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Collaborative Study for the Determination of 3-MCPD-Fatty Acid Esters in Edible Fats and Oils

Second Collaborative Study - Part I
Method Validation and Proficiency Test

Imprint

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1 Summary

With the objective to provide a method for the determination of 3-MCPD fatty acid esters in edible fats and oils, a collaborative study involving 40 laboratories was organized by Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung – BfR).

To begin with, three analytical methods, developed and validated in-house at BfR, were transmitted to the participating laboratories thus allowing familiarisation with the methods. Then, two series of samples containing 5 samples each, were dispatched which had to be analyzed on different days by applying either one of the three BfR methods or an in-house method of their own. Analytical results were obtained from 36 laboratories some of which had carried out sample analysis using more than one method.

Evaluation of the 27 datasets which were obtained by using one of the BfR methods (BfR Method 9) shows that reproducible results were obtained with this method. Thus the target to validate a method for the detection of 3-MCPD fatty acid esters was achieved.

While reproducible results were also obtained with another BfR method (BfR Method 8), a higher level of uncertainty must be taken into account in this case because only six datasets (of test results) were available.

As to the overall evaluation of the proficiency test which was based on a total of 48 datasets, it appeared that more than 90 % of the laboratories reached a z-score lower than or equal to two for all five samples.

2 Introduction

2.1 Background

In 1978, 3-chloropropane-1,2-diol (3-MCPD) was identified as a contaminant resulting from food manufacturing and processing (BfR 2003a). For a number of years already, 3-MCPD fatty acid esters have been known to occur in food (Svejkovska et al. 2004; Divinonva et al. 2004; Zelinkova et al. 2006). In samples analyzed at the end of 2007 by the Official Food Control Laboratory of the German Federal State Baden-Wuerttemberg high levels of 3-MCPD fatty acid esters were detected in refined edible fats such as margarine and oil and in fat-containing foods including infant formula and follow-up formula (BfR 2007a). These levels had been obtained using the method published by R. Weißhaar. This method relies on the release of 3-MCPD from fatty acid esters after alkaline hydrolysis (Weißhaar 2008).

So far, no toxicologically relevant data have been available to assess a possible health risk arising from the identified levels of 3-MCPD fatty acid esters. Therefore, opinions on the potential impact on health are based on the risk assessment results for free 3-MCPD (BfR 2007b). In animal experiments, free 3-MCPD led to hyperplasias on the renal tubules and, at higher levels, induced benign tumours. Both the Scientific Committee on Food (SCF) of the European Commission (EC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued assessments of a possible health risk from the substance 3-MCPD. Both committees established a tolerable daily intake (TDI) of 2 µg of 3-MCPD per kilogram body weight for humans (SCF 2001, JECFA 2002).

In December 2007, BfR was mandated by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) to set up the Working Group *"AG Analytik zur Bestimmung von 3-MCPD-Fettsäureestern in raffinierten Fetten und fetthaltigen Lebensmitteln"* (Analysis of 3-MCPD Fatty Acid Esters in Refined Edible Fats and Fat-containing Foods) and to validate an analytical method for the determination of 3-MCPD fatty acid esters (First Collaborative Study – Part I: Edible Fats and Oils). The validation study which was subsequently organized was based on the Weißhaar method.

The collaborative study showed that additional 3-MCPD was formed during analysis in some samples which had been analyzed by methods using NaCl. As a result, excessive 3-MCPD concentrations were found in these samples. It was concluded that the use of these methods leads to a sum parameter from 3-MCPD and 3-MCPD forming substances.

According to the present state of knowledge the noted 3-MCPD forming substances are considered to be fatty acid esters of glycidol which, in the presence of chloride ions, are transformed into 3-MCPD during the process of alkaline catalyzed alcoholysis of fatty acids. The method used in the validation study was published as a DGF Standard Method (C-III 18 (09)) for the determination of 3-MCPD fatty acid esters and 3-MCPD forming substances in March 2009. From the point of view of risk assessment the determination of a sum parameter is not sufficient due to the toxicological potential which is different in glycidol and 3-MCPD.

Therefore, the BfR was requested by the Working Group "*AG Analytik zur Bestimmung von 3-MCPD-Fettsäureestern in Speisefetten und fetthaltigen Lebensmitteln*" to develop an analytical method for the exclusive determination of 3-MCPD fatty acid esters and subsequently validate the method by means of a second collaborative study.

2.2 Scope of the Study

The study aims to validate an analytical method for the specific determination of 3-MCPD fatty acid esters in edible fats and oils.

In order to test the applied methods for systematic errors that might occur, the study allowed the participants to use an in-house method of their own in addition or alternatively to one of the three BfR methods validated in-house (BfR Method 8, BfR Method 9 and BfR Method 10). It was necessary to adopt such a concept since certified reference material with defined concentrations of 3-MCPD fatty acid esters is not available and all methods are based on indirect determination. In addition, it was planned to evaluate the total of the analytical results in a proficiency test.

3 Concept

3.1 Participants

The members of the Working Group "*AG Analytik"* and the participants of the First Collaborative Study for the Determination of 3-MCPD Fatty Acid Esters in Edible Oils and Fats – Part I were invited to take part in the collaborative study. The invited laboratories were given the possibility to extend the invitation to other laboratories likely to take an interest in the study. Participants were not pre-selected and participation was free of charge. A total of forty laboratories took part in the study. A list of the participating laboratories is provided in Annex 3.

3.2 Procedure

All methods provide indirect evidence of 3-MCPD fatty acid esters after hydrolysis of the esters and release of 3-MCPD. The released 3-MCPD is derivatized and quantified by means of gas chromatography-mass spectrometry (GC-MS) and an internal standardisation.

In June 2009, three analytical methods for the determination of 3-MCPD fatty acid esters were transmitted to the participants. These BfR methods had been further developed and validated in-house on the basis of suggestions of the Working Group „Analytik". They mainly differ in hydrolysis of the esters and in derivatization. To ensure the laboratories' own control, they were also provided with a solid fat sample of known 3-MCPD concentration. The method descriptions are given in Annexes 9 – 11.

3.2.1 BfR Method 8

Determination of 3-MCPD fatty acid esters in edible oils and solid fats by means of GC-MS. Indirect determination by acid hydrolysis of the esters into free 3-MCPD. Derivatization with phenylboronic acid.

Principle of method:
The fat sample is dissolved in t-BME and an internal standard (d_5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by acid hydrolysis with methanol and sulphuric acid. As a result, fatty acids and free 3-MCPD are formed. The reaction is stopped with a saturated sodium hydrogen carbonate solution. The sample is defatted with isohexane and subsequently the released 3-MCPD is derivatized with phenylboronic acid. After extraction of the derivatives with cyclohexane, the sample is evaporated to complete dryness, dissolved in isooctane and an aliquot is analyzed by means of GC-MS.

3.2.2 BfR Method 9

Determination of 3-MCPD fatty acid esters in edible oils and solid fats by means of GC-MS. Indirect determination by alkaline hydrolysis of the esters into free 3-MCPD. Derivatization with phenylboronic acid.

Principle of method:
The fat sample is dissolved in t-BME and an internal standard (d_5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by alkaline hydrolysis with a sodium methylate solution. As a result, fatty acid methyl esters and free 3-MCPD are formed. The reaction is stopped with a solution of ammonium sulphate and sulphuric acid. The sample is defatted with isohexane and, subsequently, the released 3-MCPD is extracted with ethyl acetate, de-

rivatized with phenylboronic acid and dried. The residue is dissolved in acetone and an aliquot is taken for analysis by GC-MS.

3.2.3 BfR Method 10

Determination of 3-MCPD fatty acid esters in edible oils and solid fats by means of GC-MS. Indirect determination by alkaline hydrolysis of the esters into free 3-MCPD. Derivatization with heptafluorobutyric anhydride.

Principle of method:
The fat sample is dissolved in t-BME and an internal standard (d_5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by alkaline hydrolysis with a sodium methylate solution. As a result, fatty acid methyl esters and free 3-MCPD are formed. The reaction is stopped with a solution of ammonium sulphate and sulphuric acid. The sample is defatted with isohexane and, subsequently, the released 3-MCPD is extracted with ethyl acetate, dried and derivatized with HFBA. An aliquot of the organic phase is analyzed by GC-MS.

3.3 Execution of the Study

The participants had six weeks to get familiar with the analytical methods. Before the dispatch of the samples for the proper trial, the participants were asked to report back the experiences made with the methods so far; subsequently, their annotations and questions were taken into account and corresponding comments were provided in the covering letter when the samples were dispatched. Necessary modifications and recommendations were integrated into the second version of the methods (Annexes 9–11). Apart from the BfR methods the participants had the possibility to use in-house methods of their own, alternatively.

Each laboratory received two series of samples containing 5 samples each which had to be analyzed on two different days. The participants did not know that the samples of the series were identical (fully-nested trial). Threefold determination had to be carried out on each sample. Quality control was based on a sample of known 3-MCPD concentration.

3.4 Time frame

The study was conducted within the following time frame:

• 2nd Meeting of the Working Group *"AG Analytik zur Bestimmung von 3-MCPD-Fettsäureestern in raffinierten Fetten und fetthaltigen Lebensmitteln"*	November 2008
• Development of three analytical BfR methods	January – April 2009
• Laboratories were invited to take part in the study	April 2009
• Three analytical BfR methods and a control sample with known 3-MCPD concentration were transmitted to the participants, familiarization with the methods	June 2009
• Participants were questioned on their experience made with the analytical methods and could make suggestions for modifications	June 2009
• Purchase, selection and preparation of samples	May 2009
• Aliquotation of samples	June 2009
• Homogeneity testing of the sample material, dispatch of samples	July 2009
• Return of the analytical results	September 2009
• Statistical evaluation of the analytical results	October – December 2009
• Stability testing of the samples	July 2009 – July 2010

Test results received from one laboratory in December 2009 were too late to be included in the evaluation.

4 Sample material

4.1 Preparation of sample material

In May 2009, twenty edible oils and frying fats were bought from retailers for the preparation of sample material. Oil and fat samples were analyzed repeatedly by different operators using various test methods. Depending on the required concentration range, five of the oils/fats were selected for analysis.

Tabel 1: Sample designation, ingredients and 3-MCPD concentrations of the dispatched reference materials

BfR sample name	Trade name	Indicated ingredients	3-MCPD concentration (mg/kg)*
L_OEL	Plant Oil	Sunflower oil, rape seed oil, vitamin E	0.16
B_FETT	Vegetable Fat	Vegetable fats and oils, air	0.98
F_OEL	Deep Frying / Frying/ Broiling Oil	Vegetable fats, non-hydrogenated	1.69
P_FETT	Vegetable Fat	Vegetable fats, vegetable oils, vegetable oil, hydrogenated	3.15
T_OEL	Grape seed oil	Grape seed oil	3.92

*Mean value of homogeneity determination by BfR

From the oils/fats selected for testing 3 kg each of the same batch were given into a beaker. Solid fat samples were melted in a water bath. The samples were homogenized under nitrogen for 20 minutes using a magnet stirrer. Using a multichannel pipette, sample amounts of 6 mL, respectively were placed into 93 amber glass vessels with screw caps. The residual amount of material was stored in portions of 90 mL in amber glass bottles (100 mL). The aliquots were labelled with a sample number and kept under argon. Before and after aliquotation, the material was stored at 4°C.

4.2 Homogeneity testing

In order to test homogeneity, threefold determination was carried out on every tenth sample portion. The 3-MCPD concentration was used as a homogeneity parameter.

The measuring results were evaluated by one-way analysis of variance (**An**alyis **o**f **Va**riance - ANOVA). At a significance level of 5 %, all samples were found to be homogenous and suitable as test material in the study. Both the analytical results from homogeneity testing and the calculations are presented in Annex 2.

4.3 Stability testing

Considering possible influencing factors and storage conditions, a test protocol was established in parallel with sample aliquotation to control the stability of the samples. Over the duration of the study, five sample aliquots, respectively were stored at 4°C and at room temperature. Threefold determinations of the sample aliquots were planned to be performed successively at intervals of four weeks, two, six and 12 months after dispatch of the samples. For the statistical evaluation of the stability of the samples, so far, the analytical results from homogeneity testing have been available as well as those obtained from testing after one,

two and six and twelve months of storage. The analytical results obtained provide evidence of the stability of the samples.

5 Dispatch of samples and return of analytical results

5.1 Laboratory code

Each laboratory received a laboratory code “LC00XX“. Laboratories which took part in the study using more than one analytical method received an additional number (see Annex 4, Table 21).

5.2 Sample treatment, covering documents and return of results

In July 2009, samples and covering documents were dispatched to the laboratories (0). All samples were received two days after dispatch at the latest by the participants. They were asked to transmit the test results (the analytical results and the respective sample amounts) via file templates of “Prolab“-software, version 2.11. Additionally, each participant received an Excel file for the purpose of providing annotations on the respective analytical method. When a BfR method was used, participants were asked to report modifications of analytical parameters or, in case an in-house method of their own was used, to indicate the analytical parameters.

