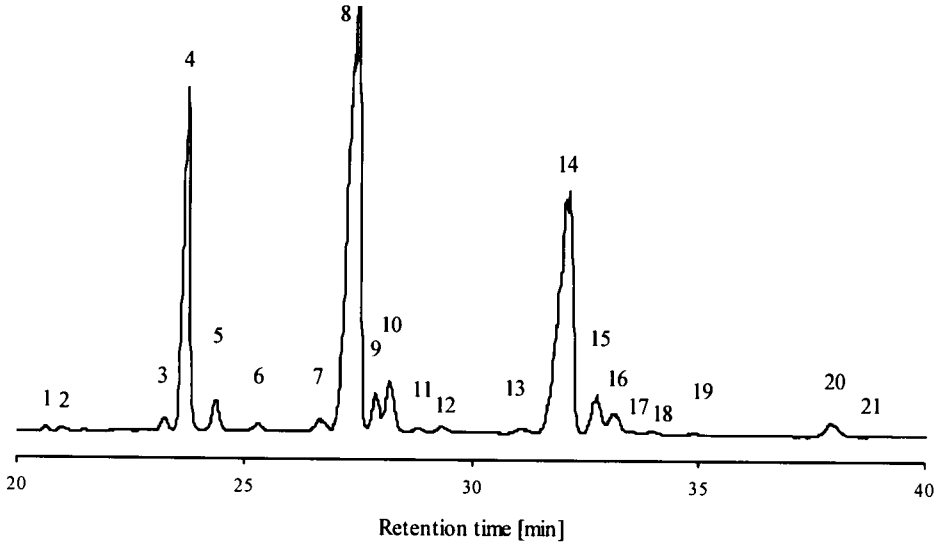
9.1.2 Determination of detector response factors

Experience has shown that for a properly functioning chromatographic system the response factors for the five main triglycerides (POP, POS, POO, SOS, SOO) vary within a range of 0.80 – 1.20.

Annex 1: Suitable GC conditions to be used for triglyceride analyses of cocoa butter

Method possibility	*1*	*2*	*3*	*4*	*5*
Column characteristics					
stationary phase	DB-17HT	RTx-65TG	CB-TAP	RTx-65TG	CB-TAP
length [m]	30	30	25	30	25
i.d. [mm]	0.25	0.25	0.25	0.25	0.25
film thickness [µm]	0.15	0.1	0.1	0.1	0.1
Temperature mode					
- oven					
injection temperature [°C] / hold time [min]	80 / 2	340 / 1	280 / 0	100 / 0.5	340 / 0
programme rate 1 [°C/min]	50	1	10	50	1
temperature 1 [°C] / hold time [min]	300 / 0	-	320 / 0	330 / 2	-
programme rate 2 [°C/min]	30	-	2	1	-
temperature 2 [°C] / hold time [min]	-	-	-	-	-
programme rate 3 [°C/min]	-	-	-	-	-
final temperature [°C] / hold time [min]	350 / 30	360 / 3	360 / 6	350 /5	360 / 10
- injector temperature [°C]	oven track	390	370	oven track	360
- detector temperature [°C]	360	370	370	355	360
Injection mode	OCI	split	split	OCI	split
Carrier gas					
type	H_2	H_2	H_2	He	He
pressure [kPa]	-	120	100	-	150
flow [mL/min]	0.8	-	-	0.8	-
Sample					
concentration [mg/mL]	0.3	50	12.5	0.3	
volume injected [µl]	0.5	0.1	0.6	0.5	1

Method possibility	6	7	8	9	10	11
Column characteristics						
stationary phase	RTx-65TG	CB-TAP	DB-17HT	CB-TAP	CB-TAP	CB-TAP
length [m]	30	25	30	25	25	25
i.d. [mm]	0.25	0.25	0.25	0.25	0.25	0.25
film thickness [µm]	0.1	0.1	0.15	0.1	0.1	0.1
Temperature mode						
- oven						
injection temperature [°C] / hold time [min]	200 / 0	100 / 0.1	50 / 2	200 /2	100 / 1	200 / 2
programme rate 1 [°C/min]	15	70	50	20	30	12
temperature 1 [°C] / hold time [min]	360 / 0	-	300 / 1	320 / 0	300 / 2	-
programme rate 2 [°C/min]	1	-	10	1	30	-
temperature 2 [°C] / hold time [min]	-	-	340 / 2	-	-	-
programme rate 3 [°C/min]	-	-	0.5	-	-	-
final temperature [°C] / hold time [min]	370	350 /21	345 /26	360 / 10	340 / 35	350 / 10
- injector temperature [°C]	390	oven track	50	65-220-370	100	
- detector temperature [°C]	390	360	360	370	360	360
Injection mode	split	OCI	OCI	OCI	OCI	hot OCI
Carrier gas						
type	H_2	H_2	H_2	He	H_2	H_2
pressure [kPa]	150	-	120	90	150	-
flow [mL/min]	-	1	-	-	-	2.4
Sample						
concentration [mg/mL]	10	15	0.5	1 - 2	0.5	0.65
volume injected [µl]	0.5	0.5	0.5	0.1	0.4	0.3



Annex 2: Triglyceride profile of the cocoa butter CRM

Peak identification: 1, PPP; 2, MOP; 3, PPS; 4, POP; 5, PLP; 6, unidentified; 7, PSS; 8, POS; 9, POO; 10, PLS; 11, PLO; 12, unidentified; 13, SSS; 14, SOS; 15, SOO; 16, SLS + OOO; 17, SLO; 18, unidentified; 19, unidentified; 20, SOA; 21, AOO

Experimental conditions:

GC column:	25 m x 0.25 mm fused silica capillary column coated with 0.1 µm Chrompack TAP
Oven temperature	100 °C held for min, 30 °C/min to 340 ° held for 35 min
Injector	Cold on-column
Detector (FID)	360 °C
Carrier gas	H_2 at 1.6 bar head pressure
Amount injected	0.5 µl of a 0.4 mg/mL solution

Annex 3: Abbreviations

PPP: tripalmitin
MOP: 1-margaroyl-2-oleoyl-3-palmitoylglycerol
PPS: 1,2-dipalmitoyl-3-stearoylglycerol
POP: 1,3-dipalmitoyl-2-oleoylglycerol
PLP: 1,3-dipalmitoyl-2-linoleoylglycerol
PSS: 1-palmitoyl-2,3-distearoylglycerol
POS: 1-palmitoyl-2-oleoyl-3-stearoylglycerol
POO: 1-palmitoyl-2,3-dioleoylglycerol
PLS: 1-palmitoyl-2-linoleoyl-3-stearoylglycerol
PLO: 1-palmitoyl-2-linoleoyl-3-oleoylglycerol
SSS: tristaerin
SOS: 1,3-distearoyl-2-oleoylglycerol
SOO: 1-stearoyl-2,3-dioleoylglycerol
SLS: 1,3-distearoyl-2- linoleoyl glycerol
OOO: triolein
SLO: 1- stearoyl -2-linoleoyl-3-oleoylglycerol
SOA: 1-stearoyl-2-oleoyl-arachidoylglycerol
AOO: 1-arachidoyl-2,3- dioleoylglycerol

ANNEX C

Tables C 1 – C 13: Results accepted on technical grounds

Figures C 1 – C 13: Bar-charts of results accepted on technical grounds

Table C14: Statistical evaluation of results accepted on technical grounds

Table C15: Statistical evaluation of results accepted on technical and statistical grounds

Table C 1: Results accepted on technical grounds for sample 1 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.43	20.43	47.70	47.69	31.87	31.88	20.67	20.67	pure CB	pure CB
2	20.41	20.46	47.80	47.78	31.79	31.75	20.73	20.76	pure CB	pure CB
3	20.35	20.36	47.55	47.58	32.10	32.06	20.51	20.54	pure CB	pure CB
4	20.61	20.72	47.66	47.73	31.73	31.54	20.77	20.91	pure CB	pure CB
5	20.52	20.41	47.68	47.64	31.80	31.96	20.72	20.61	pure CB	pure CB
6	20.37	20.43	47.91	47.98	31.71	31.58	20.79	20.88	pure CB	pure CB
7	20.51	20.52	47.77	47.78	31.72	31.70	20.78	20.80	pure CB	pure CB
8	20.17	20.62	47.57	47.72	32.26	31.66	20.39	20.83	pure CB	pure CB
9	20.33	20.42	47.73	47.75	31.94	31.83	20.62	20.70	pure CB	pure CB
10	20.26	20.28	47.56	47.76	32.19	31.96	20.44	20.61	pure CB	pure CB
11	20.35	20.25	47.76	47.78	31.89	31.97	20.66	20.60	pure CB	pure CB
12	20.43	20.42	47.58	47.72	31.99	31.86	20.58	20.68	pure CB	pure CB
13	20.20	20.03	47.85	47.80	31.95	32.17	20.61	20.46	pure CB	pure CB

Table C 2: Results accepted on technical grounds for sample 2 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	19.44	19.44	47.44	47.45	33.11	33.12	19.76	19.76	pure CB	pure CB
2	19.56	19.53	47.14	47.12	33.30	33.35	19.63	19.59	pure CB	pure CB
3	19.58	19.57	47.32	47.44	33.09	33.00	19.78	19.85	pure CB	pure CB
4	19.11	19.19	47.39	47.44	33.50	33.37	19.48	19.57	pure CB	pure CB
5	19.48	19.53	47.42	47.64	33.10	32.83	19.77	19.97	pure CB	pure CB
6	19.43	19.57	47.43	47.44	33.14	32.99	19.74	19.85	pure CB	pure CB
7	19.45	19.38	47.49	47.54	33.05	33.07	19.81	19.79	pure CB	pure CB
8	19.49	19.29	47.29	47.35	33.22	33.36	19.68	19.58	pure CB	pure CB
9	19.51	19.42	47.10	47.40	33.39	33.18	19.56	19.71	pure CB	pure CB
10	19.53	19.39	47.39	47.47	33.08	33.14	19.79	19.74	pure CB	pure CB
11	19.47	19.53	47.61	47.39	32.93	33.08	19.90	19.79	pure CB	pure CB
12	19.44	19.53	47.54	47.53	33.03	32.94	19.83	19.89	pure CB	pure CB
13	19.66	19.57	47.60	47.54	32.74	32.89	20.04	19.93	pure CB	pure CB