6 Results

6.1 Results returned

Results were received from a total of 36 laboratories. Some laboratories analyzed the samples using more than one method (see Annex 4, Table 21). Thus, evaluation could be based on 48 datasets obtained from 36 laboratories. An overview of the number of datasets received from the participants and of the analytical methods applied is given in Table 2.

Table 2: Number of datasets received for the respective analytical method

Analytical method used	Number of datasets
BfR Method 8	6
BfR Method 9	27
BfR Method 10	6
In-house method	9

The datasets were used for both method validation and for evaluation within the scope of proficiency testing.

6.2 Method validation

According to DIN ISO 5725-1, the reliability of a statement concerning the precision of a method under investigation within the scope of a method validation, depends on both the number of participating laboratories and the number of repeated measurement. Consequently, due to 27 datasets received for “BfR Method 9“, good estimates of precision which are sufficient may be expected for the standard deviation of repeatability and reproducibility. BfR Method 8 and BfR Method 10 were applied by 6 laboratories. The respective number of datasets received is not sufficient to make reliable statements on the precision of the method. Statistical values relating to Method 8 are given below. Data of precision relating to BfR Method 10 have been omitted, because results from two laboratories highly deviated from those obtained from the other laboratories.

By way of a questionnaire, all laboratories were requested to specify conditions of analysis in order to allow to identify and to evaluate modifications of the described BfR method instructions. As to GC-MS measurements, for example, modified temperatures as well as the use of a backflush unit were accepted. Also accepted were the use of different solvents for resolution and increase of the number of extraction steps. Hydrolysis of esters was modified by one laboratory in a way which did not conform to the conditions of analysis of “BfR Method 9” and, subsequently, this dataset was evaluated as an in-house method of the laboratory.

6.2.1 Evaluation procedure

Evaluation was carried out according to DIN ISO 5725-2 and DIN ISO 5725-3 as a fully-nested experiment. The fact that not all participating laboratories provided three analytical results for each sample material as required was taken into account for the calculation of the statistical values. The sample material used was oils and fats bought from retailers for which no certified 3-MCPD concentrations were available. The calculated mean value of the laboratories was used as reference value for statistical evaluation after exclusion of outliers. The individual results are shown in Annex 4, Tables 22-28.

The analytical results received from the laboratories were tested for outliers by means of numerical tests and graphical techniques at a significance level of 1 %. For each sample

material, the six individual results of each laboratory were tested for single outliers within the laboratory by means of the Grubbs' test. Values identified as outliers were excluded. Subsequently, the standard deviations and mean values of the laboratories were examined. Based on results from the Cochran test and Mandel's *k* statistics, the standard deviation of one laboratory was eliminated as an outlier, because it proved to be significantly different from the standard deviations of the other laboratories. Laboratory mean values were tested for deviations from the total mean value of all laboratories using Grubbs' test and Mandel's *h* statistics. Laboratory mean values which, in both tests, showed significant deviations from the total mean value of all laboratories were also excluded.

6.2.2 Method validation BfR Method 9

The test results of the laboratories were subject to the outlier tests mentioned under 6.2.1 which were performed with numerical tests and graphical techniques.

The consistency values (*k* and *h* statistics) are illustrated by graphical representations, in Fig. 7 and 8 (Annex 4). The yellow line represents the critical value at a significance level of 5 % while the red line represents the critical limit at the 1 % significance level. The critical limit was determined under the assumption that all laboratories had provided three test results for each sample material in both series (day 1 and day 2).

The *h* values established for Laboratory LC0032 on the basis of test results from four out of five samples (B_Fett, P_Fett, F_Oel, T_Oel) as well as from the control sample exceeded the critical value of Mandel's *h* statistics. Additionally, the mean values of this laboratory were identified as outliers in the Grubbs' test. The assumption that these findings were due to difficulties encountered during the analytical procedure was confirmed later by the laboratory. Therefore, the test results of this laboratory were excluded from further evaluation. Laboratories with test results classified as outliers are shown in Table 3.

Table 3: Laboratories with test results identified as outliers (BfR Method 9)

Material	Numerical tests			Graphical testing	
	Grubbs' A	Cochan	Grubbs' B	h statistics	k statistics
L_Oel	-	LC0020 LC0010	-	-	LC0020 LC0010
B_Fett	LC0021	LC0005	-	-	LC0005
F_Oel	-	LC0007	-	-	LC0007
P_Fett	-	LC0202	-	-	LC0202
T_Oel	LC0039	LC0038	-	-	LC0038

In two cases (Laboratories LC0021 and LC0039), the Grubbs' test revealed one individual value each deviating extremely from the mean value within the laboratory in samples "B_Fett" and "T_Oel", respectively. These values were excluded. On the basis of the adjusted data (after exclusion of outliers) the mean values of the laboratories and the measures of precision, i.e. repeatability standard deviation (s_r) and reproducibility standard deviation (s_R) as well as the Horwitz standard deviation (sH) were calculated for the respective sample materials (levels). Under the experimental conditions of a nested trial chosen in this case, it was possible to determine simultaneously the influence of the time interval between the analyses as an additional measure of precision for the intermediate condition (s_Z). The results are shown in Table 4.

Table 4: Statistical values for all sample materials (BfR Method 9)

	L_Oel	B_Fett	F_Oel	P_Fett	T_Oel	Cont
Mean value of 3-MCPD [mg/kg]	**0.30**	**0.91**	**1.72**	**3.46**	**4.04**	**2.96****
Rel. SD according to Horwitz (rel. sH) [%]	19.17	16.23	14.74	13.27	12.96	13.59
Reproducibility SD (s_R) [mg/kg]	0.17	0.21	0.28	0.55	0.62	0.38
Rel. reproducibility SD (rel. s_R) [%]	55.22	22.87	16.38	15.77	15.26	12.82
Repeatability SD (s_r) [mg/kg]	0.05	0.10	0.14	0.25	0.28	0.18
Rel. repeatability SD (rel. s_r) [%]	16.23	11.43	8.08	7.37	6.85	6.13
Intermediate SD (s_Z) [mg/kg]	0.09	0.11	0.20	0.34	0.37	0.38
Rel. intermediate SD (rel. s_Z) [%]	31.52	12.52	11.52	9.82	9.11	12.82
Number of datasets (after elimination of outliers)	19	25	25	25	25	23
Total of datasets	27***	27	27	27	27	27
Number of outliers****	2	1	1	1	1	3
Ratio sr/sR	0.29	0.50	0.49	0.47	0.45	0.48
HorRat	2.9	1.4	1.1	1.2	1.2	0.9

** The concentration of the control sample was previously specified to be 3.0 ± 0.5 mg/kg.
*** Five laboratories stated values as "<LOD" or "<LOQ".
**** One data set was completely excluded from evaluation due to the laboratory's systematic deviation from the method.

The 3-MCPD concentration of sample L_Oel was found to be within the range of the quantification limit of the method which explains the high relative reproducibility standard deviation of 55.2 %. Within a concentration range from 0.9 mg/kg to 4.0 mg/kg relative reproducibility standard deviations between 22.9 % and 15.3 % were obtained which is an indication of a good reproducibility of the method. The relative reproducibility standard deviation as well as both the relative repeatability standard deviation and the relative intermediate standard deviation decrease with increasing 3-MCPD concentrations.

The criterion generally used for the evaluation of methods of chemical analysis is the HorRat value which is considered to be independent of the analyte, method and matrix. The HorRat value is the quotient from the reproducibility standard deviation and the Horwitz standard deviation. Results from a collaborative trial performed to test a method are accepted, if the HorRat values obtained range between 0.5 and 2.0. At concentrations within the range of the quantification limit the HorRat value of 2 can be exceeded (Horwitz und Albert 2006). The HorRat values calculated for BfR Method 9 were within a range between 1.1 and 1.4. An exception to this was sample L_Oel with a 3-MCPD concentration close to the method's quantification limit.

The values of precision give evidence of a good reproducibility of the method.

6.2.3 Method validation BfR Method 8

BfR Method 8 was applied by six laboratories. Due to the small number of laboratories, the calculated measures of precision, i.e. repeatability and reproducibility, obtained with this method show a higher degree of uncertainty than results obtained with "BfR Method 9". The statistical values were established as described under 6.2.1. Table 5 gives an overview of the laboratories whose results were identified as outliers.

Table 5: Laboratories with test results classified as outliers (BfR Method 8)

Material	Numerical tests			Graphical testing	
	Grubbs' A	Cochran	Grubbs' B	*h* statistics	*k* statistics
L_Oel	-	-	-	-	-
B_Fett	-	-	-	-	-
F_Oel	LC0019	-	-	-	-
P_Fett	LC0102	LC0029	-	-	LC0029
T_Oel	-	-	-	-	-

Table 6 presents the statistical values for the respective sample materials adjusted after exclusion of outliers (for BfR Method 8). Although quantified test results for sample material L_Oel had been provided only by four laboratories, the corresponding statistical values are quoted for the sake of completeness. The relative reproducibility standard deviations were calculated to be within a range from 6.2 % to 27.4 %.

Table 6: Statistical values for all sample materials (BfR Method 8)

	L_Oel	B_Fett	F_Oel	P_Fett	T_Oel	Cont
Mean value of 3-MCPD [mg/kg]	**(0.26)**	**0.83**	**1.49**	**3.06**	**3.51**	**2.71***
Rel. SD according to Horwitz (rel. sH) [%]	(19.63)	16.46	15.07	13.52	13.24	13.77
Reproducibility SD (s_R) [mg/kg]	(0.12)	0.23	0.23	0.19	0.37	0.46
Rel. reproducibility SD (rel. s_R) [%]	(45.04)	27.43	15.25	6.23	10.52	17.11
Repeatability SD (s_r) [mg/kg]	(0.03)	0.12	0.15	0.17	0.31	0.46
Rel. repeatability SD (rel. s_r) [%]	(11.11)	14.74	9.97	5.54	8.88	17.11
Intermediate SD (s_Z) [mg/kg]	(0.04)	0.14	0.17	0.19	0.33	0.46
Rel. intermediate SD (rel. s_Z) [%]	(16.73)	17.04	11.36	6.23	9.28	17.11
Number of datasets (after elimination of outliers)	(4)	6	6	5	6	6
Total of datasets	(6**)	6	6	6	6	6
Number of outliers				1		
Ratio sr/sR	(0.25)	0.54	0.65	0.89	0.84	1.00
HorRat	(2.3)	1.7	1.0	0.5	0.8	1.2

* The concentration of the control sample was previously specified to be 3.0 ± 0.5 mg/kg.
**Two laboratories stated values as “<LOD“ or “<LOQ“.

6.3 Proficiency testing

Both the test results obtained by using the BfR Methods 8, 9 and 10 and the results obtained using own in-house methods were subject to proficiency testing (PT) according to ISO 13528, DIN ISO 5725-2 und DIN ISO 5725-3. To ensure comparison of the calculated values with those relating to BfR Method 9, robust method of data analysis was decided to be abandoned. To evaluate the performance of the laboratories, z-scores were used.

6.3.1 Procedure of evaluation

Outliers were identifed and excluded as described under 6.2.1. In accordance with DIN ISO 5725-2 and DIN ISO 5725-3, the arithmetic mean value was calculated as assigned value and the reproducibility standard deviation as target standard deviation from the mean values of the laboratories adjusted after exclusion of outliers. The different number of individual values for each laboratory was taken into account when calculating the repeatability and reproducibility standard deviation.

6.3.2 Results from proficiency testing

The single test results of all laboratories are summarized in Table 22–28, Annex 4.
Laboratory LC0032 was not included in the statistical calculation of the mean values and reproducibility standard deviations (see 6.2.2).

The laboratories with test results classified as outliers in the proficiency test are shown in Table 7. The result obtained by laboratory LC0020 for the L_Oel sample was identified as an outlier according to the Cochran test. Although the identification as an outlier was not confirmed by Mandel’s *k* statistics, the value was eliminated due to the high deviation between the subsamples A and B (analyzed on two different days).

Table 7: Laboratories with test results identified as outliers in the proficiency test after numerical testing and confirmation by graphical techniques

Material	Numerical tests			Graphical testing	
	Grubbs' A	Cochran	Grubbs' B	*h* statistics	*h* statistics
L_Oel	LC0302	LC0432 LC0020		LC0432	LC0432 LC0302
B_Fett	LC0021	LC0005 LC0015		LC0020	LC0005 LC0015 LC0021
F_Oel	LC0019 LC0432	LC0302			LC0302 LC0432
P_Fett	LC0102	LC0202 LC0302			LC0202 LC0302
T_Oel	LC0039	LC0038 LC0302 LC0015			LC0038 LC0015 LC0302

The statistical values underlying in the evaluation of the laboratory results are shown in Table 8.

As the evaluation within the scope of method validation has shown, sample material L_Oel has a high relative reproducibility standard deviation of 55.7 % and consequently a high HorRat value of 2.9 (see 6.2.2). This sample material has a 3-MCPD concentration close to the quantification limit. The relative reproducibility standard deviations for the other materials were found to be between 22.6 % and 14.4 % which corresponds to a range accepted for precision between different laboratories. They correspond approximately to the values that can theoretically be expected according to Horwitz which is confirmed by the HorRat values calculated to range between 1.4 and 1.1.

Based on the mean values and reproducibility standard deviations quoted in Table 8, z-scores were established using the following formula:

$$z = \frac{MV_L - X}{s}$$

Z z-score
MV_L Mean value of the laboratory
X Reference value (here: Mean value of all laboratories, adjusted after elimination of outliers)
s Target standard deviation (here: reproducibility standard deviation)

Table 8: Statistical values obtained in the proficiency test calculated on the basis of results from all laboratories, adjusted after exclusion of outliers

	L_Oel	B_Fett	F_Oel	P_Fett	T_Oel	Cont
Mean value of 3-MCPD [mg/kg]	0.30	0.87	1.69	3.39	3.90	2.88*
Rel. SD according to Horwitz (rel. sH) [%]	19.17	16.33	14.78	13.31	13.03	13.64
Reproducibility SD (s_R) [mg/kg]	0.167	0.197	0.288	0.503	0.561	0.351
Rel. reproducibility SD (rel. s_R) [%]	55.73	22.59	16.99	14.84	14.37	12.17
Repeatability SD (s_r) [mg/kg]	0.046	0.106	0.151	0.268	0.266	0.215
Rel. repeatability SD (rel. s_r) [%]	15.32	12.19	8.91	7.89	6.80	7.45
Intermediate SD (s_Z) [mg/kg]	0.098	0.115	0.199	0.320	0.341	0.215
Rel. intermediate SD (rel. s_Z) [%]	32.56	13.21	11.73	9.42	8.74	7.45
Number of datasets (after elimination of outliers)	37	45	46	45	44	44
Total of datasets	48**	48	48	48	48	48
Number of outliers***	2	2	1	2	3	3
HorRat	2.9	1.4	1.2	1.1	1.1	0.9

* The 3-MCPD concentration of the control sample was previously specified to be 3.0 ± 0.5 mg/kg.
** Eight laboratories stated values as "<LOD" or "<LOQ".
***One dataset was completely excluded from evaluation due to the laboratory's systematic deviation from the method.

The calculated z-scores are given in Table 29, Annex 4, tories had provided test results obtained with more than one method, z-scores were calculated for each dataset. The results relating to the respective method were evaluated as individual test results. Laboratories which gave indications such as “<LOD“ or “<LOQ“ for samples were not evaluated separately.

A z-score of < |2| was reached by 41 laboratories (85%) for all samples. This z-score of |2| was exceeded by five laboratories in the case of one material out of five. For four out of these five laboratories the z-score ranged between |2| and |3|, while for one laboratory a z-score of > |3| was calculated. One laboratory reached a z-score of |2| < z < |3| for two materials out of five.

The number of datasets (n) in the respective z-score ranges and their relative portions of the total of data sets are shown in Table 9.

Table 9: Summary of results from proficiency testing

	L_Oel		B_Fett		F_Oel		P_Fett		T_Oel	
	n	rel. portion (%)	n	rel. portion (%)	n	rel. portion (%)	n	rel. portion (%)	n	rel. portion (%)
Total	40		48		48		48		48	
|z| ≤ 2	36	90	46	96	47	98	47	98	45	94
2 < |z| < 3	3	8	2	4	0	0	1	2	1	2
|z| ≥ 3	1	2	0	0	1	2	0	0	2	4

Fig.13 to Fig.17, Annex 4, raphical representation of the z-scores, classified by samples. Fig. 18, Annex 4, summarizes the z-scores of all samples.

6.4 Summary of results for BfR Method 8, BfR Method 9 and the proficiency test

Table 10 summarizes the statistical values relating to BfR Method 8 and BfR Method 9 as well as to proficiency testing. The results of BfR Method 9 and the results from proficiency testing give evidence of conformity of the statistical values of all materials. As to the control material with a known 3-MCPD concentration, reproducibility standard deviations were calculated to be slightly lower than those calculated for the other materials. When using BfR Method 8, lower 3-MCPD concentrations were found, though not significantly lower than those determined by using the other methods.

Table 10: Results for BfR Method 8, BfR Method 9 and proficiency testing

Sample	L_OEL	B_FETT	F_OEL	P_FETT	T_OEL	CONT
All methods						
Mean value [mg/kg]	0.30	0.87	1.69	3.39	3.90	2.88
Rel. SD according to Horwitz (rel. sH) [%]	19.17	16.33	14.78	13.31	13.03	13.64
Rel. repeatability SD (rel. s_r) [%]	55.73	22.59	16.99	14.84	14.37	12.17
Rel. reproducibility SD (rel s_R) [%]	15.32	12.19	8.91	7.89	6.80	7.45
Number of datasets after exclusion of outliers	37	45	46	45	44	44
HorRat	2.9	1.4	1.2	1.1	1.1	0.9
BfR Method 9						
Mean value [mg/kg]	0.30	0.91	1.72	3.46	4.04	2.96
Rel. SD according to Horwitz (rel. sH) [%]	19.17	16.23	14.74	13.27	12.96	13.59
Rel. repeatability SD (rel. s_r) [%]	55.22	22.87	16.38	15.77	15.26	12.82
Rel. reproducibility SD (rel s_R) [%]	16.23	11.43	8.08	7.37	6.85	6.13
Number of datasets after exclusion of outliers	19	25	25	25	25	23
HorRat	2.9	1.4	1.1	1.2	1.2	0.9
BfR Method 8						
Mean value [mg/kg]	(0.26)	0.83	1.49	3.06	3.51	2.71
Rel. SD according to Horwitz (rel. sH) [%]	(19.63)	16.46	15.07	13.52	13.24	13.77
Rel. repeatability SD (rel. s_r) [%]	(45.04)	27.43	15.25	6.23	10.52	17.11
Rel. reproducibility SD (rel s_R) [%]	(11.11)	14.74	9.97	5.54	8.88	17.11
Number of datasets after exclusion of outliers	(4)	6	6	5	6	6
HorRat	(2.3)	1.7	1.0	0.5	0.8	1.2

The mean values and the reproducibility standard deviations of the different sample materials obtained after evaluation are given in Fig. 1.

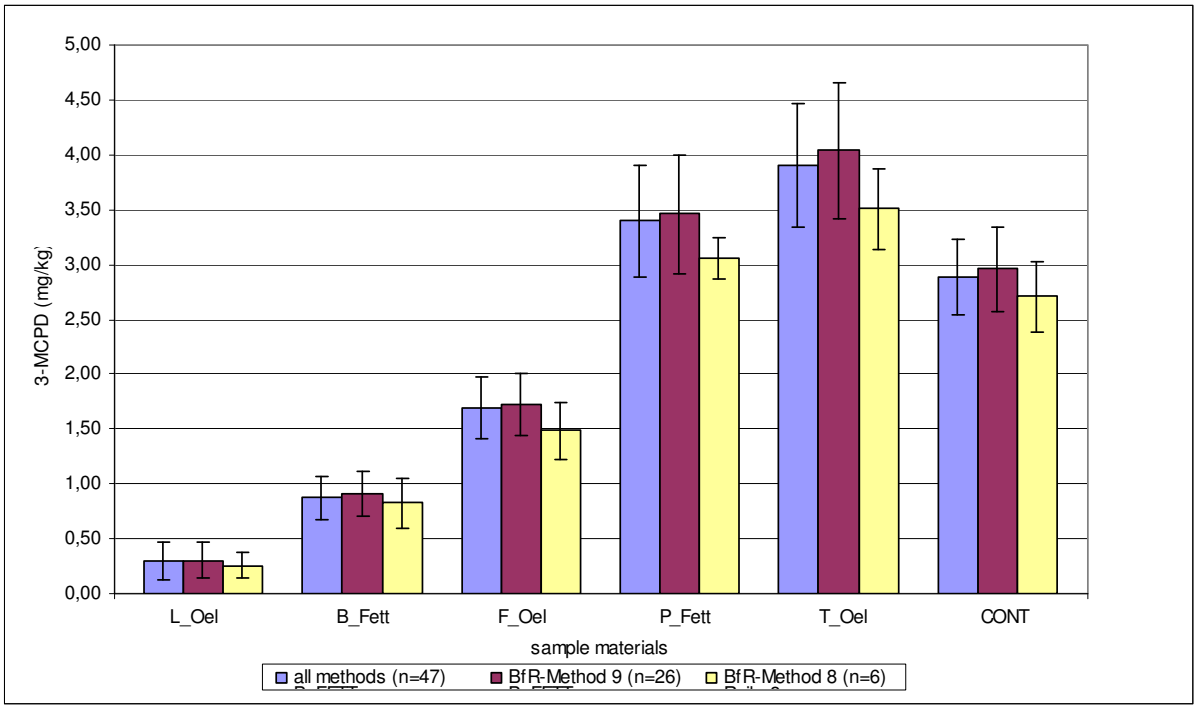

Figure 1: Mean values and reproducibility standard deviations of the different sample materials obtained for BfR Method 8, „BfR Method 9“ and the proficiency test (all methods)

7 Annex

7.1 Bibliography

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7.2 Results obtained from homogeneity testing of the sample material

Sample L_Oel

Table 11: 3-MCPD Measuring results obtained from homogeneity testing of sample L_Oel in sample aliquots

3-MCPD measuring results (mg/kg)						
Sample number	Value 1	Value 2	Value 3	MV (mg/kg)	SD	CV (%)
L_OEL_10	0.20	0.16	0.18	**0.18**	**0.02**	**12.87**
L_OEL_20	0.15	0.19	0.14	**0.16**	**0.03**	**17.50**
L_OEL_30	0.17	0.15	0.16	**0.16**	**0.01**	**7.14**
L_OEL_40	0.13	0.18	0.16	**0.16**	**0.03**	**16.15**
L_OEL_50	0.14	0,12	0.15	**0.14**	**0.01**	**8.28**
L_OEL_60	0,15	0.20	0.14	**0.16**	**0.03**	**18.19**
L_OEL_70	0.14	0.15	0.14	**0.14**	**0.00**	**2.69**
L_OEL_80	0.15	0.16	0.17	**0.16**	**0.01**	**4.49**
L_OEL_90	0.19	0.17	0.10	**0.15**	**0.05**	**32.19**
				MV	SD	CV (%)
Total (all samples)				**0.157**	**0.024**	**15.41**

Table 12: Results of one-way analysis of variance based on analytical results given in Table

ANOVA						
Source of variation	*Sum of squares (SS)*	*Degrees of freedom (df)*	*Mean square (MS)*	*Test static (F)*	*P-Value*	*Critical value F*
Differences between the groups	0.004	8	0.00050	0.81	0,60	2,51
Within the groups	0.011	18	0.00062			
Total	0.015	26				

0,81 < 2.51	**Null hypothesis is true, samples are homogeneous**

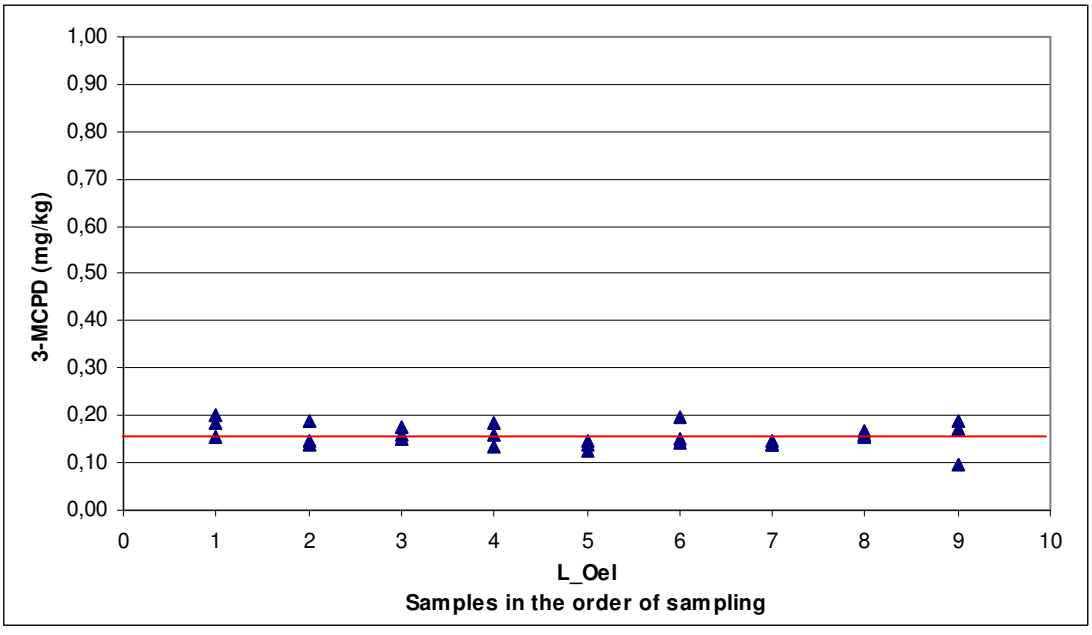

Figure 2: Graphical plot of threefold determinations of L_Oel samples for homogeneity testing