Table C 3: Results accepted on technical grounds for sample 3 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	18.99	18.98	47.26	47.26	33.74	33.76	19.30	19.29	pure CB	pure CB
2	19.09	19.19	47.22	47.18	33.68	33.62	19.34	19.39	pure CB	pure CB
3	18.95	18.81	47.32	47.26	33.72	33.93	19.31	19.16	pure CB	pure CB
4	18.74	19.03	47.13	47.20	34.13	33.77	19.02	19.28	pure CB	pure CB
5	18.99	18.97	47.09	47.13	33.91	33.89	19.18	19.19	pure CB	pure CB
6	19.80	19.29	47.62	47.62	32.58	33.09	20.15	19.78	pure CB	pure CB
7	19.04	18.99	47.32	47.34	33.65	33.67	19.37	19.36	pure CB	pure CB
8	18.80	19.02	47.20	47.29	34.00	33.69	19.11	19.34	pure CB	pure CB
9	18.95	18.95	47.19	47.18	33.86	33.86	19.22	19.21	pure CB	pure CB
10	18.93	18.85	47.27	47.03	33.80	34.12	19.26	19.02	pure CB	pure CB
11	19.12	19.03	47.30	47.00	33.59	33.97	19.42	19.13	pure CB	pure CB
12	19.02	18.95	47.14	47.17	33.85	33.88	19.22	19.20	pure CB	pure CB
13	19.25	19.13	47.30	47.23	33.45	33.64	19.52	19.38	pure CB	pure CB

Table C 4: Results accepted on technical grounds for sample 4 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.67	20.66	47.08	47.07	32.25	32.27	20.40	20.38	CB/CBE	CB/CBE
2	20.57	20.67	46.98	46.83	32.44	32.50	20.26	20.21	CB/CBE	CB/CBE
3	20.59	20.66	47.00	46.98	32.41	32.37	20.28	20.31	CB/CBE	CB/CBE
4	20.76	20.14	47.12	47.08	32.12	32.77	20.49	20.01	CB/CBE	CB/CBE
5	20.55	20.65	47.08	47.19	32.37	32.15	20.31	20.47	CB/CBE	CB/CBE
6	20.82	20.69	47.39	47.22	31.79	32.09	20.73	20.51	CB/CBE	CB/CBE
7	20.84	20.82	47.18	47.15	31.98	32.03	20.59	20.56	CB/CBE	CB/CBE
8	20.09	20.68	46.96	47.04	32.96	32.28	19.88	20.37	CB/CBE	CB/CBE
9	20.58	20.61	46.97	47.07	32.45	32.32	20.25	20.34	CB/CBE	CB/CBE
10	20.56	20.55	47.19	47.12	32.26	32.33	20.39	20.33	CB/CBE	CB/CBE
11	20.55	20.59	46.77	47.14	32.68	32.27	20.08	20.38	CB/CBE	CB/CBE
12	20.42	20.47	47.20	47.25	32.39	32.28	20.29	20.37	CB/CBE	CB/CBE
13	20.70	21.02	47.08	46.99	32.22	31.99	20.42	20.59	CB/CBE	CB/CBE

Table C 5: Results accepted on technical grounds for sample 5 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.99	21.00	46.51	46.50	32.50	32.50	20.21	20.21	CB/CBE	CB/CBE
2	20.80	20.82	46.34	46.34	32.86	32.84	19.95	19.96	CB/CBE	CB/CBE
3	20.81	20.77	46.35	46.42	32.84	32.81	19.96	19.99	CB/CBE	CB/CBE
4	20.51	20.77	46.13	46.27	33.36	32.96	19.58	19.88	CB/CBE	CB/CBE
5	20.53	20.48	46.15	46.01	33.32	33.51	19.61	19.47	CB/CBE	CB/CBE
6	20.53	20.90	46.45	46.38	33.02	32.71	19.83	20.06	CB/CBE	CB/CBE
7	20.84	20.81	46.56	46.50	32.60	32.69	20.14	20.08	CB/CBE	CB/CBE
8	20.95	20.46	46.30	46.15	32.75	33.39	20.03	19.56	CB/CBE	CB/CBE
9	20.83	20.82	46.45	46.47	32.73	32.71	20.05	20.06	CB/CBE	CB/CBE
10	20.79	20.68	46.52	46.53	32.69	32.79	20.07	20.00	CB/CBE	CB/CBE
11	20.75	20.86	46.17	46.20	33.07	32.94	19.79	19.89	CB/CBE	CB/CBE
12	20.59	20.65	46.58	46.46	32.83	32.88	19.97	19.93	CB/CBE	CB/CBE
13	20.84	20.65	45.92	46.17	33.24	33.18	19.67	19.72	CB/CBE	CB/CBE

Table C 6: Results accepted on technical grounds for sample 6 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.00	20.00	46.82	46.82	33.18	33.18	19.71	19.72	CB/CBE	CB/CBE
2	20.00	20.03	46.79	46.80	33.21	33.17	19.69	19.72	CB/CBE	CB/CBE
3	19.82	19.84	46.74	46.68	33.44	33.47	19.52	19.50	CB/CBE	CB/CBE
4	19.88	19.68	46.76	46.72	33.36	33.60	19.58	19.40	CB/CBE	CB/CBE
5	19.89	19.95	46.68	46.61	33.43	33.44	19.53	19.52	CB/CBE	CB/CBE
6	19.99	20.02	46.84	46.76	33.17	33.22	19.72	19.69	CB/CBE	CB/CBE
7	20.08	20.06	46.90	46.88	33.02	33.06	19.83	19.81	CB/CBE	CB/CBE
8	19.28	19.92	46.52	46.82	34.20	33.26	18.97	19.66	CB/CBE	CB/CBE
9	19.94	19.96	46.79	46.79	33.27	33.26	19.65	19.66	CB/CBE	CB/CBE
10	19.97	19.96	46.90	46.90	33.13	33.14	19.75	19.75	CB/CBE	CB/CBE
11	19.79	19.84	46.84	46.71	33.37	33.44	19.57	19.52	CB/CBE	CB/CBE
12	19.73	19.83	46.87	46.71	33.40	33.46	19.55	19.51	CB/CBE	CB/CBE
13	20.09	20.13	46.77	47.02	33.14	32.85	19.74	19.96	CB/CBE	CB/CBE

Table C 7: Results accepted on technical grounds for sample 7 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.42	20.43	46.17	46.15	33.40	33.42	19.55	19.54	CB/CBE	CB/CBE
2	20.42	20.52	46.17	46.11	33.42	33.37	19.54	19.58	CB/CBE	CB/CBE
3	20.14	20.16	46.18	46.24	33.68	33.60	19.35	19.41	CB/CBE	CB/CBE
4	19.96	20.63	46.05	46.06	33.99	33.31	19.12	19.62	CB/CBE	CB/CBE
5	20.45	20.43	46.17	46.16	33.38	33.41	19.57	19.54	CB/CBE	CB/CBE
6	19.96	19.80	46.26	46.25	33.79	33.95	19.27	19.15	CB/CBE	CB/CBE
7	20.53	20.54	46.25	46.27	33.22	33.20	19.69	19.70	CB/CBE	CB/CBE
8	20.56	20.50	46.22	46.11	33.22	33.39	19.69	19.56	CB/CBE	CB/CBE
9	20.48	20.42	46.25	46.22	33.26	33.36	19.65	19.58	CB/CBE	CB/CBE
10	20.38	20.37	46.24	46.10	33.38	33.52	19.57	19.46	CB/CBE	CB/CBE
11	20.37	20.45	46.07	46.09	33.56	33.45	19.44	19.51	CB/CBE	CB/CBE
12	20.36	20.57	46.23	46.08	33.41	33.35	19.54	19.59	CB/CBE	CB/CBE
13	19.95	20.14	46.15	46.09	33.90	33.77	19.18	19.28	CB/CBE	CB/CBE

Table C 8: Results accepted on technical grounds for sample 8 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	19.78	19.77	46.68	46.68	33.54	33.55	19.45	19.44	CB/CBE	CB/CBE
2	19.76	19.76	46.52	46.48	33.73	33.76	19.31	19.29	CB/CBE	CB/CBE
3	19.82	19.69	46.67	46.64	33.52	33.66	19.47	19.36	CB/CBE	CB/CBE
4	19.56	19.75	46.16	46.80	34.27	33.45	18.91	19.52	CB/CBE	CB/CBE
5	19.79	19.74	46.46	46.27	33.75	33.99	19.30	19.12	CB/CBE	CB/CBE
6	19.54	19.71	46.85	46.84	33.61	33.45	19.40	19.52	CB/CBE	CB/CBE
7	19.43	19.52	46.70	46.68	33.87	33.80	19.21	19.26	CB/CBE	CB/CBE
8	19.55	19.81	46.57	46.61	33.88	33.59	19.20	19.42	CB/CBE	CB/CBE
9	19.75	19.73	46.51	46.56	33.74	33.71	19.30	19.32	CB/CBE	CB/CBE
10	19.62	19.72	46.75	46.73	33.62	33.55	19.39	19.44	CB/CBE	CB/CBE
11	19.55	19.77	46.60	46.79	33.85	33.44	19.22	19.53	CB/CBE	CB/CBE
12	19.84	19.73	46.55	46.48	33.61	33.79	19.40	19.27	CB/CBE	CB/CBE
13	18.60	18.58	46.63	46.61	34.77	34.81	18.55	18.52	CB/CBE	CB/CBE

Table C 9: Results accepted on technical grounds for sample 9 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.48	20.49	46.08	46.10	33.44	33.41	19.52	19.54	CB/CBE	CB/CBE
2	20.46	20.51	45.90	45.80	33.64	33.69	19.37	19.34	CB/CBE	CB/CBE
3	20.41	20.39	45.98	46.12	33.61	33.48	19.40	19.49	CB/CBE	CB/CBE
4	20.26	20.66	46.27	46.10	33.47	33.23	19.50	19.67	CB/CBE	CB/CBE
5	20.53	20.60	46.04	45.94	33.43	33.46	19.53	19.51	CB/CBE	CB/CBE
6	20.23	20.31	46.50	46.10	33.27	33.59	19.65	19.41	CB/CBE	CB/CBE
7	20.35	20.32	46.25	46.28	33.40	33.40	19.55	19.55	CB/CBE	CB/CBE
8	20.89	20.56	46.19	46.04	32.93	33.40	19.90	19.55	CB/CBE	CB/CBE
9	20.37	20.41	46.07	46.11	33.56	33.48	19.44	19.49	CB/CBE	CB/CBE
10	20.32	20.48	46.00	45.99	33.68	33.53	19.35	19.46	CB/CBE	CB/CBE
11	20.27	20.32	46.05	46.01	33.69	33.67	19.34	19.36	CB/CBE	CB/CBE
12	20.22	20.27	45.99	45.90	33.79	33.83	19.27	19.23	CB/CBE	CB/CBE
13	18.72	18.43	45.84	45.89	35.44	35.68	18.06	17.88	CB/CBE	CB/CBE