Sample B_Fett

Table 13: 3-MCPD measuring results obtained from homogeneity testing of sample B_Fett in sample aliquots

3-MCPD measuring results (mg/kg)						
Sample number	Value 1	Value 2	Value 3	MV (mg/kg)	SD	CV (%)
B_Fett_10	0.89	1.02	0.92	**0.94**	**0.07**	**7.38**
B_Fett_20	0.93	1.04	0.95	**0.97**	**0.06**	**6.28**
B_Fett_30	1.07	1.03	1.05	**1.05**	**0.02**	**2.01**
B_Fett_40	0.96	0.97	0.96	**0.96**	**0.00**	**0.16**
B_Fett_50	0.95	1.09	1.02	**1.02**	**0.07**	**7.12**
B_Fett_60	0.97	0.96	0.88	**0.94**	**0.05**	**5.63**
B_Fett_70	1.08	0.97	0.95	**1.00**	**0.07**	**6.88**
B_Fett_80	0.86	1.02	0.95	**0.95**	**0.08**	**8.52**
B_Fett_90	0.97	0.98	1.04	**1.00**	**0.04**	**3.62**
				MV	SD	CV (%)
Total (all samples)				**0.980**	**0.060**	**6.10**

Table 14: Results of one-way analysis of variance based on analytical results given in Table

ANOVA						
Source of variation	*Sum of Squares (SS)*	*Degrees of freedom (df)*	*Mean Square (MS)*	*Test Static (F)*	*P-Value*	*Critical value F*
Differences between the groups	0.14	8	0.02	1.29	0.31	2.51
Within the groups	0.24	18	0.01			
Total	0.37	26				

1.29 < 2.51	**Null hypothesis is true, samples are homogeneous**

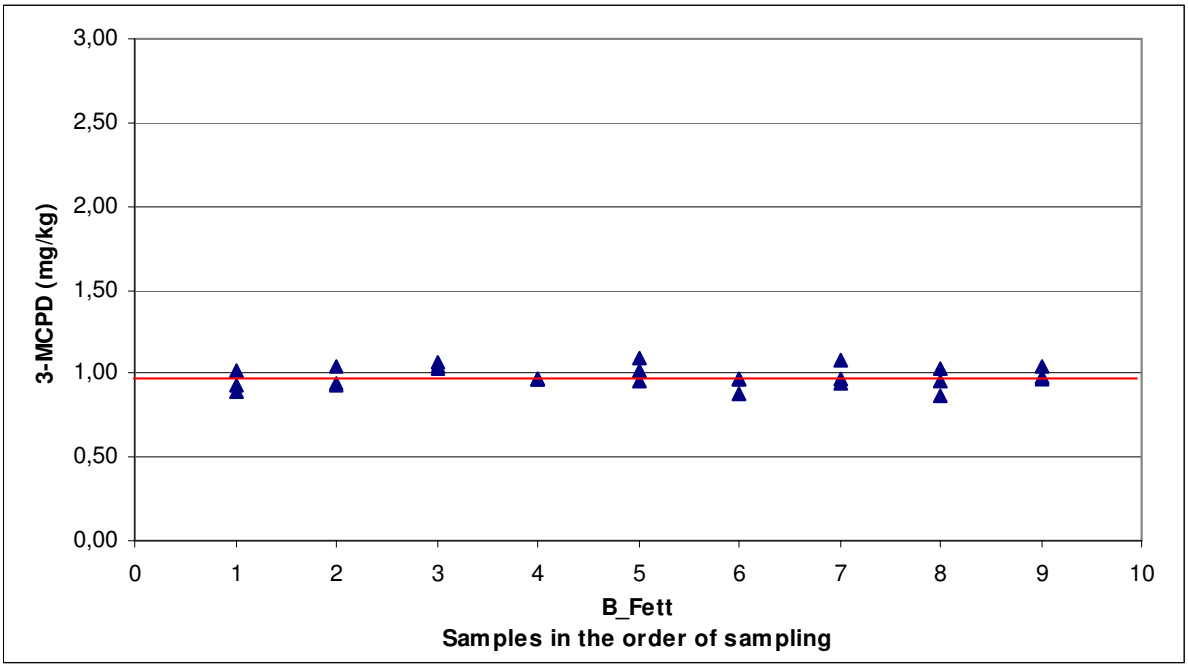

Figure 3: Graphical plot of threefold determinations of samples B_Fett for homogeneity testing

Sample F_Oel

Table 15: 3-MCPD measuring results obtained from homogeneity testing of sample F_Oel in sample aliquots

3-MCPD measuring results (mg/kg)						
Sample number	Value 1	Value 2	Value 3	MV (mg/kg)	SD	CV (%)
F_OEL_10	1.42	1.62	1.52	**1.52**	**0.10**	**6.44**
F_OEL_20	1.78	1.59	1.79	**1.72**	**0.12**	**6.75**
F_OEL_30	1.12	1.71	1.50	**1.44**	**0.30**	**20.63**
F_OEL_40	1.76	1.81	1.50	**1.69**	**0.16**	**9.74**
F_OEL_50	1.42	1.42	1.25	**1.36**	**0.10**	**7.32**
F_OEL_60	1.36	2.44	1.62	**1.81**	**0.56**	**31.17**
F_OEL_70	1.83	1.70	1.58	**1.70**	**0.13**	**7.36**
F_OEL_80	1.30	1.78	2.27	**1.78**	**0.48**	**27.24**
F_OEL_90	1.57	1.46	3.61	**2.21**	**1.21**	**54.78**
				MV	SD	CV (%)
Total (all samples)				**1.69**	**0.47**	**28.03**

Table16: Results of one-way analysis of variance based on analytical results given in Table

ANOVA						
Source of variation	*Sum of squares (SS)*	*Degrees of freedom (df)*	*Mean square (MS)*	*Test static (F)*	*P-Value*	*Critical value F*
Differences between the groups	1.480	8	0.18	0.76	0.64	2.51
Within the groups	4.375	18	0.24			
Total	5.855	26	5.855			

0.76 < 2.51	**Null hypothesis is true, samples are homogeneous**

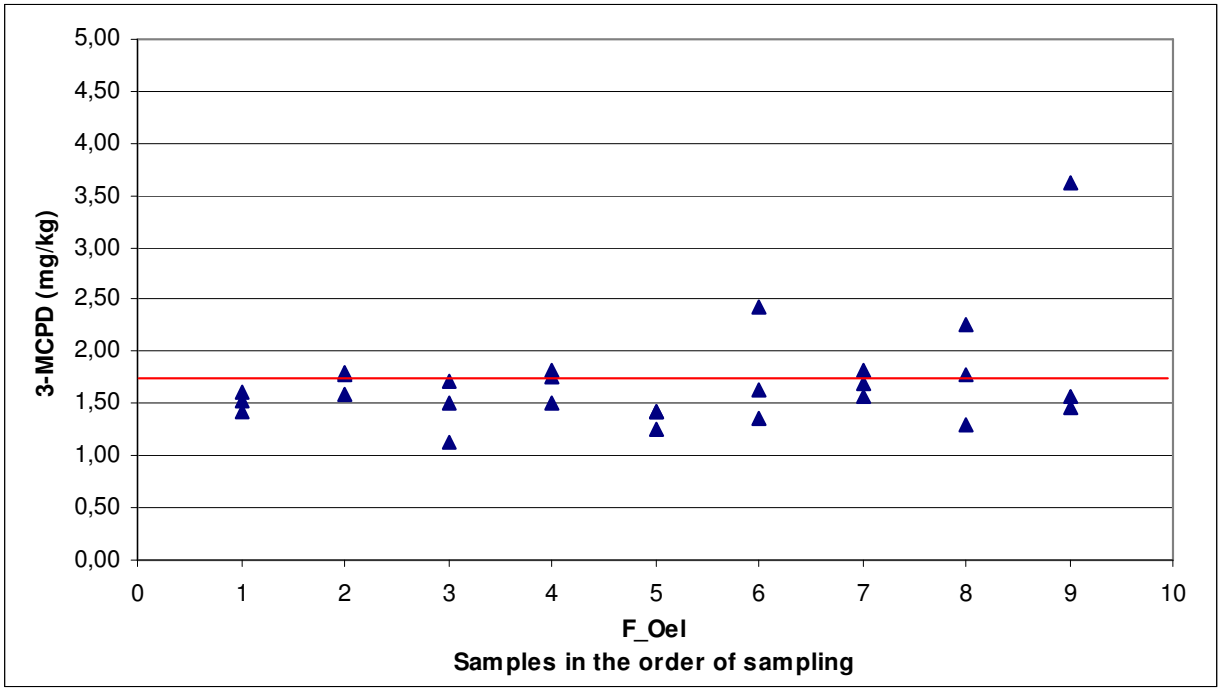

Figure 4: Graphical plot of threefold determinations of samples F_Oel for homogeneity testing

Sample P_Fett

Table 17: 3-MCPD Measuring results obtained from homogeneity testing of sample P_Fett in sample aliquots

3-MCPD measuring results (mg/kg)						
Sample number	Value 1	Value 2	Value 3	MV (mg/kg)	SD	CV (%)
P_Fett_20	2,58	3,09	3,29	**2,98**	**0,36**	**12,15**
P_Fett_30	3,52	3,29	2,93	**3,25**	**0,30**	**9,18**
P_Fett_40	3,17	2,84	3,74	**3,25**	**0,45**	**13,98**
P_Fett_50	2,83	3,05	3,06	**2,98**	**0,13**	**4,46**
P_Fett_60	3,17	3,31	3,54	**3,34**	**0,19**	**5,62**
P_Fett_70	2,95	3,10	3,08	**3,04**	**0,08**	**2,59**
P_Fett_80	3,08	3,27	3,12	**3,15**	**0,10**	**3,19**
P_Fett_90	3,11	3,32	3,25	**3,23**	**0,11**	**3,30**
				MW	SD	VK (%)
Total (all samples)				**3,15**	**0,25**	**7,83**

Table 18: Results of one-way analysis of variance based on analytical results given in Table

ANOVA						
Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	Test static (F)	P-Value	Critical value F
Differences between the groups	0,39	7	0,06	0,87	0,55	2,66
Within the groups	1,01	16	0,06			
Total	1,40	23				

0.87 < 2.66	**Null hypothesis is true, samples are homogeneous**

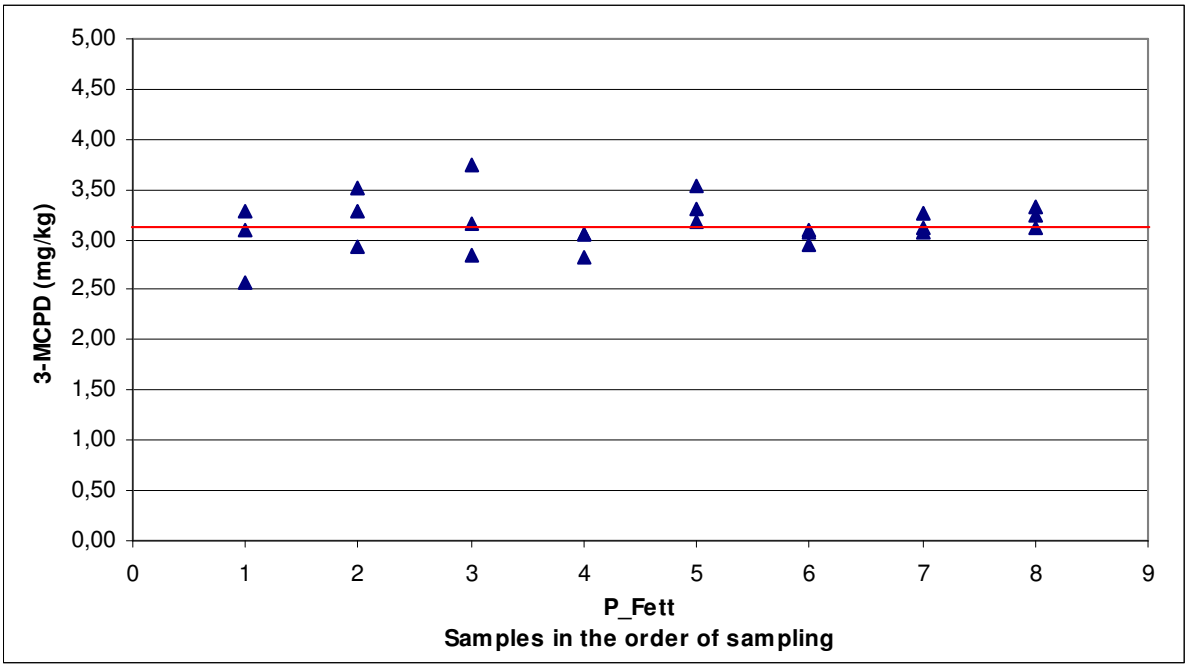

Figure 5: Graphical plot of threefold determinations of samples P_Fett for homogeneity testing

Sample T_Oel

Table 19: 3-MCPD Measuring results obtained from homogeneity testing of sample T_Oel in sample aliquots

3-MCPD measuring results (mg/kg)						
Sample number	Value 1	Value 2	Value 3	MV (mg/kg)	SD	CV (%)
T_OEL_10	3.64	3.18	3.86	**3.56**	**0.35**	**9.74**
T_OEL_20	3.75	3.70	3.88	**3.77**	**0.09**	**2.49**
T_OEL_30	3.84	4.04	4.36	**4.08**	**0.26**	**6.44**
T_OEL_40	3.62	4.29	4.66	**4.19**	**0.53**	**12.65**
T_OEL_50	3.58	3.58	3.91	**3.69**	**0.19**	**5.07**
T_OEL_60	4.38	3.48	3.70	**3.85**	**0.47**	**12.11**
T_OEL_70	3.79	4.37	3.89	**4.02**	**0.31**	**7.71**
T_OEL_80	4.35	4.08	3.69	**4.04**	**0.33**	**8.20**
T_OEL_90	3.98	4.11	4.15	**4.08**	**0.09**	**2.17**
				MW	SD	VK (%)
Total (all samples)				**3.920**	**0.337**	**8.60**

Table 20: Results of one-way analysis of variance based on analytical results given in Table

ANOVA						
Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	Test static (F)	P-Value	Critical value F
Differences between the groups	1.06	8	0.13	1.26	0.32	2.51
Within the groups	1.89	18	0.11			
Total	2.95	26				

1.26 < 2.51	Null hypothesis is true, samples are homogeneous

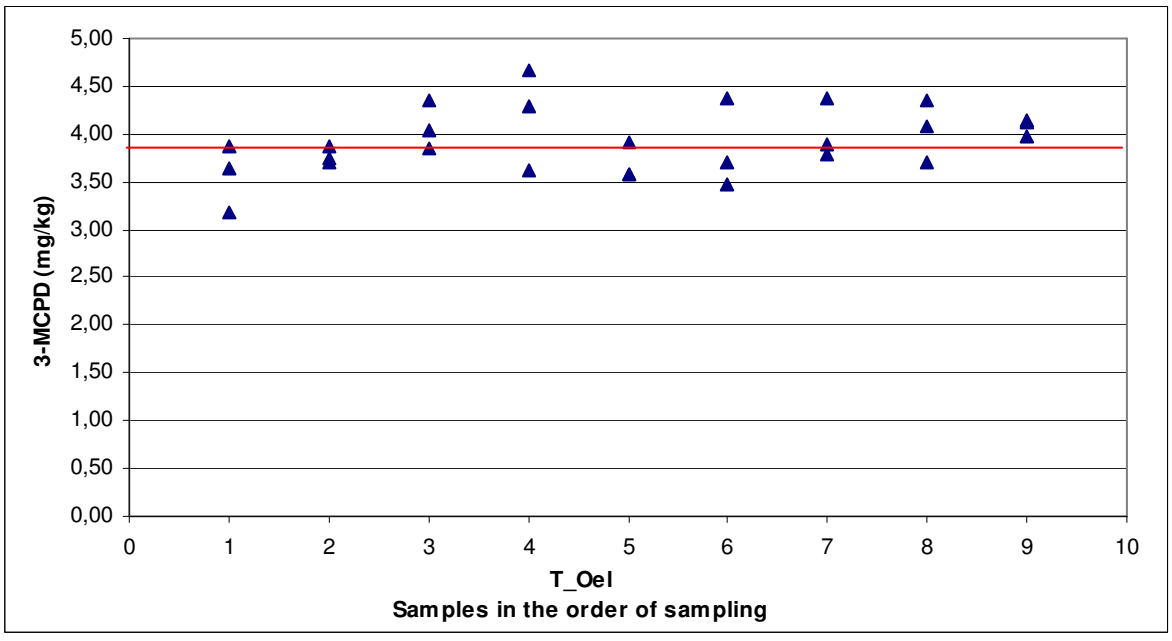

Figure 6: Graphical plot of threefold determinations of samples T_Oel for homogeneity testing

7.3 List of Participants

Institution	Location	Country
ANALYTEC – Labor für Lebensmitteluntersuchung und Umwelt-analytik	Salzburg	Austria
Institut Dr. Appelt Hilter GmbH & Co. KG Warenprüfung & Qualitätskontrolle	Hilter a. T.W.	Germany
Amt für Verbraucherschutz Kreisverwaltung Mettmann 39-4	Mettmann	Germany
Bundesinstitut für Risikobewertung, Fachgruppe 82 Kontaminanten	Berlin	Germany
Bilacon	Berlin	Germany
CHELAB, Chemisches Laboratorium	Hemmingen	Germany
Chemisches Untersuchungsamt der Stadt Hagen	Hagen	Germany
Chemisches Untersuchungsinstitut Leverkusen	Leverkusen	Germany
Chemische und Veterinäruntersuchungsamt Ostwestfalen-Lippe Dez. 6.4 Kontaminanten	Detmold	Germany
Chemisches und Veterinäruntersuchungsamt Stuttgart	Fellbach	Germany
Eurofins WEJ Contaminants	Hamburg	Germany
Ferrero S.p.A.	Alba (CN)	Italy
SGS Germany GmbH, Consumer Testing Services Food	Hamburg	Germany
Institut für Getreideverarbeitung GmbH	Nuthetal OT Bergholz-Rehbrücke	Germany
Institut für Qualitätsforschung in der Süßwarenwirtschaft e.V.	Köln	Germany
Institut Kirchhoff Berlin GmbH	Berlin	Germany
Labor Kneißler	Burglengenfeld	Germany
Institut Prof. Dr. Georg Kurz GmbH	Köln	Germany
LAVES Lebensmittelinstitut Braunschweig	Braunschweig	Germany
Lebensmittelchemisches Institut des Bundesverbandes der Süßwarenindustrie	Köln	Germany
Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit	Erlangen	Germany
Landeslabor Berlin-Brandenburg	Berlin	Germany
Labor für Umweltanalytik GmbH	Schwerin	Germany
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen	Dresden	Germany
Landesuntersuchungsamt Rheinland-Pfalz, Institut für Lebensmittelchemie	Trier	Germany
LUFA-ITL GmbH	Kiel	Germany
Landeslabor Schleswig-Holstein (Lebensmittel- Veterinär- und Umweltuntersuchungsamt) - Außenstelle Lübeck -	Lübeck	Germany
Max Rubner-Institut Bundesforschungs-institut für Ernährung und Lebensmittel	Münster	Germany
Institut Nehring GmbH	Braunschweig	Germany
Neotron SPA	Modena	Italy
Nestlé Deutschland AG, NQAC Weiding	Polling-Weiding	Germany
SGS Belgium-IAC	Antwerpen	Belgium
SQTS Swiss Quality Testing Services	Dietikon 1	Switzerland
Unilever R&D Vlaardingen	Vlaardingen	Netherlands
CLUS/Unilever Schweiz GmbH	Thayngen	Switzerland
Chemisches Labor Dr. Wirts und Partner	Hannover	Germany

7.4 Individual Data

Table 21: Laboratories which used more than one analytical method

Laboratory code	BfR Method 8	BfR Method 9	BfR Method 10	Own in-house method
LC0001	x (LC0001)	x (LC0101)		
LC0002	x (LC0102)	x (LC0202)	x (LC0302)	x (LC0002)
LC0003				x (LC0003) x (LC0203)*
LC0011	x (LC0111)	x (LC0211)	x (LC0311)	x (LC0011)
LC0025		x (LC0225)	x (LC0325)	x (LC0025)
LC0032		x (LC0232)		x (LC0034)

*BfR Method 9 was modified to such an extent that it was assessed as a method of its own.

Table 22: Individual results obtained for Plant Oil Sample "L_Oel"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD	MV	Type of outlier
				1	2	3			1-3	1-3	sample	sample	
LC0002	L_OEL_1	IM	mg/kg	0.33	0.46	0.55			0.447	0.111			
LC0002	L_OEL_2	IM	mg/kg	0.48	0.45	0.27			0.400	0.114	0.093	0.42	
LC0003	L_OEL_1	IM	mg/kg	0.19	0.19	0.19	0.15	0.11	0.190	0.000			
LC0003	L_OEL_2	IM	mg/kg	0.20	0.20	0.21	0.15	0.11	0.203	0.006	0.006	0.20	
LC0011	L_OEL_1	IM	mg/kg	0.18	0.16	0.16			0.167	0.012			
LC0011	L_OEL_2	IM	mg/kg	0.21	0.19	0.20			0.200	0.010	0.016	0.18	
LC0013	L_OEL_1	IM	mg/kg	NB	NB	NB	0.28						
LC0013	L_OEL_2	IM	mg/kg	NB	NB	NB	0.28						
LC0024	L_OEL_1	IM	mg/kg	0.23	0.25	0.26	0.1	0.05	0.247	0.015			
LC0024	L_OEL_2	IM	mg/kg	0.26	0.26	0.22	0.1	0.05	0.247	0.023	0.020	0.25	
LC0025	L_OEL_1	IM	mg/kg	0.17	0.17		0.15	0.05	0.170	0.000			
LC0025	L_OEL_2	IM	mg/kg	0.17	0.18		0.15	0.05	0.175	0.007	0.004	0.17	
LC0033	L_OEL_1	IM	mg/kg	0.59	0.63	0.45			0.557	0.095			
LC0033	L_OEL_2	IM	mg/kg	0.54	0.44	0.45			0.477	0.055	0.071	0.52	
LC0203	L_OEL_1	IM	mg/kg	0.19	0.18	0.20	0.15	0.05	0.190	0.010			
LC0203	L_OEL_2	IM	mg/kg	0.18	0.20	0.20	0.15	0.05	0.193	0.012	0.009	0.19	
LC0432	**L_OEL_1**	**IM**	**mg/kg**	**0.14**	**0.65**	**0.38**			**0.390**	**0.255**			
LC0432	**L_OEL_2**	**IM**	**mg/kg**		**0,29**	**3,47**			**1,880**	**2,249**	**1,228**	**1,14**	**C (PT)**
LC0001	L_OEL_1	8	mg/kg					0,1					
LC0001	L_OEL_2	8	mg/kg					0,1					
LC0019	L_OEL_1	8	mg/kg	0.13	0.15	0.14			0.140	0.010			
LC0019	L_OEL_2	8	mg/kg	0.13	0.14	0.15			0.140	0.010	0.010	0.14	
LC0028	L_OEL_1	8	mg/kg	0.34	0.41	0.39			0.380	0.036			
LC0028	L_OEL_2	8	mg/kg	0.40	0.43	0.43			0.420	0.017	0.028	0.40	
LC0029	L_OEL_1	8	mg/kg	NB	NB	NB	0.3	0.1					
LC0029	L_OEL_2	8	mg/kg		NB		0.3	0.1					
LC0102	L_OEL_1	8	mg/kg	0.39	0.29	0.27			0.317	0.064			
LC0102	L_OEL_2	8	mg/kg	0.23	0.23	0.24			0.233	0.006	0.050	0.28	
LC0111	L_OEL_1	8	mg/kg	0.21	0.25	0.24			0.233	0.021			
LC0111	L_OEL_2	8	mg/kg	0.19	0.20	0.18			0.190	0.010	0.022	0.21	
LC0004	L_OEL_1	9	mg/kg	NB	NB	NB	0.25	0.1					
LC0004	L_OEL_2	9	mg/kg	NB	NB	NB	0.25	0.1					
LC0005	L_OEL_1	9	mg/kg	NB	NB	NB	0.8	0.4					
LC0005	L_OEL_2	9	mg/kg	NB	NB		0.8	0.4					
LC0006	L_OEL_1	9	mg/kg	0.27	0.25	0.19			0.237	0.042			