Table C 10: Results accepted on technical grounds for sample 10 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	19.93	19.93	46.68	46.67	33.39	33.40	19.56	19.56	CB/CBE	CB/CBE
2	19.82	19.91	46.66	46.57	33.52	33.52	19.47	19.47	CB/CBE	CB/CBE
3	19.86	19.88	46.64	46.62	33.49	33.50	19.48	19.48	CB/CBE	CB/CBE
4	19.70	19.70	46.52	46.41	33.78	33.90	19.27	19.19	CB/CBE	CB/CBE
5	19.83	19.79	46.57	46.87	33.60	33.35	19.41	19.59	CB/CBE	CB/CBE
6	19.92	19.93	46.75	46.62	33.33	33.44	19.61	19.52	CB/CBE	CB/CBE
7	19.99	20.06	46.85	46.84	33.16	33.10	19.73	19.77	CB/CBE	CB/CBE
8	19.91	19.91	46.66	46.61	33.43	33.47	19.53	19.50	CB/CBE	CB/CBE
9	19.92	19.90	46.60	46.67	33.48	33.44	19.50	19.53	CB/CBE	CB/CBE
10	19.73	19.75	46.70	46.60	33.57	33.64	19.43	19.37	CB/CBE	CB/CBE
11	19.84	19.79	46.78	46.63	33.39	33.58	19.56	19.42	CB/CBE	CB/CBE
12	19.81	19.80	46.49	46.47	33.70	33.73	19.33	19.31	CB/CBE	CB/CBE
13	21.29	21.28	47.08	47.18	31.63	31.54	20.85	20.92	CB/CBE	CB/CBE

Table C 11: Results accepted on technical grounds for sample 11 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	20.80	20.80	46.09	46.08	33.10	33.12	19.77	19.76	CB/CBE	CB/CBE
2	20.86	20.83	46.27	46.06	32.87	33.12	19.94	19.76	CB/CBE	CB/CBE
3	20.69	20.72	46.00	46.04	33.31	33.24	19.62	19.67	CB/CBE	CB/CBE
4	20.41	20.63	46.02	46.02	33.58	33.34	19.42	19.60	CB/CBE	CB/CBE
5	19.75	19.70	46.57	46.68	33.68	33.62	19.35	19.39	CB/CBE	CB/CBE
6	20.89	20.71	46.01	46.21	33.10	33.08	19.77	19.79	CB/CBE	CB/CBE
7	21.01	21.02	46.31	46.30	32.68	32.68	20.08	20.08	CB/CBE	CB/CBE
8	21.00	20.83	46.11	46.07	32.89	33.10	19.92	19.77	CB/CBE	CB/CBE
9	20.74	20.83	46.07	46.15	33.19	33.03	19.70	19.82	CB/CBE	CB/CBE
10	20.67	20.85	46.19	46.12	33.13	33.03	19.75	19.82	CB/CBE	CB/CBE
11	20.62	20.57	46.08	45.88	33.31	33.55	19.62	19.44	CB/CBE	CB/CBE
12	20.84	20.88	45.97	45.85	33.20	33.27	19.70	19.65	CB/CBE	CB/CBE
13	20.41	20.76	46.27	46.25	33.33	32.99	19.60	19.85	CB/CBE	CB/CBE

Table C 12: Results accepted on technical grounds for sample 12 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	25.30	25.19	43.79	43.69	30.91	31.12	21.37	21.22	CB/CBE	CB/CBE
2	25.35	25.38	43.88	43.61	30.77	31.01	21.48	21.30	CB/CBE	CB/CBE
3	25.27	25.29	43.70	43.61	31.03	31.11	21.29	21.23	CB/CBE	CB/CBE
4	25.51	25.36	43.77	43.71	30.72	30.94	21.52	21.36	CB/CBE	CB/CBE
5	25.33	25.27	43.63	43.59	31.03	31.15	21.29	21.20	CB/CBE	CB/CBE
6	24.84	25.36	44.56	43.91	30.60	30.73	21.60	21.51	CB/CBE	CB/CBE
7	24.34	24.08	43.74	43.57	31.92	32.34	20.63	20.33	CB/CBE	CB/CBE
8	25.58	25.95	43.63	43.66	30.79	30.39	21.46	21.76	CB/CBE	CB/CBE
9	25.36	25.35	43.74	43.78	30.91	30.87	21.38	21.41	CB/CBE	CB/CBE
10	25.84	25.64	43.38	43.58	30.78	30.78	21.48	21.48	CB/CBE	CB/CBE
11	25.02	25.27	43.87	44.15	31.11	30.58	21.23	21.62	CB/CBE	CB/CBE
12	24.92	24.93	44.25	44.17	30.83	30.90	21.43	21.39	CB/CBE	CB/CBE
13	25.43	25.71	43.72	43.75	30.85	30.54	21.42	21.65	CB/CBE	CB/CBE

Table C 13: Results accepted on technical grounds for sample 13 (two replicates)

Lab code	$POP_{measured}$ A	$POP_{measured}$ B	$POS_{measured}$ A	$POS_{measured}$ B	$SOS_{measured}$ A	$SOS_{measured}$ B	$POP_{calculated}$ (1) A	$POP_{calculated}$ (1) B	Decision (2) A	Decision (2) B
1	19.67	19.66	47.39	47.42	32.94	32.92	19.89	19.90	pure CB	pure CB
2	19.77	19.79	47.13	47.32	33.09	32.89	19.78	19.93	pure CB	pure CB
3	19.78	19.80	47.36	47.32	32.86	32.87	19.95	19.94	pure CB	pure CB
4	19.68	19.57	47.35	47.52	32.97	32.91	19.87	19.91	pure CB	pure CB
5	19.67	19.80	47.55	47.47	32.77	32.73	20.01	20.04	pure CB	pure CB
6	19.38	19.67	47.66	47.47	32.96	32.86	19.87	19.95	pure CB	pure CB
7	18.56	18.50	47.11	47.06	34.33	34.44	18.87	18.79	pure CB	pure CB
8	19.84	19.79	47.40	47.45	32.76	32.76	20.02	20.02	pure CB	pure CB
9	19.77	19.74	47.42	47.41	32.81	32.85	19.99	19.96	pure CB	pure CB
10	19.61	19.77	47.38	47.35	33.01	32.88	19.84	19.93	pure CB	pure CB
11	19.92	19.90	47.39	47.40	32.68	32.70	20.08	20.07	pure CB	pure CB
12	19.86	19.71	47.48	47.42	32.66	32.87	20.09	19.94	pure CB	pure CB
13	19.88	19.72	47.24	47.30	32.88	32.98	19.93	19.86	pure CB	pure CB

(1) Results based on equation 1 (see page 7)
(2) Decision based on equation 2 (see page 7) (CB/CBE = CBE admixture to CB)

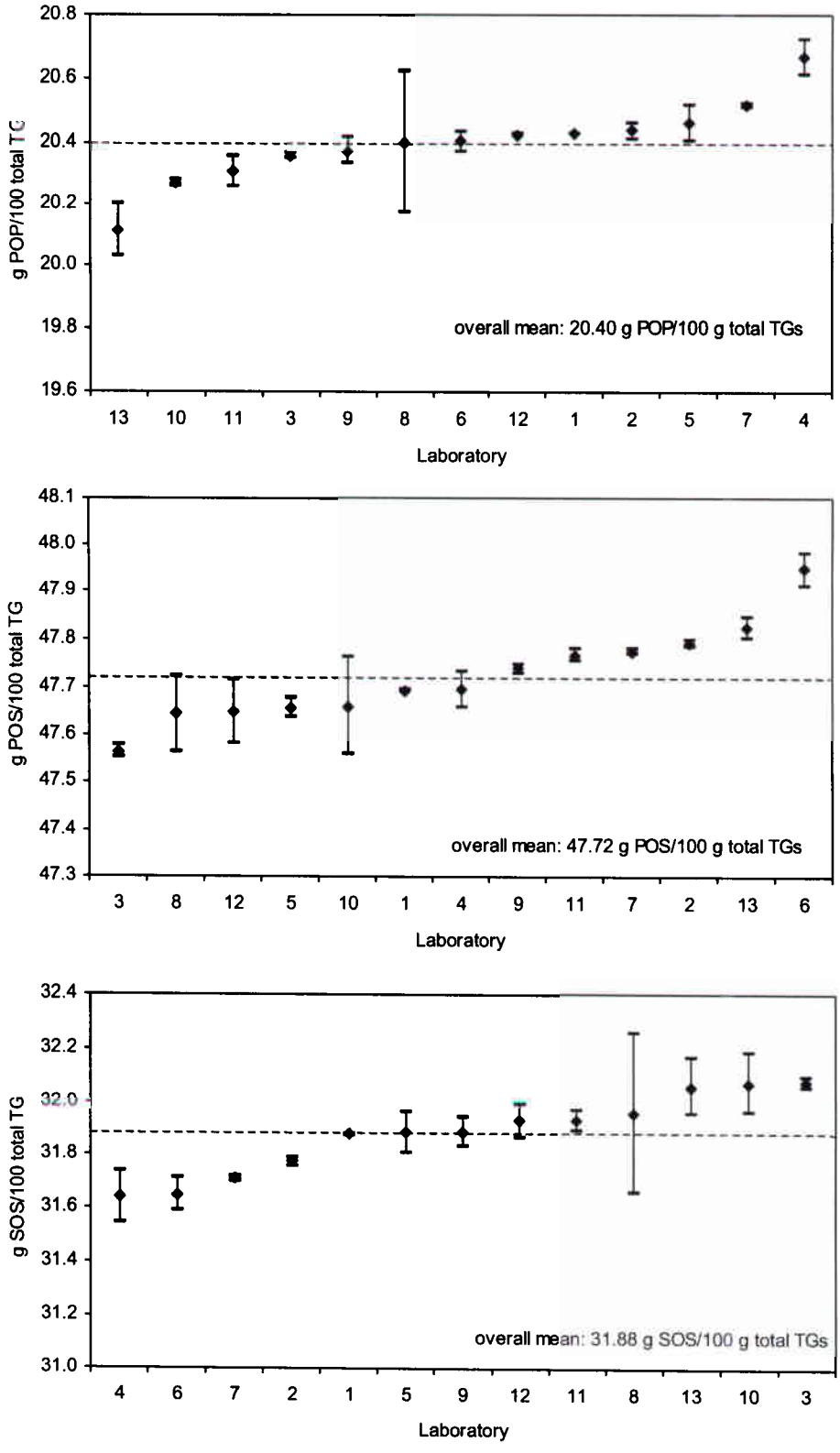


Figure C 1: Bar charts of results accepted on technical grounds for sample 1 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

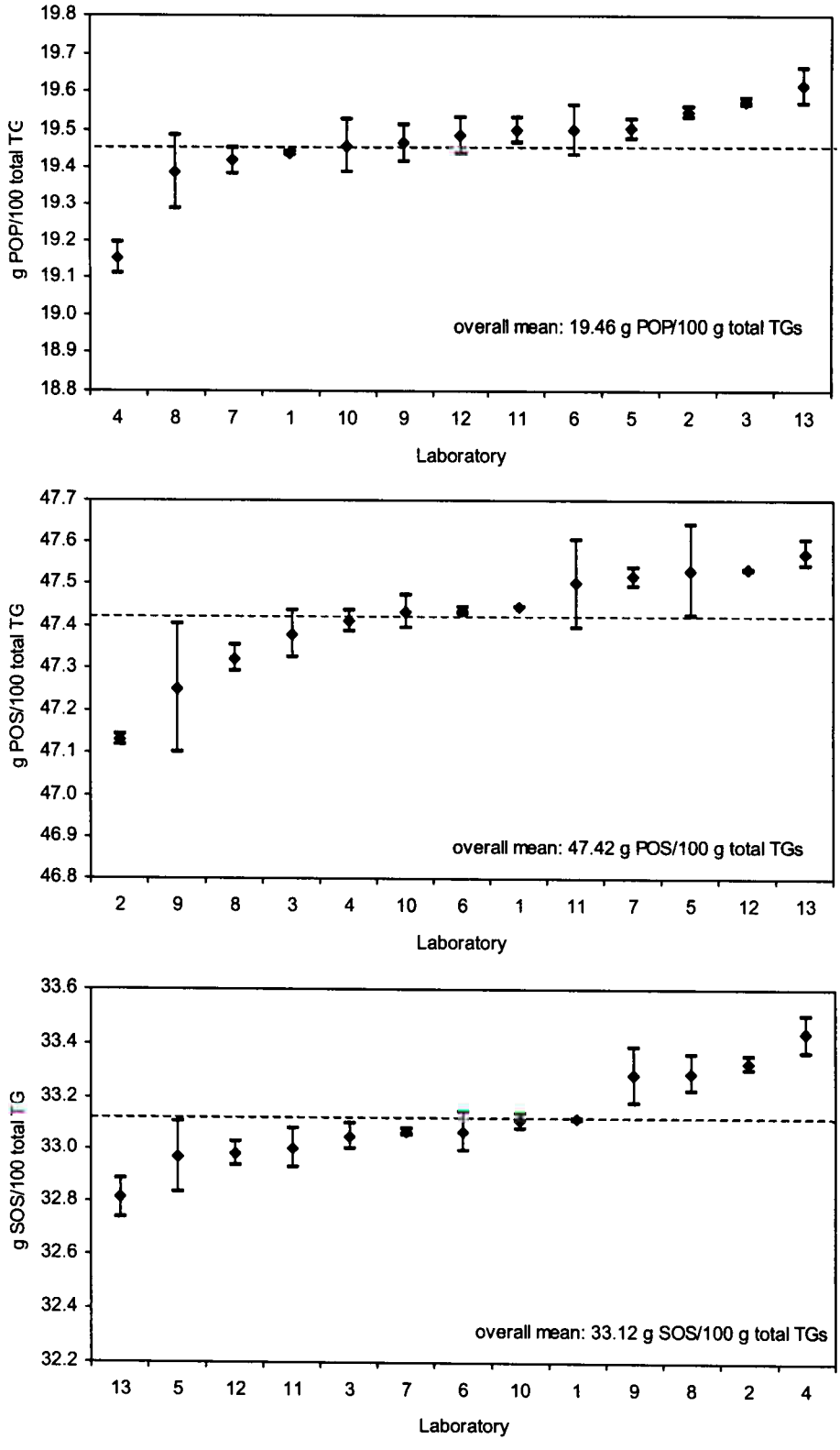


Figure C 2: Bar charts of results accepted on technical grounds for sample 2 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

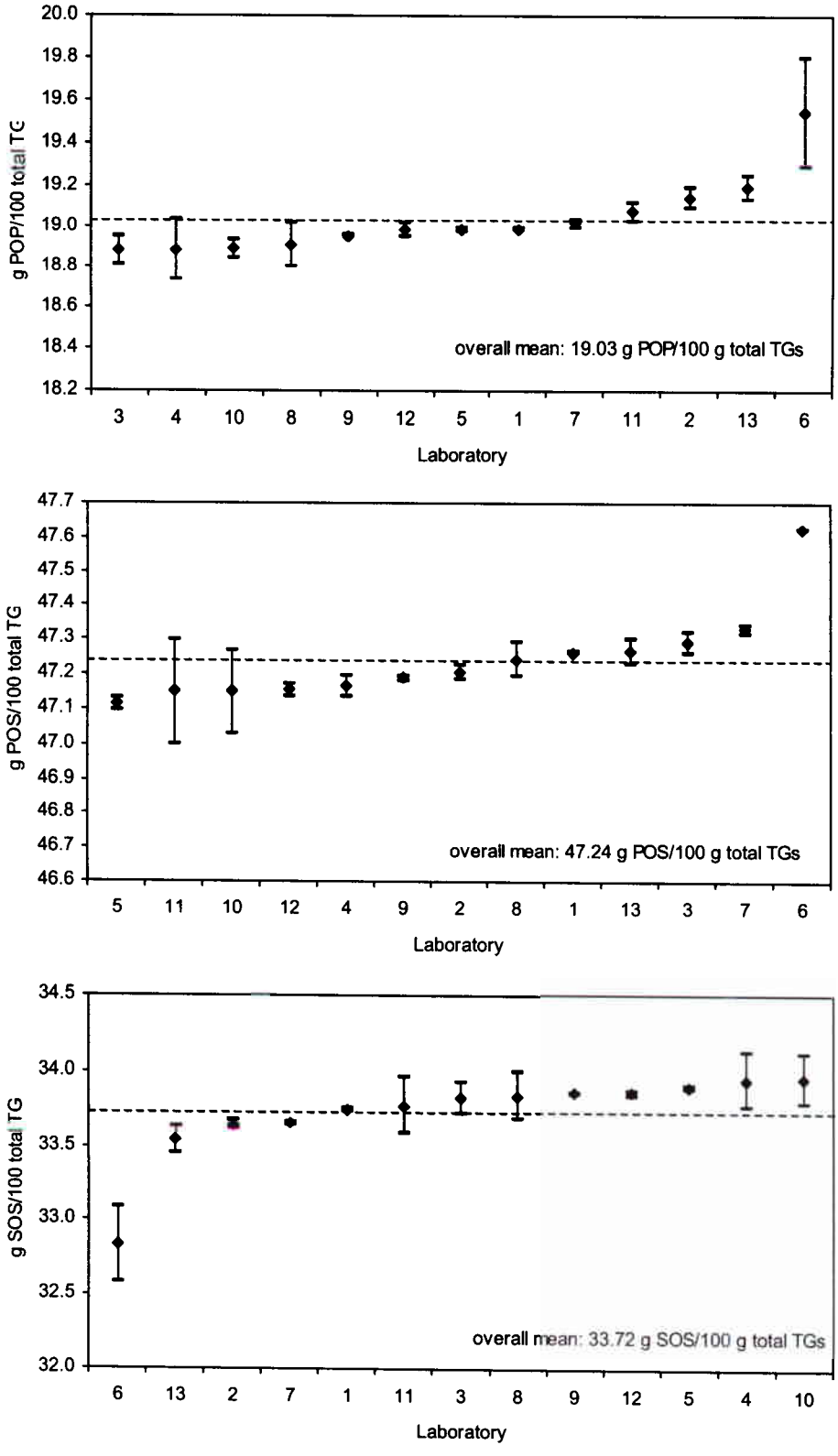


Figure C 3: Bar charts of results accepted on technical grounds for sample 3 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

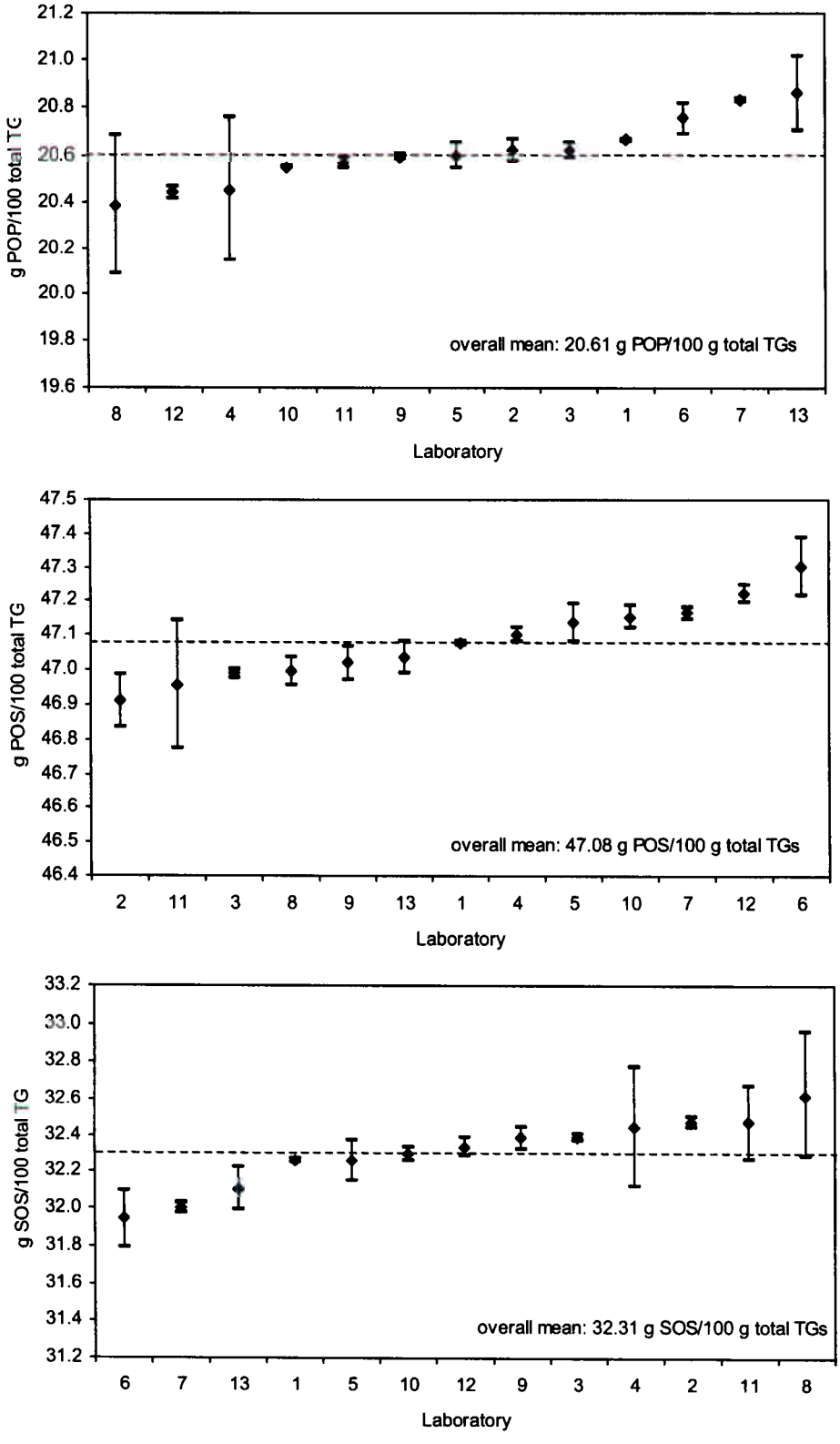


Figure C 4: Bar charts of results accepted on technical grounds for sample 4 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