Table 22: Individual results obtained for Plant Oil Sample "L_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD	MV	Type of outlier
				1	2	3			1-3	1-3	sample	sample	
LC0006	L_OEL_2	9	mg/kg	0.16	0.19	0.22			0.190	0.030	0.035	0.21	
LC0007	L_OEL_1	9	mg/kg	0.49	0.60	0.66	0.19	0.05	0.583	0.086			
LC0007	L_OEL_2	9	mg/kg	0.97	0.91	0.78	0.19	0.05	0.887	0.097	0.145	0.74	
LC0009	L_OEL_1	9	mg/kg	0.20	0.20	0.17	0.17	0.05	0.190	0.017			
LC0009	L_OEL_2	9	mg/kg	0.17	0.18	0.19	0.17	0.05	0.180	0.010	0.012	0.19	
LC0010	**L_OEL_1**	**9**	**mg/kg**	**0.96**	**0.98**	**1.03**			**0.990**	**0.036**			
LC0010	**L_OEL_2**	**9**	**mg/kg**	**0.51**	**0.56**	**0.47**			**0.513**	**0.045**	**0.197**	**0.75**	**C (M9)**
LC0012	L_OEL_1	9	mg/kg	0.23	0.22	0.22			0.223	0.006			
LC0012	L_OEL_2	9	mg/kg	0.22	0.22	0.23			0.223	0.006	0.006	0.22	
LC0016	L_OEL_1	9	mg/kg	NB	NB	NB	0.24						
LC0016	L_OEL_2	9	mg/kg	NB	NB	0.27	0.24		0.270			0.27	
LC0017	L_OEL_1	9	mg/kg	0.25	0.19	0.17			0.203	0.042			
LC0017	L_OEL_2	9	mg/kg	0.19	0.22	0.15			0.187	0.035	0.032	0.20	
LC0018	L_OEL_1	9	mg/kg	0.37	0.29	0.28			0.313	0.049			
LC0018	L_OEL_2	9	mg/kg	0.28	0.28	0.57			0.377	0.167	0.104	0.35	
LC0020	**L_OEL_1**	**9**	**mg/kg**	**1.02**	**1.12**	**1.05**	**0.15**	**0.05**	**1.063**	**0.051**			
LC0020	**L_OEL_2**	**9**	**mg/kg**	**0.31**	**0.47**	**0.62**	**0.15**	**0.05**	**0.467**	**0.155**	**0.261**	**0.76**	**C (M9, PT)**
LC0021	L_OEL_1	9	mg/kg		NB		0.12	0.04					
LC0021	L_OEL_2	9	mg/kg	NB	NB		0.12	0.04					
LC0022	L_OEL_1	9	mg/kg	0.28	0.27	0.29			0.280	0.010			
LC0022	L_OEL_2	9	mg/kg	0.28	0.28	0.26			0.273	0.012	0.009	0.28	
LC0026	L_OEL_1	9	mg/kg	0.35	0.30	0.28			0.310	0.036			
LC0026	L_OEL_2	9	mg/kg	0.31	0.33	0.28			0.307	0.025	0.025	0.31	
LC0027	L_OEL_1	9	mg/kg	0.17	0.24	0.19			0.200	0.036			
LC0027	L_OEL_2	9	mg/kg	0.17	0.16	0.12			0.150	0.026	0.033	0.18	
LC0030	L_OEL_1	9	mg/kg	0.49	0.40	0.43			0.440	0.046			
LC0030	L_OEL_2	9	mg/kg	0.50	0.42	0.32			0.413	0.090	0.059	0.43	
LC0032	L_OEL_1	9	mg/kg		0.09	0.13	0.09	0.05	0.110	0.028			
LC0032	L_OEL_2	9	mg/kg								0.028	0.11	
LC0034	L_OEL_1	9	mg/kg	0.22	0.21	0.20	0.2	0.1	0.210	0.010			
LC0034	**L_OEL_2**	**9**	**mg/kg**	**0.22**	**0.22**	**0.20**	**0.2**	**0.1**	**0.213**	**0.012**	**0.009**	**0.21**	
LC0035	L_OEL_1	9	mg/kg	0.33	0.39	0.34			0.353	0.032			
LC0035	L_OEL_2	9	mg/kg	0.41	0.35	0.42			0.393	0.038	0.033	0.37	
LC0038	L_OEL_1	9	mg/kg	NB	NB	NB	0.15	0.075					
LC0038	L_OEL_2	9	mg/kg	NB	0.16	NB	0.15	0.075	0.160			0.16	

Table 22: Individual results obtained for Plant Oil Sample "L_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD	MV	Type of outlier
				1	2	3			1-3	1-3	sample	sample	
LC0039	L_OEL_1	9	mg/kg	0.22	NB	NB	0.2		0.220				
LC0039	L_OEL_2	9	mg/kg	0.31	NB	0.24	0.2		0.275	0.049	0.045	0.25	
LC0040	L_OEL_1	9	mg/kg	0.42	0.37	0.38			0.390	0.026			
LC0040	L_OEL_2	9	mg/kg	0.77	0.75	0.73			0.750	0.020	0.148	0.57	
LC0101	L_OEL_1	9	mg/kg					0.1					
LC0101	L_OEL_2	9	mg/kg					0.1					
LC0202	L_OEL_1	9	mg/kg	0.31	0.34	0.32			0.323	0.015			
LC0202	L_OEL_2	9	mg/kg	0.20	0.24	0.25			0.230	0.026	0.042	0.28	
LC0211	L_OEL_1	9	mg/kg	0.24	0.24	0.25			0.243	0.006			
LC0211	L_OEL_2	9	mg/kg	0.25	0.25	0.25			0.250	0.000	0.004	0.25	
LC0213	L_OEL_1	9	mg/kg	NB	NB	NB	0.28						
LC0213	L_OEL_2	9	mg/kg	NB	NB	NB	0.28						
LC0225	L_OEL_1	9	mg/kg	0.33	0.27	0.25	0.15	0.05	0.283	0.042			
LC0225	L_OEL_2	9	mg/kg	0.23	0.30	0.22	0.15	0.05	0.250	0.044	0.037	0.27	
LC0008	L_OEL_1	10	mg/kg	0.28	0.26	0.29			0.277	0.015			
LC0008	L_OEL_2	10	mg/kg	0.27	0.26	0.27			0.267	0.006	0.010	0.27	
LC0015	L_OEL_1	10	mg/kg	0.37	NB		0.34	0.23	0.370				
LC0015	L_OEL_2	10	mg/kg			NB	0.34	0.23				0.37	
LC0031	L_OEL_1	10	mg/kg	0.29	0.29	0.27	0.15	0.1	0.283	0.012			
LC0031	L_OEL_2	10	mg/kg	0.19	0.17	0.19	0.15	0.1	0.183	0.012	0.042	0.23	
LC0302	**L_OEL_1**	**10**	**mg/kg**	**1.25**	0.31	0.20			**0.587 (0.265)**	**0.577 (0.078)**			
LC0302	L_OEL_2	10	mg/kg	0.28	0.20	0.24			0.240	0.040	**0.363 (0.044)**	**0.41 (0.25)**	**A (a)**
LC0311	L_OEL_1	10	mg/kg	0.23	0.25	0.18			0.220	0.036			
LC0311	L_OEL_2	10	mg/kg	0.21	0.17	0.21			0.197	0.023	0.026	0.21	
LC0325	L_OEL_1	10	mg/kg	0.26	0.28	0.24	0.15	0.05	0.260	0.020			
LC0325	L_OEL_2	10	mg/kg	0.43	0.52	0.39	0.15	0.05	0.447	0.067	0.086	0.35	

Table 23: Individual results obtained for Vegetable Fat Sample "B_Fett"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0002	B_FETT_1	IM	mg/kg	0.80	0.89	1.08			0.923	0.143			
LC0002	B_FETT_2	IM	mg/kg	0.95	1.09	0.73			0.923	0.181	0.133	0.92	
LC0003	B_FETT_1	IM	mg/kg	0.86	0.83	0.82	0.15	0.11	0.837	0.021			
LC0003	B_FETT_2	IM	mg/kg	0.82	0.85	0.92	0.15	0.11	0.863	0.051	0.034	0.85	
LC0011	B_FETT_1	IM	mg/kg	0.75	0.75	0.78			0.760	0.017			
LC0011	B_FETT_2	IM	mg/kg	0.78	0.72	0.77			0.757	0.032	0.021	0.76	
LC0013	B_FETT_1	IM	mg/kg	0.82	0.78	0.87	0.28		0.823	0.045			
LC0013	B_FETT_2	IM	mg/kg	0.64	0.64	0.71	0.28		0.663	0.040	0.074	0.74	
LC0024	B_FETT_1	IM	mg/kg	0.77	0.81	0.84	0.1	0.05	0.807	0.035			
LC0024	B_FETT_2	IM	mg/kg	0.97	0.88	0.84	0.1	0.05	0.897	0.067	0.057	0.85	
LC0025	B_FETT_1	IM	mg/kg	0.75	0.90		0.15	0.05	0.825	0.106			
LC0025	B_FETT_2	IM	mg/kg	0.89	0.77		0.15	0.05	0.830	0.085	0.068	0.83	
LC0033	B_FETT_1	IM	mg/kg	1.33	1.11	1.07			1.170	0.140			
LC0033	B_FETT_2	IM	mg/kg	1.08	1.32	1.18			1.193	0.121	0.107	1.18	
LC0203	B_FETT_1	IM	mg/kg	0.76	0.90	0.76	0.15	0.05	0.807	0.081			
LC0203	B_FETT_2	IM	mg/kg	0.82	0.87	0.79	0.15	0.05	0.827	0.040	0.053	0.82	
LC0432	B_FETT_1	IM	mg/kg	0.63	0.86	0.77			0.753	0.116			
LC0432	B_FETT_2	IM	mg/kg	0.74	0.81	0.92			0.823	0.091	0.090	0.79	
LC0001	B_FETT_1	8	mg/kg	0.77	0.74	1.03		0.1	0.847	0.159			
LC0001	B_FETT_2	8	mg/kg	0.84	1.05	0.94		0.1	0.943	0.105	0.117	0.90	
LC0019	B_FETT_1	8	mg/kg	0.81	0.82	0.88			0.837	0.038			
LC0019	B_FETT_2	8	mg/kg	0.79	0.83	0.91			0.843	0.061	0.042	0.84	
LC0028	B_FETT_1	8	mg/kg	1.14	0.90	1.16			1.067	0.145			
LC0028	B_FETT_2	8	mg/kg	1.41	1.25	1.01			1.223	0.201	0.157	1.15	
LC0029	B_FETT_1	8	mg/kg	0.58	0.37	0.52	0.3	0.1	0.490	0.108			
LC0029	B_FETT_2	8	mg/kg	0.68	0.71	0.62	0.3	0.1	0.668	0.047	0.100	0.58	
LC0102	B_FETT_1	8	mg/kg	1.04	0.86	0.79			0.897	0.129			
LC0102	B_FETT_2	8	mg/kg	0.80	0.43	0.77			0.667	0.206	0.169	0.78	
LC0111	B_FETT_1	8	mg/kg	0.74	0.73	0.70			0.723	0.021			
LC0111	B_FETT_2	8	mg/kg	0.73	0.66	0.72			0.703	0.038	0.026	0.71	
LC0004	B_FETT_1	9	mg/kg	0.67	0.71	0.71	0.25	0.1	0.697	0.023			
LC0004	B_FETT_2	9	mg/kg	0.68	0.70	0.68	0.25	0.1	0.687	0.012	0.015	0.69	
LC0005	**B_FETT_1**	**9**	**mg/kg**	**1.67**	**0.76**	**0.89**	0.8	0.4	**1.107**	**0.492**			
LC0005	**B_FETT_2**	**9**	**mg/kg**	**1.12**	**1.02**	**0.98**	0.8	0.4	**1.040**	**0.072**	**0.288**	**1.07**	**C (M9, PT)**
LC0006	B_FETT_1	9	mg/kg	0.99	1.10	1.01			1.033	0.059			