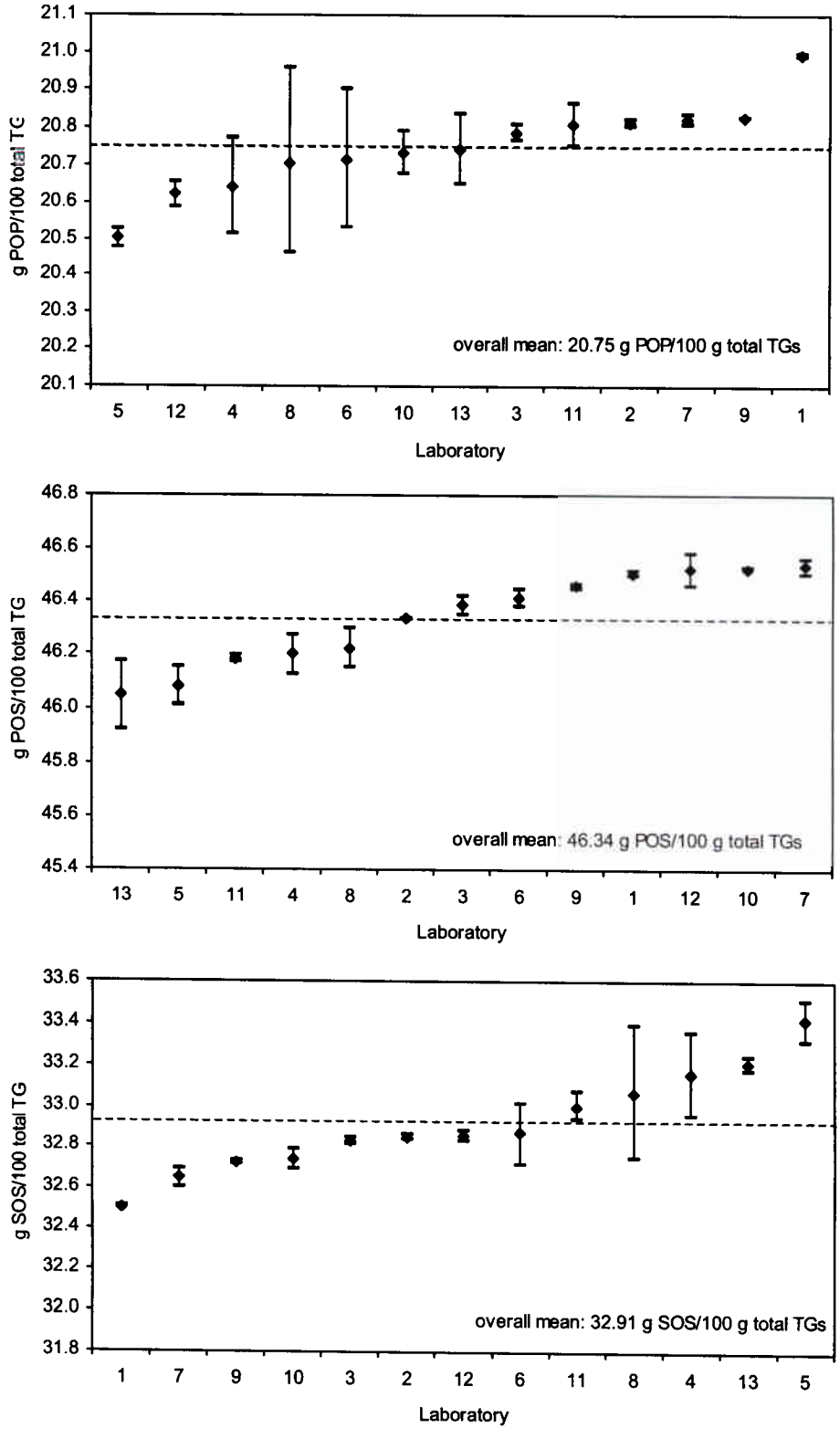


Figure C 5: Bar charts of results accepted on technical grounds for sample 5 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

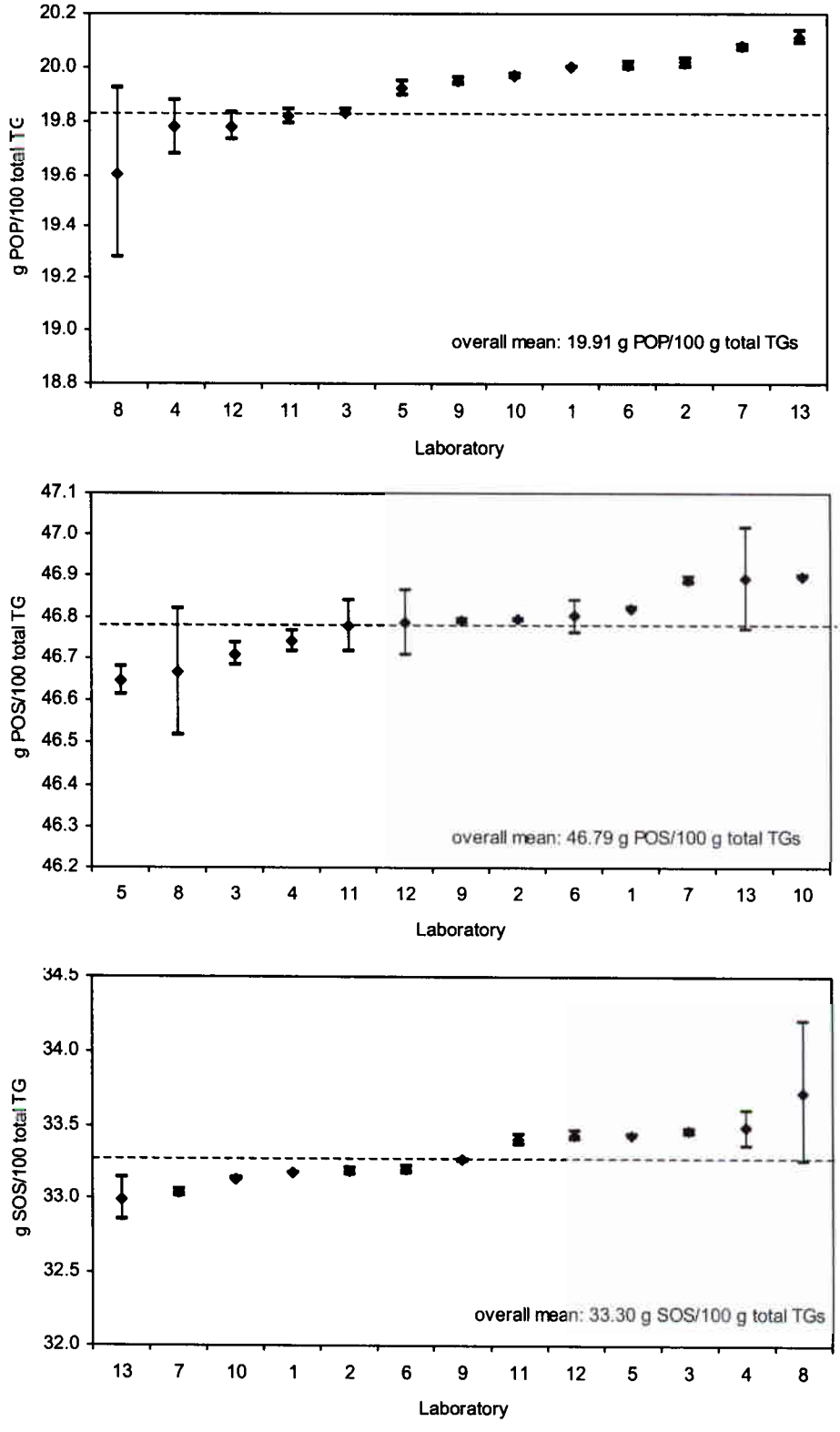


Figure C 6: Bar charts of results accepted on technical grounds for sample 6 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

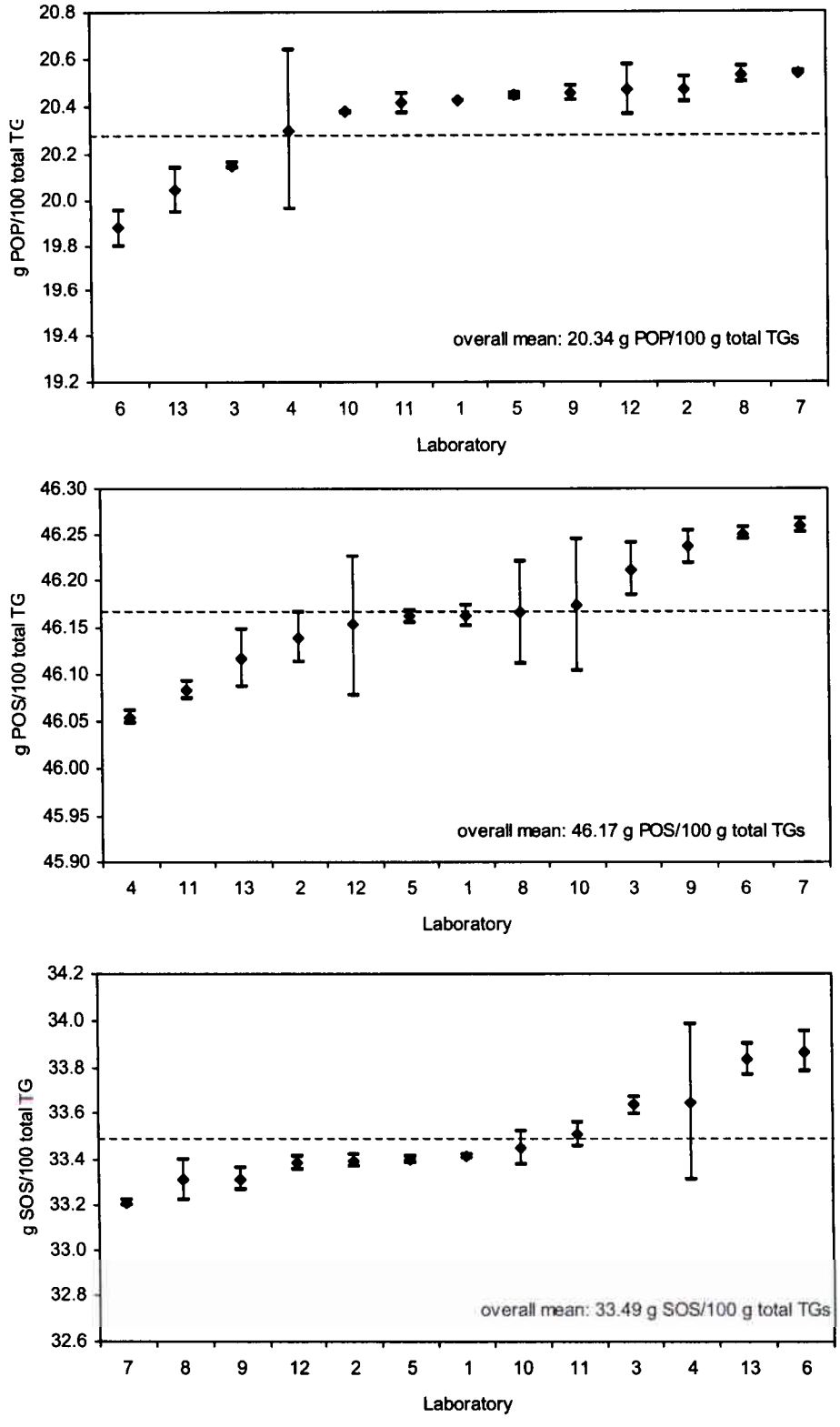


Figure C 7: Bar charts of results accepted on technical grounds for sample 7 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

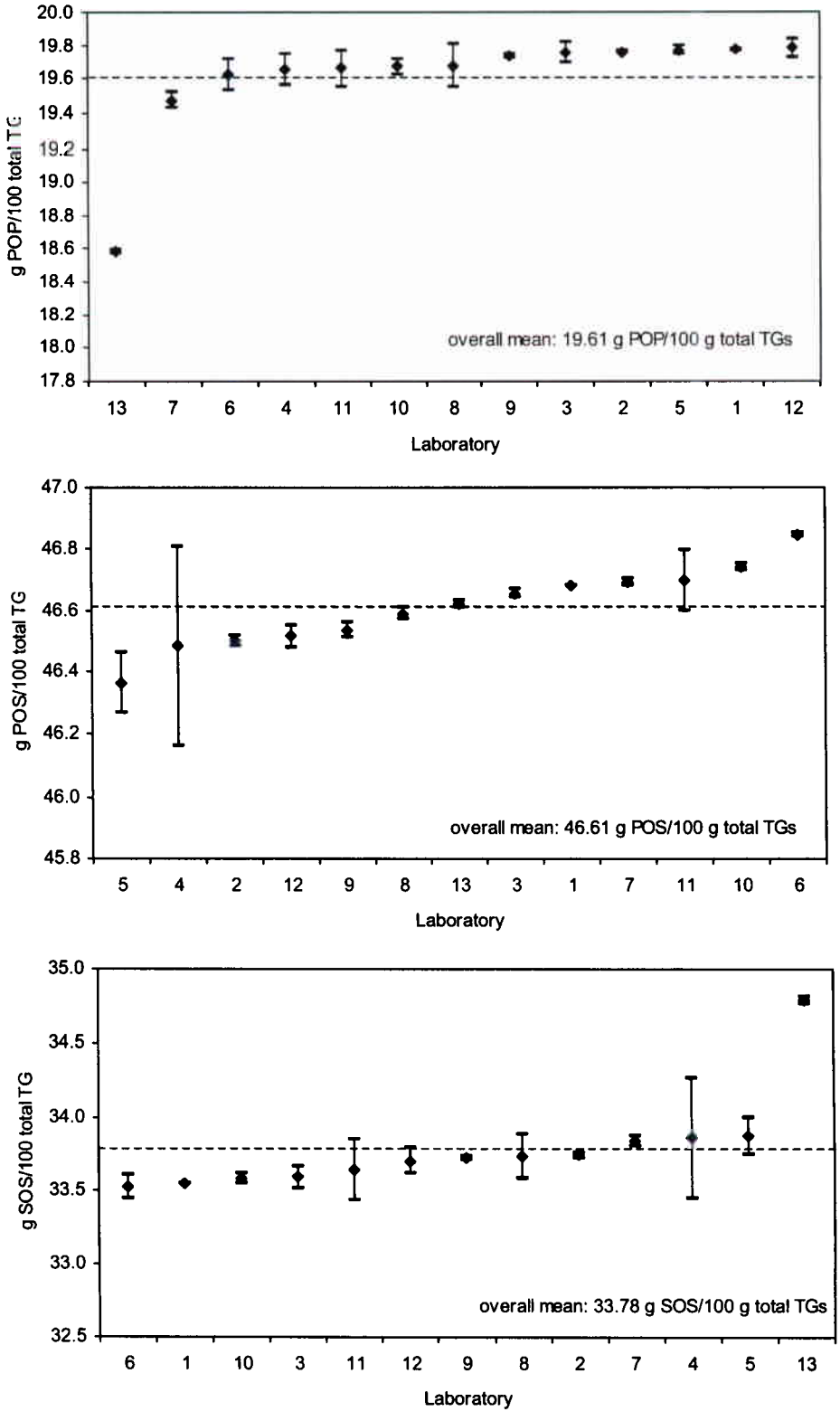


Figure C 8: Bar charts of results accepted on technical grounds for sample 8 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

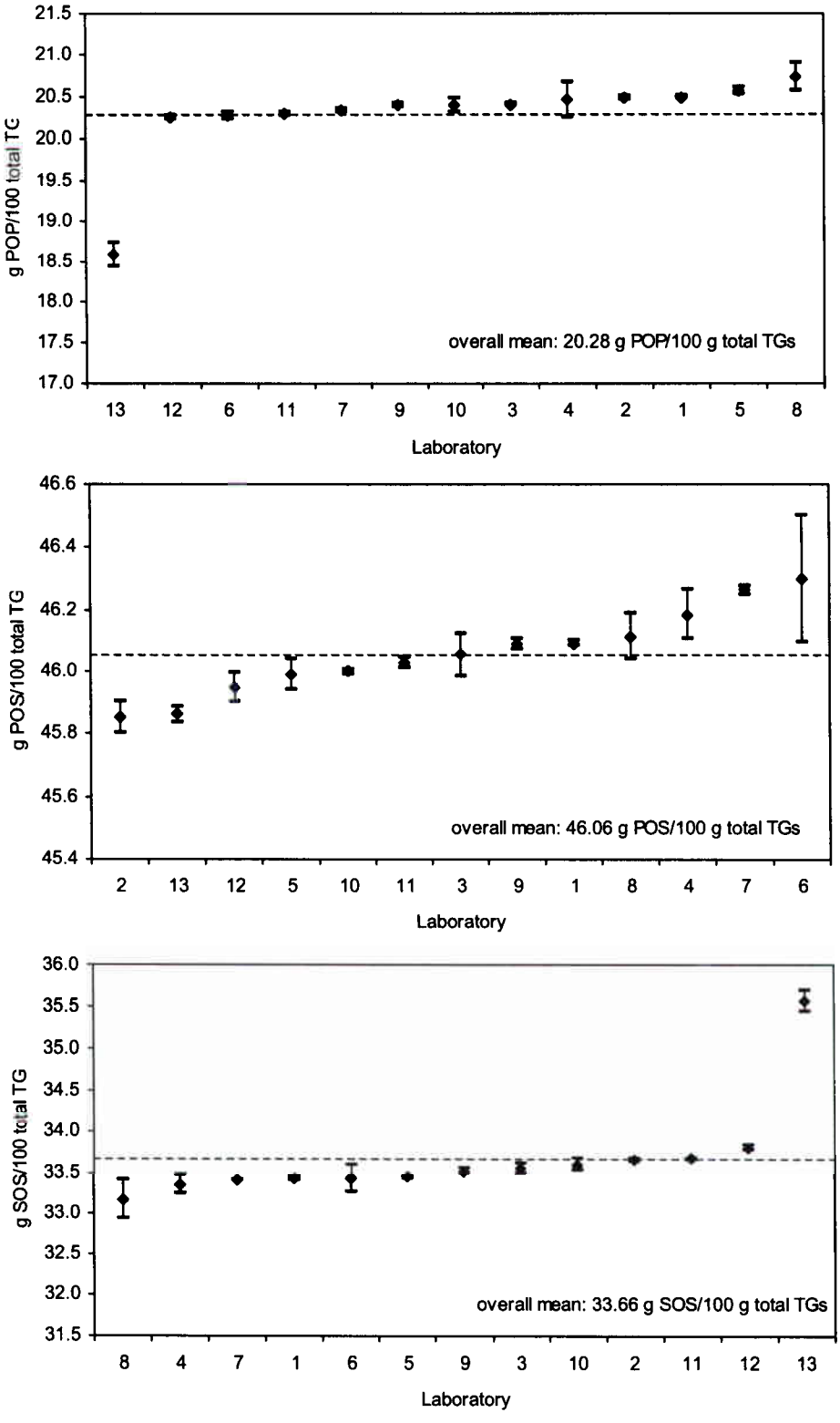


Figure C 9: Bar charts of results accepted on technical grounds for sample 9 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

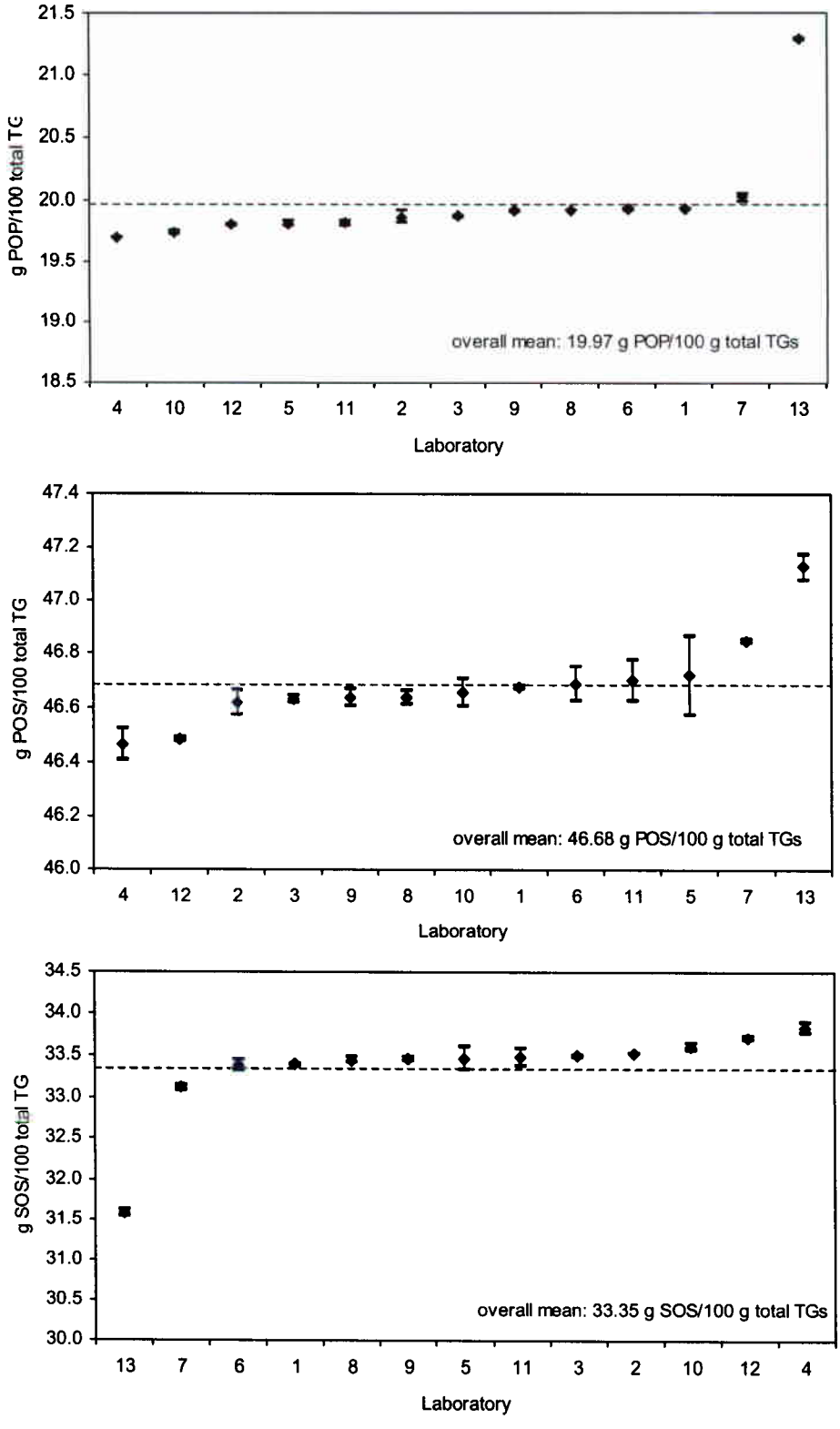


Figure C 10: Bar charts of results accepted on technical grounds for sample 10 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