Table 23: Individual results obtained for Vegetable Fat Sample "B_Fett" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0006	B_FETT_2	9	mg/kg	0.93	0.90	1.01			0.947	0.057	0.059	0.99	
LC0007	B_FETT_1	9	mg/kg	0.98	0.83	0.96	0.19	0.05	0.923	0.081			
LC0007	B_FETT_2	9	mg/kg	0.70	0.89	1.08	0.19	0.05	0.890	0.190	0.120	0.91	
LC0009	B_FETT_1	9	mg/kg	0.84	0.70	0.70	0.17	0.05	0.747	0.081			
LC0009	B_FETT_2	9	mg/kg	0.76	0.89	0.78	0.17	0.05	0.810	0.070	0.067	0.78	
LC0010	B_FETT_1	9	mg/kg	1.41	1.37	1.00			1.260	0.226			
LC0010	B_FETT_2	9	mg/kg	1.10	1.10	1.29			1.163	0.110	0.150	1.21	
LC0012	B_FETT_1	9	mg/kg	0.84	0.85	0.85			0.847	0.006			
LC0012	B_FETT_2	9	mg/kg	0.87	0.75	0.85			0.823	0.064	0.038	0.84	
LC0016	B_FETT_1	9	mg/kg	0.75	0.77	0.82	0.24		0.779	0.037			
LC0016	B_FETT_2	9	mg/kg	0.84	0.81	0.76	0.24		0.803	0.040	0.033	0.79	
LC0017	B_FETT_1	9	mg/kg	0.76	0.79	0.82			0.790	0.030			
LC0017	B_FETT_2	9	mg/kg	0.78	0.75	0.78			0.770	0.017	0.022	0.78	
LC0018	B_FETT_1	9	mg/kg	0.64	0.66	0.88			0.727	0.133			
LC0018	B_FETT_2	9	mg/kg	0.73	0.75	0.74			0.740	0.010	0.077	0.73	
LC0020	B_FETT_1	9	mg/kg	1.38	1.83	1.50	0.15	0.05	1.570	0.233			
LC0020	B_FETT_2	9	mg/kg	1.29	1.20	1.32	0.15	0.05	1.270	0.062	0.185	1.42	
LC0021	**B_FETT_1**	**9**	**mg/kg**	**1.67**	0.58	0.68	0.12	0.04	**0.977 (0.63)**	**0.602 (0.071)**			
LC0021	B_FETT_2	9	mg/kg	0.65	0.76	0.75	0.12	0.04	0.72	0.061	**0.365 (0.064)**	**0.85 (0.68)**	**A(a)**
LC0022	B_FETT_1	9	mg/kg	0.87	0.86	0.88			0.870	0.010		0	
LC0022	B_FETT_2	9	mg/kg	1.03	1.07	1.10			1.067	0.035	0.083	0.97	
LC0026	B_FETT_1	9	mg/kg	1.06	1.09	1.04			1.063	0.025			
LC0026	B_FETT_2	9	mg/kg	1.02	1.18	1.01			1.070	0.095	0.057	1.07	
LC0027	B_FETT_1	9	mg/kg	0.93	0.83	0.83			0.863	0.058			
LC0027	B_FETT_2	9	mg/kg	0.71	0.73	0.74			0.727	0.015	0.066	0.80	
LC0030	B_FETT_1	9	mg/kg	1.11	1.22	0.89			1.073	0.168			
LC0030	B_FETT_2	9	mg/kg	0.88	0.91	0.84			0.877	0.035	0.128	0.98	
LC0032	B_FETT_1	9	mg/kg	0.60	0.53	0.38	0.09	0.05	0.503	0.112			
LC0032	B_FETT_2	9	mg/kg	0.37	0.35	0.34			0.353	0.015	0.090	0.43	
LC0034	B_FETT_1	9	mg/kg	0.96	0.95	0.89	0.2	0.1	0.933	0.038			
LC0034	B_FETT_2	9	mg/kg	1.10	0.98	0.90	0.2	0.1	0.993	0.101	0.067	0.96	
LC0035	B_FETT_1	9	mg/kg	0.84	0.89	0.91			0.880	0.036			
LC0035	B_FETT_2	9	mg/kg	0.98	0.91	0.93			0.940	0.036	0.038	0.91	

Table 23: Individual results obtained for Vegetable Fat Sample "B_Fett" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0038	B_FETT_1	9	mg/kg	0.74	0.68	1.09	0.15	0.075	0.837	0.221			
LC0038	B_FETT_2	9	mg/kg	0.95	0.79	0.96	0.15	0.075	0.900	0.095	0.142	0.87	
LC0039	B_FETT_1	9	mg/kg	0.90	0.79	0.87	0.2		0.853	0.057			
LC0039	B_FETT_2	9	mg/kg	0.93	0.83	0.73	0.2		0.830	0.100	0.067	0.84	
LC0040	B_FETT_1	9	mg/kg	0.91	0.96	0.99			0.953	0.040			
LC0040	B_FETT_2	9	mg/kg	1.13	1.08	1.19			1.133	0.055	0.083	1.04	
LC0101	B_FETT_1	9	mg/kg	0.91	1.25	1.51		0.1	1.223	0.301			
LC0101	B_FETT_2	9	mg/kg	1.34	1.11	1.45		0.1	1.300	0.173	0.203	1.26	
LC0202	B_FETT_1	9	mg/kg	1.12	0.98	0.79			0.963	0.166			
LC0202	B_FETT_2	9	mg/kg	0.83	1.05	0.73			0.870	0.164	0.140	0.92	
LC0211	B_FETT_1	9	mg/kg	0.87	0.86	0.82			0.850	0.026			
LC0211	B_FETT_2	9	mg/kg	0.78	0.70	0.80			0.760	0.053	0.050	0.81	
LC0213	B_FETT_1	9	mg/kg	0.73	0.71	0.78	0.28		0.740	0.036			
LC0213	B_FETT_2	9	mg/kg	0.77	0.85	0.79	0.28		0.803	0.042	0.041	0.77	
LC0225	B_FETT_1	9	mg/kg	0.73	0.72	0.76	0.15	0.05	0.737	0.021			
LC0225	B_FETT_2	9	mg/kg	0.73	0.66	0.78	0.15	0.05	0.723	0.060	0.037	0.73	
LC0008	B_FETT_1	10	mg/kg	0.78	0.78	0.75			0.770	0.017			
LC0008	B_FETT_2	10	mg/kg	0.77	0.76	0.74			0.757	0.015	0.014	0.76	
LC0015	**B_FETT_1**	**10**	**mg/kg**	**1.42**	**1.58**	**1.52**	0.34	0.23	**1.507**	**0.081**			
LC0015	**B_FETT_2**	**10**	**mg/kg**		**0.56**	**0.90**	0.34	0.23	**0.730**	**0.240**	**0.368**	**1.20**	**C (PT)**
LC0031	B_FETT_1	10	mg/kg	0.86	1.10	0.79	0.15	0.1	0.917	0.163			
LC0031	B_FETT_2	10	mg/kg	0.73	0.75	0.75	0.15	0.1	0.743	0.012	0.118	0.83	
LC0302	B_FETT_1	10	mg/kg	0.67	0.88	0.48			0.677	0.200			
LC0302	B_FETT_2	10	mg/kg	0.44	0.88	0.64			0.653	0.220	0.172	0.67	
LC0311	B_FETT_1	10	mg/kg	0.68	0.65	0.74			0.690	0.046			
LC0311	B_FETT_2	10	mg/kg	0.80	0.75	0.78			0.777	0.025	0.047	0.73	
LC0325	B_FETT_1	10	mg/kg	0.69	0.86	0.83	0.15	0.05	0.793	0.091			
LC0325	B_FETT_2	10	mg/kg	1.07	0.79	1.04	0.15	0.05	0.967	0.154	0.125	0.88	

Table 24: Individual results obtained for Frying Oil Sample "F_Oel"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0002	F_OEL_1	IM	mg/kg	1.58	1.72	2.01			1.770	0.219			
LC0002	F_OEL_2	IM	mg/kg	1.87	1.93	1.55			1.783	0.204	0.173	1.78	
LC0003	F_OEL_1	IM	mg/kg	1.64	1.61	1.64	0.15	0.11	1.630	0.017			
LC0003	F_OEL_2	IM	mg/kg	1.66	1.65	1.62	0.15	0.11	1.643	0.021	0.017	1.64	
LC0011	F_OEL_1	IM	mg/kg	1.65	1.50	1.62			1.590	0.079			
LC0011	F_OEL_2	IM	mg/kg	1.46	1.48	1.51			1.483	0.025	0.065	1.54	
LC0013	F_OEL_1	IM	mg/kg	1.65	1.48	1.58	0.28		1.570	0.085			
LC0013	F_OEL_2	IM	mg/kg	1.46	1.44	1.39	0.28		1.430	0.036	0.078	1.50	
LC0024	F_OEL_1	IM	mg/kg	1.72	1.77	1.86	0.1	0.05	1.783	0.071			
LC0024	F_OEL_2	IM	mg/kg	1.81	1.80	1.69	0.1	0.05	1.767	0.067	0.057	1.78	
LC0025	F_OEL_1	IM	mg/kg	1.90	2.00		0.15	0.05	1.950	0.071			
LC0025	F_OEL_2	IM	mg/kg	1.72	1.52		0.15	0.05	1.620	0.141	0.183	1.79	
LC0033	F_OEL_1	IM	mg/kg	2.23	2.29	1.86			2.127	0.233			
LC0033	F_OEL_2	IM	mg/kg	2.14	1.78	2.17			2.030	0.217	0.188	2.08	
LC0203	F_OEL_1	IM	mg/kg	1.60	1.61	1.65	0.15	0.05	1.620	0.026			
LC0203	F_OEL_2	IM	mg/kg	1.72	1.57	1.62	0.15	0.05	1.637	0.076	0.047	1.63	
LC0432	**F_OEL_2**	IM	**mg/kg**	**1.63**	**1.53**	**3.86**			**2.34 (1.58)**	**1.317 (0.707)**			
LC0432	F_OEL_2	IM	mg/kg	2.04	1.72	1.84			1.867	0.162	**0.790 (0.168)**	**2.10 (1.75)**	**A (a)**
LC0001	F_OEL_1	8	mg/kg	1.37	1.46	1.23		0.1	1.353	0.116			
LC0001	F_OEL_2	8	mg/kg	1.24	1.12	1.59		0.1	1.317	0.244	0.157	1.34	
LC0019	F_OEL_1	8	mg/kg	1.59	1.71	1.63			1.643	0.061			
LC0019	**F_OEL_2**	**8**	**mg/kg**	**1.58**	**2.28**	**1.63**			**1.83 (1.605)**	**0.390 (0.035)**	**0.241 (0.045)**	**1.74 (1.63)**	**A (a)**
LC0028	F_OEL_1	8	mg/kg	1.67	1.52	1.60			1.597	0.075			
LC0028	F_OEL_2	8	mg/kg	1.68	1.65	1.72			1.683	0.035	0.060	1.64	
LC0029	F_OEL_1	8	mg/kg	1.12	1.02	1.07	0.3	0.1	1.070	0.050			
LC0029	F_OEL_2	8	mg/kg	1.21	1.32	1.65	0.3	0.1	1.393	0.229	0.189	1.23	
LC0102	F_OEL_1	8	mg/kg	1.95	1.31	1.69			1.650	0.322			
LC0102	F_OEL_2	8	mg/kg	1.55	1.66	1.53			1.580	0.070	0.192	1.62	
LC0111	F_OEL_1	8	mg/kg	1.52	1.61	1.58			1.570	0.046			
LC0111	F_OEL_2	8	mg/kg	1.42	1.33	1.33			1.360	0.052	0.095	1.47	
LC0004	F_OEL_1	9	mg/kg	1.47	1.41	1.46	0.25	0.1	1.447	0.032			
LC0004	F_OEL_2	9	mg/kg	1.46	1.50	1.39	0.25	0.1	1.450	0.056	0.037	1.45	