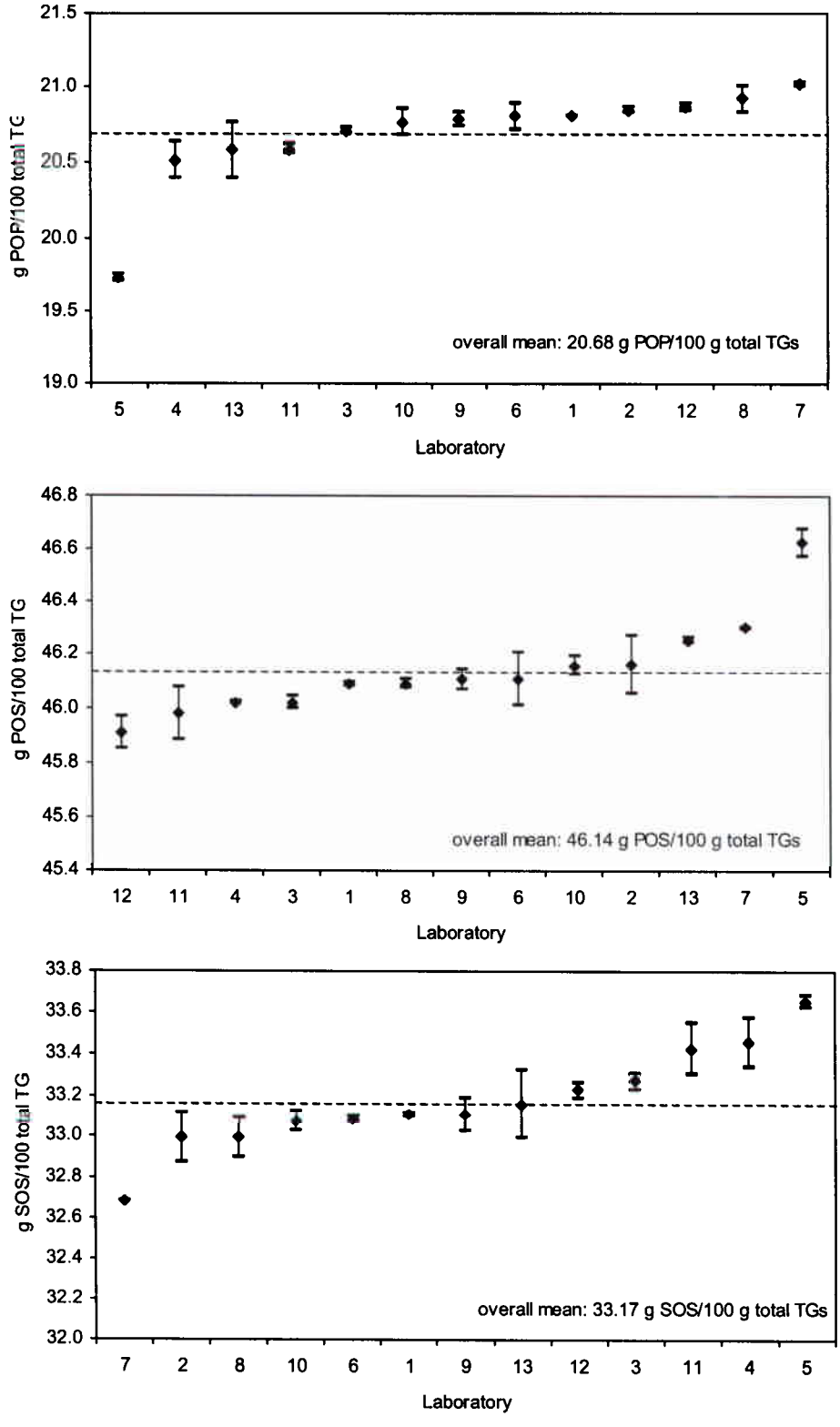


Figure C 11: Bar charts of results accepted on technical grounds for sample 11 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

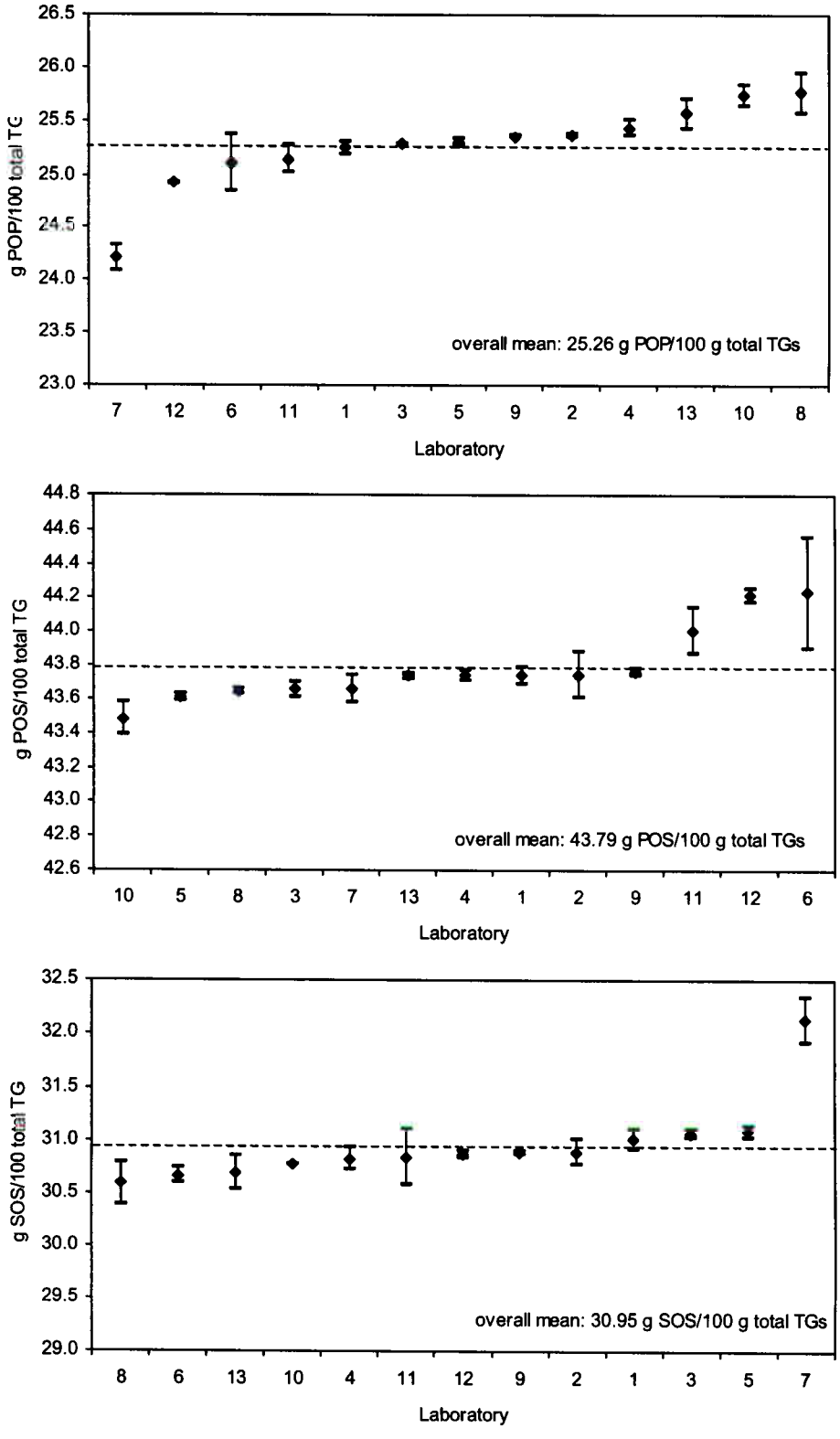


Figure C 12: Bar charts of results accepted on technical grounds for sample 12 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

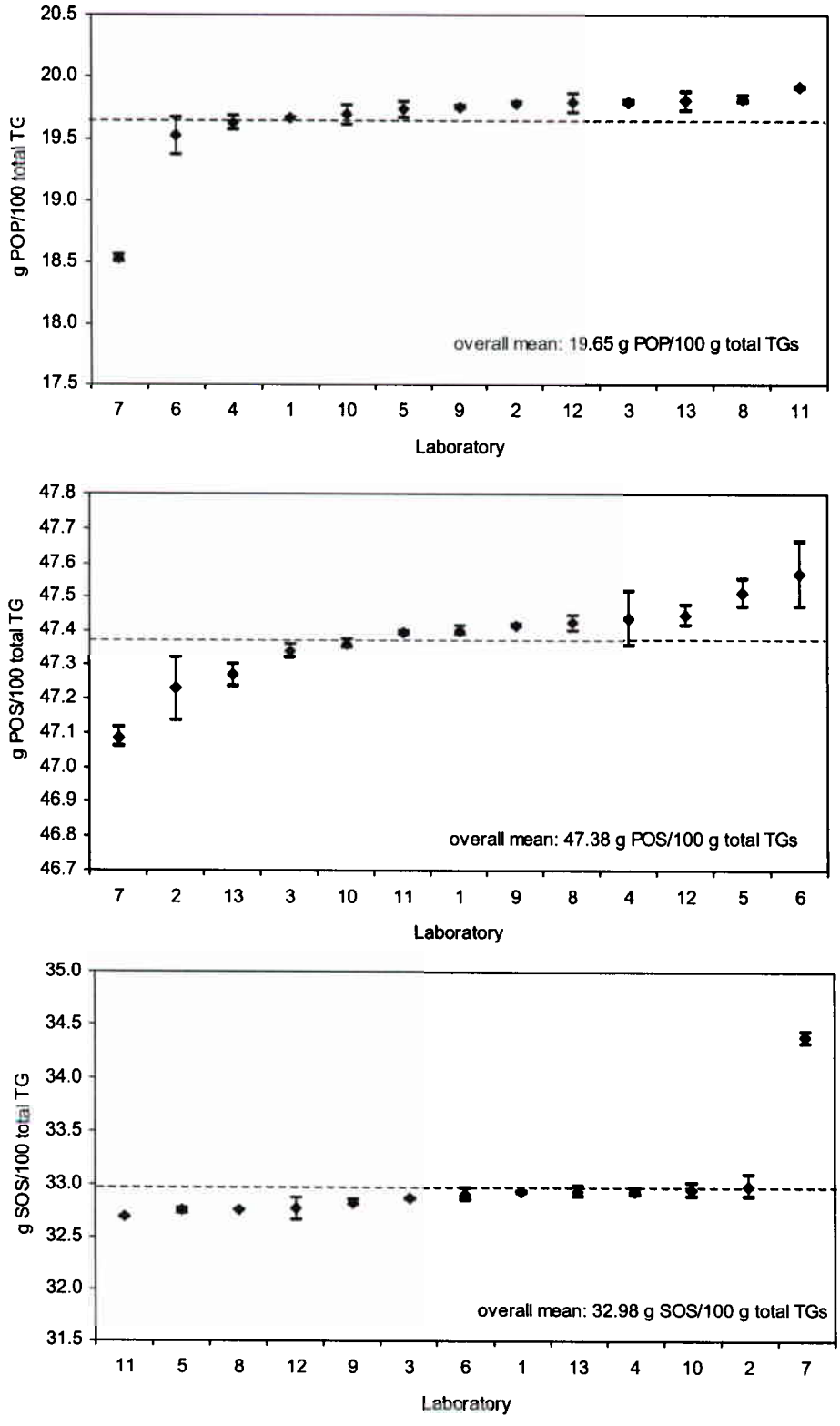


Figure C 13: Bar charts of results accepted on technical grounds for sample 13 (Laboratory means, minima and maxima; total TGs = POP, POS and SOS)

Table C 14: Statistical evaluation of the results accepted on technical grounds (Results reported as g TG / 100 g total TGs (= POP+POS+SOS=100 %))

Sa.No.	p [1]	mean	min	max	r	s_r	RSD_r	R	s_R	RSD_R
						POP				
1	13	20.40	20.11	20.67	0.291	0.104	0.5	0.418	0.149	0.7
2	13	19.46	19.15	19.62	0.192	0.069	0.4	0.343	0.122	0.6
3	13	19.03	18.88	19.54	0.374	0.133	0.7	0.573	0.205	1.1
4	13	20.61	20.38	20.86	0.514	0.184	0.9	0.540	0.193	0.9
5	13	20.75	20.50	20.99	0.395	0.141	0.7	0.437	0.156	0.8
6	13	19.91	19.60	20.11	0.377	0.135	0.7	0.481	0.172	0.9
7	13	20.34	19.88	20.54	0.419	0.150	0.7	0.632	0.226	1.1
8	13	19.61	18.59	19.78	0.261	0.093	0.5	0.911	0.325	1.7
9	13	20.28	18.58	20.72	0.348	0.124	0.6	1.498	0.535	2.6
10	13	19.97	19.70	21.29	0.077	0.028	0.1	1.138	0.406	2.0
11	13	20.68	19.72	21.01	0.295	0.105	0.5	0.920	0.329	1.6
12	13	25.26	24.21	25.77	0.456	0.163	0.6	1.157	0.413	1.6
13	13	19.65	18.53	19.91	0.242	0.086	0.4	0.993	0.355	1.8
						POS				
1	13	47.72	47.57	47.95	0.174	0.062	0.1	0.303	0.108	0.2
2	13	47.42	47.13	47.57	0.256	0.092	0.2	0.396	0.142	0.3
3	13	47.24	47.11	47.62	0.227	0.081	0.2	0.403	0.144	0.3
4	13	47.08	46.91	47.31	0.267	0.095	0.2	0.368	0.132	0.3
5	13	46.34	46.05	46.53	0.215	0.077	0.2	0.510	0.182	0.4
6	13	46.79	46.64	46.90	0.251	0.089	0.2	0.288	0.103	0.2
7	13	46.17	46.05	46.26	0.142	0.051	0.1	0.200	0.071	0.2
8	13	46.61	46.36	46.85	0.388	0.139	0.3	0.454	0.162	0.3
9	13	46.06	45.85	46.30	0.283	0.101	0.2	0.430	0.154	0.3
10	13	46.68	46.46	47.13	0.229	0.082	0.2	0.492	0.176	0.4
11	13	46.14	45.91	46.62	0.220	0.079	0.2	0.530	0.189	0.4
12	13	43.79	43.48	44.23	0.451	0.161	0.4	0.709	0.253	0.6
13	13	47.38	47.08	47.57	0.190	0.068	0.1	0.377	0.135	0.3
						SOS				
1	13	31.88	31.64	32.08	0.416	0.149	0.5	0.515	0.184	0.6
2	13	33.12	32.81	33.44	0.271	0.097	0.3	0.523	0.187	0.6
3	13	33.72	32.83	33.96	0.498	0.178	0.5	0.898	0.321	1.0
4	13	32.31	31.94	32.62	0.621	0.222	0.7	0.701	0.250	0.8
5	13	32.91	32.50	33.42	0.478	0.171	0.5	0.782	0.279	0.8
6	13	33.30	33.00	33.73	0.562	0.201	0.6	0.706	0.252	0.8
7	13	33.49	33.21	33.87	0.425	0.152	0.5	0.643	0.230	0.7
8	13	33.78	33.53	34.79	0.575	0.205	0.6	0.996	0.356	1.1
9	13	33.66	33.26	35.56	0.386	0.138	0.4	1.686	0.602	1.8
10	13	33.35	31.58	33.84	0.214	0.076	0.2	1.566	0.559	1.7
11	13	33.17	32.68	33.65	0.341	0.122	0.4	0.727	0.260	0.8
12	13	30.95	30.59	32.13	0.521	0.186	0.6	1.140	0.407	1.3
13	13	32.98	32.69	34.38	0.209	0.075	0.2	1.222	0.437	1.3

(1) no. of laboratories

Table C 15: Statistical evaluation of the results accepted on technical and statistical grounds (Results reported as g TG / 100 g total TGs (= POP+POS+SOS=100 %))

Sa.no.	p (1)	(2)	(3)	(4)	mean	r	s_r	RSD_r	R	s_R	RSD_R
						POP					
1	12	1	8	C	20.40	0.160	0.057	0.3	0.397	0.142	0.7
2	12	1	4	SG	19.49	0.194	0.069	0.4	0.227	0.081	0.4
3	12	1	6	SG	18.99	0.258	0.092	0.5	0.337	0.120	0.6
4	13	0			20.61	0.514	0.184	0.9	0.540	0.193	0.9
5	13	0			20.75	0.395	0.141	0.7	0.437	0.156	0.8
6	12	1	8	C	19.94	0.140	0.050	0.3	0.331	0.118	0.6
7	12	1	4	C	20.35	0.209	0.075	0.4	0.601	0.214	1.1
8	11	2	13; 4	SG; C	19.70	0.261	0.093	0.5	0.317	0.113	0.6
9	12	1	13	SG	20.42	0.322	0.115	0.6	0.441	0.157	0.8
10	12	1	13	SG	19.86	0.080	0.029	0.1	0.262	0.094	0.5
11	12	1	5	SG	20.76	0.306	0.109	0.5	0.459	0.164	0.8
12	11	2	7; 6	SG; C	25.37	0.358	0.128	0.5	0.741	0.265	1.0
13	12	1	7	SG	19.74	0.250	0.089	0.5	0.335	0.120	0.6
						POS					
1	12	1	8	C	47.73	0.157	0.056	0.1	0.302	0.108	0.2
2	12	1	4	SG	47.42	0.265	0.095	0.2	0.413	0.148	0.3
3	12	1	6	SG	47.21	0.236	0.084	0.2	0.253	0.090	0.2
4	13	0			47.08	0.267	0.095	0.2	0.368	0.132	0.3
5	13	0			46.34	0.215	0.077	0.2	0.510	0.182	0.4
6	12	1	8	C	46.80	0.196	0.070	0.1	0.254	0.091	0.2
7	12	1	4	C	46.18	0.148	0.053	0.1	0.183	0.066	0.1
8	11	2	13; 4	SG; C	46.62	0.177	0.063	0.1	0.399	0.142	0.3
9	12	1	13	SG	46.08	0.293	0.105	0.2	0.414	0.148	0.3
10	12	1	13	SG	46.65	0.233	0.083	0.2	0.330	0.118	0.3
11	12	1	5	SG	46.10	0.221	0.079	0.2	0.351	0.125	0.3
12	11	2	7; 6	SG; C	43.76	0.282	0.101	0.2	0.588	0.210	0.5
13	12	1	7	SG	47.40	0.195	0.070	0.1	0.298	0.106	0.2
						SOS					
1	12	1	8	C	31.87	0.261	0.093	0.3	0.473	0.169	0.5
2	12	1	4	SG	33.09	0.271	0.097	0.3	0.464	0.166	0.5
3	12	1	6	SG	33.80	0.430	0.154	0.5	0.471	0.168	0.5
4	13	0			32.31	0.621	0.222	0.7	0.701	0.250	0.8
5	13	0			32.91	0.478	0.171	0.5	0.782	0.279	0.8
6	12	1	8	C	33.27	0.230	0.082	0.2	0.506	0.181	0.5
7	12	1	4	C	33.48	0.209	0.075	0.2	0.596	0.213	0.6
8	11	2	13; 4	SG; C	33.68	0.383	0.137	0.4	0.417	0.149	0.4
9	12	1	13	SG	33.50	0.377	0.134	0.4	0.549	0.196	0.6
10	12	1	13	SG	33.50	0.217	0.077	0.2	0.517	0.185	0.6
11	12	1	5	SG	33.14	0.354	0.126	0.4	0.633	0.226	0.7
12	11	2	7; 6	SG; C	30.87	0.503	0.180	0.6	0.553	0.198	0.6
13	12	1	7	SG	32.86	0.208	0.074	0.2	0.310	0.111	0.3

(1) no. of accepted laboratories
(2) no. of outliers removed
(3) Identity of outlying laboratories
(4) Reason for removal (C = Cochran, SG = Single Grubbs)

15

LA-NA-20685-EN-C

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ISBN 92-894-5510-1

9 789289 455107

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