Table 24: Individual results obtained for Frying Oil Sample "F_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0005	F_OEL_1	9	mg/kg	1.63	1.69	1.73	0.8	0.4	1.683	0.050			
LC0005	F_OEL_2	9	mg/kg	2.07	2.04	2.32	0.8	0.4	2.143	0.154	0.210	1.91	
LC0006	F_OEL_1	9	mg/kg	1.76	2.02	1.97			1.917	0.138			
LC0006	F_OEL_2	9	mg/kg	1.70	1.81	1.95			1.820	0.125	0.115	1.87	
LC0007	**F_OEL_1**	**9**	**mg/kg**	**1.93**	**1.49**	**2.37**	0.19	0.05	**1.930**	**0.440**			
LC0007	**F_OEL_2**	**9**	**mg/kg**	**2.57**	**1.83**	**1.89**	0.19	0.05	**2.097**	**0.411**	**0.354**	**2.01**	**C (M9)**
LC0009	F_OEL_1	9	mg/kg	1.82	1.91	1.54	0.17	0.05	1.757	0.193			
LC0009	F_OEL_2	9	mg/kg	1.57	1.64	1.89	0.17	0.05	1.700	0.168	0.150	1.73	
LC0010	F_OEL_1	9	mg/kg	2.20	2.09	2.13			2.140	0.056			
LC0010	F_OEL_2	9	mg/kg	2.12	2.24	2.01			2.123	0.115	0.074	2.13	
LC0012	F_OEL_1	9	mg/kg	1.75	1.69	1.77			1.737	0.042			
LC0012	F_OEL_2	9	mg/kg	1.64	1.62	1.62			1.627	0.012	0.051	1.68	
LC0016	F_OEL_1	9	mg/kg	1.53	1.57	1.64	0.24		1.580	0.056			
LC0016	F_OEL_2	9	mg/kg	1.71	1.65	1.59	0.24		1.650	0.060	0.055	1.62	
LC0017	F_OEL_1	9	mg/kg	1.70	1.71	2.03			1.813	0.188			
LC0017	F_OEL_2	9	mg/kg	1.86	1.78	2.04			1.893	0.133	0.137	1.85	
LC0018	F_OEL_1	9	mg/kg	0.97	0.90	0.93			0.933	0.035			
LC0018	F_OEL_2	9	mg/kg	1.45	1.52	1.53			1.500	0.044	0.234	1.22	
LC0020	F_OEL_1	9	mg/kg	1.84	1.98	1.96	0.15	0.05	1.927	0.076			
LC0020	F_OEL_2	9	mg/kg	2.11	1.96	1.87	0.15	0.05	1.980	0.121	0.085	1.95	
LC0021	F_OEL_1	9	mg/kg	1.93	1.39	1.37	0.12	0.04	1.563	0.318			
LC0021	F_OEL_2	9	mg/kg	1.37	0.81	1.41	0.12	0.04	1.197	0.335	0.306	1.38	
LC0022	F_OEL_1	9	mg/kg	1.55	1.49	1.73			1.590	0.125			
LC0022	F_OEL_2	9	mg/kg	1.81	2.04	1.92			1.923	0.115	0.168	1.76	
LC0026	F_OEL_1	9	mg/kg	2.51	2.31	2.41			2.410	0.100			
LC0026	F_OEL_2	9	mg/kg	2.04	2.13	1.89			2.020	0.121	0.183	2.22	
LC0027	F_OEL_1	9	mg/kg	1.95	1.65	1.77			1.790	0.151			
LC0027	F_OEL_2	9	mg/kg	1.74	1.70	1.64			1.693	0.050	0.100	1.74	
LC0030	F_OEL_1	9	mg/kg	1.64	1.69	1.42			1.583	0.144			
LC0030	F_OEL_2	9	mg/kg	1.44	1.44	1.47			1.450	0.017	0.100	1.52	
LC0032	F_OEL_1	9	mg/kg	0.86	0.79	0.67	0.09	0.05	0.773	0.096			
LC0032	F_OEL_2	9	mg/kg	0.81	0.83	0.87			0.837	0.031	0.064	0.81	
LC0034	F_OEL_1	9	mg/kg	1.91	2.07	1.92	0.2	0.1	1.967	0.090			
LC0034	F_OEL_2	9	mg/kg	1.99	2.00	2.13	0.2	0.1	2.040	0.078	0.075	2.00	
LC0035	F_OEL_1	9	mg/kg	1.63	1.72	1.77			1.707	0.071			

Table 24: Individual results obtained for Frying Oil Sample "F_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0035	F_OEL_2	9	mg/kg	2.17	1.97	1.85			1.997	0.162	0.156	1.85	
LC0038	F_OEL_1	9	mg/kg	1.61	1.88	2.227	0.15	0.075	1.920	0.332			
LC0038	F_OEL_2	9	mg/kg	2.02	1.74	1.53	0.15	0.075	1.763	0.246	0.247	1.84	
LC0039	F_OEL_1	9	mg/kg	1.70	1.56	1.53	0.2		1.597	0.091			
LC0039	F_OEL_2	9	mg/kg	1.48	1.52	1.54	0.2		1.513	0.031	0.065	1.56	
LC0040	F_OEL_1	9	mg/kg	1.88	1.79	1.90			1.857	0.059			
LC0040	F_OEL_2	9	mg/kg	1.73	1.70	1.70			1.710	0.017	0.069	1.78	
LC0101	F_OEL_1	9	mg/kg	1.61	1.81	1.72		0.1	1.713	0.100			
LC0101	F_OEL_2	9	mg/kg	1.74	1.59	1.88		0.1	1.737	0.145	0.102	1.73	
LC0202	F_OEL_1	9	mg/kg	1.81	2.03	1.49			1.777	0.272			
LC0202	F_OEL_2	9	mg/kg	1.63	1.63	1.40			1.553	0.133	0.197	1.67	
LC0211	F_OEL_1	9	mg/kg	1.73	1.70	1.60			1.677	0.068			
LC0211	F_OEL_2	9	mg/kg	1.55	1.50	1.42			1.490	0.066	0.094	1.58	
LC0213	F_OEL_1	9	mg/kg	1.54	1.47	1.47	0.28		1.493	0.040			
LC0213	F_OEL_2	9	mg/kg	1.69	1.78	1.62	0.28		1.697	0.080	0.098	1.60	
LC0225	F_OEL_1	9	mg/kg	1.51	1.59	1.70	0.15	0.05	1.600	0.095			
LC0225	F_OEL_2	9	mg/kg	1.27	1.19	1.60	0.15	0.05	1.353	0.217	0.170	1.48	
LC0008	F_OEL_1	10	mg/kg	1.42	1.48	1.45			1.450	0.030			
LC0008	F_OEL_2	10	mg/kg	1.40	1.46	1.40			1.420	0.035	0.029	1.44	
LC0015	F_OEL_1	10	mg/kg	2.47	2.85	2.42	0.34	0.23	2.580	0.235			
LC0015	F_OEL_2	10	mg/kg		2.00	1.65	0.34	0.23	1.825	0.247	0.386	2.20	
LC0031	F_OEL_1	10	mg/kg	1.62	1.72	1.66	0.15	0.1	1.667	0.050			
LC0031	F_OEL_2	10	mg/kg	1.56	1.60	1.54	0.15	0.1	1.567	0.031	0.053	1.62	
LC0302	**F_OEL_1**	**10**	**mg/kg**	**1.48**	**1.76**	**1.39**			**1.543**	**0.193**			
LC0302	**F_OEL_2**	**10**	**mg/kg**	**2.91**	**0.97**	**1.14**			**1.673**	**1.074**	**0.632**	**1.61**	**C (PT)**
LC0311	F_OEL_1	10	mg/kg	1.55	1.67	1.55			1.590	0.069			
LC0311	F_OEL_2	10	mg/kg	1.41	1.40	1.44			1.417	0.021	0.082	1.50	
LC0325	F_OEL_1	10	mg/kg	1.48	1.59	1.60	0.15	0.05	1.557	0.067			
LC0325	F_OEL_2	10	mg/kg	1.50	1.45	1.99	0.15	0.05	1.647	0.298	0.180	1.60	

Table 25: Individual results obtained for Vegetable Fat Sample "P_Fett"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0002	P_FETT_1	IM	mg/kg	3.24	3.24	3.54			3.340	0.173			
LC0002	P_FETT_2	IM	mg/kg	3.41	3.43	3.20			3.347	0.127	0.124	3.34	
LC0003	P_FETT_1	IM	mg/kg	3.62	3.85	3.52	0.15	0.11	3.663	0.169			
LC0003	P_FETT_2	IM	mg/kg	3.63	3.70	3.62	0.15	0.11	3.650	0.044	0.101	3.66	
LC0011	P_FETT_1	IM	mg/kg	3.05	3.11	2.98			3.047	0.065			
LC0011	P_FETT_2	IM	mg/kg	2.99	2.59	3.19			2.923	0.306	0.187	2.99	
LC0013	P_FETT_1	IM	mg/kg	3.38	3.38	3.35	0.28		3.370	0.017			
LC0013	P_FETT_2	IM	mg/kg	2.93	2.98	2.78	0.28		2.897	0.104	0.203	3.13	
LC0024	P_FETT_1	IM	mg/kg	3.10	3.02	3.19	0.1	0.05	3.103	0.085			
LC0024	P_FETT_2	IM	mg/kg	2.96	2.75	2.62	0.1	0.05	2.777	0.172	0.173	2.94	
LC0025	P_FETT_1	IM	mg/kg	3.65	3.84		0.15	0.05	3.745	0.134			
LC0025	P_FETT_2	IM	mg/kg	3.41	3.68		0.15	0.05	3.545	0.191	0.154	3.65	
LC0033	P_FETT_1	IM	mg/kg	3.56	3.57	4.14			3.757	0.332			
LC0033	P_FETT_2	IM	mg/kg	3.60	4.00	3.48			3.693	0.272	0.249	3.73	
LC0203	P_FETT_1	IM	mg/kg	3.27	3.90	3.26	0.15	0.05	3.477	0.367			
LC0203	P_FETT_2	IM	mg/kg	3.42	3.97	3.27	0.15	0.05	3.553	0.369	0.302	3.52	
LC0432	P_FETT_1	IM	mg/kg	3.60	3.62	3.42			3.547	0.110			
LC0432	P_FETT_2	IM	mg/kg	3.20	3.37	4.27			3.613	0.575	0.339	3.58	
LC0001	P_FETT_1	8	mg/kg	3.17	3.26	2.88		0.1	3.103	0.199			
LC0001	P_FETT_2	8	mg/kg	2.99	3.07	3.27		0.1	3.110	0.144	0.142	3.11	
LC0019	P_FETT_1	8	mg/kg	3.08	2.91	3.16			3.050	0.128			
LC0019	P_FETT_2	8	mg/kg	2.92	3.19	3.27			3.127	0.183	0.133	3.09	
LC0028	P_FETT_1	8	mg/kg	2.82	2.86	3.08			2.920	0.140			
LC0028	P_FETT_2	8	mg/kg	3.39	3.26	2.75			3.133	0.338	0.229	3.03	
LC0029	**P_FETT_1**	**8**	**mg/kg**	**2.86**	**3.56**	**2.85**	0.3	0.1	**3.090**	**0.407**			
LC0029	**P_FETT_2**	**8**	**mg/kg**	**3.05**	**3.71**	**2.08**	0.3	0.1	**2.947**	**0.820**	**0.532**	**3.02**	**C (M8)**
LC0102	**P_FETT_1**	**8**	**mg/kg**	**4.01**	3.02	3.08			**3.37 (3.050)**	**0.555 (0.042)**			
LC0102	P_FETT_2	8	mg/kg	3.23	3.11	2.95			3.097	0.140	**0.349 (0.094)**	**3.23 (3.08)**	**A (a)**
LC0111	P_FETT_1	8	mg/kg	3.12	3.21	3.18			3.170	0.046			
LC0111	P_FETT_2	8	mg/kg	2.79	2.78	2.92			2.830	0.078	0.148	3.00	
LC0004	P_FETT_1	9	mg/kg	3.00	2.97	2.98	0.25	0.1	2.983	0.015			
LC0004	P_FETT_2	9	mg/kg	2.80	3.01	3.00	0.25	0.1	2.937	0.118	0.072	2.96	
LC0005	P_FETT_1	9	mg/kg	3.40	3.13	3.45	0.8	0.4	3.327	0.172			

Table 25: Individual results obtained for Vegetable Fat Sample "P_Fett" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
LC0005	P_FETT_2	9	mg/kg	3.30	3.43	3.11	0.8	0.4	3.280	0.161	0.137	3.30	
LC0006	P_FETT_1	9	mg/kg	3.97	3.60	4.05			3.873	0.240			
LC0006	P_FETT_2	9	mg/kg	3.54	3.53	3.82			3.630	0.165	0.195	3.75	
LC0007	P_FETT_1	9	mg/kg	3.80	3.51	3.86	0.19	0.05	3.723	0.187			
LC0007	P_FETT_2	9	mg/kg	3.82	3.55	3.85	0.19	0.05	3.740	0.165	0.144	3.73	
LC0009	P_FETT_1	9	mg/kg	3.65	3.31	3.11	0.17	0.05	3.357	0.273			
LC0009	P_FETT_2	9	mg/kg	3.24	3.11	3.60	0.17	0.05	3.317	0.254	0.216	3.34	
LC0010	P_FETT_1	9	mg/kg	4.14	4.36	4.30			4.267	0.114			
LC0010	P_FETT_2	9	mg/kg	3.71	3.68	3.99			3.793	0.171	0.227	4.03	
LC0012	P_FETT_1	9	mg/kg	3.47	3.46	3.43			3.453	0.021			
LC0012	P_FETT_2	9	mg/kg	3.13	3.40	3.24			3.257	0.136	0.113	3.36	
LC0016	P_FETT_1	9	mg/kg	3.16	2.84	3.31	0.24		3.103	0.240			
LC0016	P_FETT_2	9	mg/kg	3.37	3.37	3.32	0.24		3.353	0.029	0.173	3.23	
LC0017	P_FETT_1	9	mg/kg	3.20	3.30	3.32			3.273	0.064			
LC0017	P_FETT_2	9	mg/kg	3.14	3.08	3.11			3.110	0.030	0.078	3.19	
LC0018	P_FETT_1	9	mg/kg	2.86	2.56	2.65			2.690	0.154			
LC0018	P_FETT_2	9	mg/kg	2.74	2.86	2.79			2.797	0.060	0.105	2.74	
LC0020	P_FETT_1	9	mg/kg	3.23	3.32	2.72	0.15	0.05	3.090	0.324			
LC0020	P_FETT_2	9	mg/kg	2.94	3.31	3.20	0.15	0.05	3.150	0.190	0.218	3.12	
LC0021	P_FETT_1	9	mg/kg	2.60	3.00	2.33	0.12	0.04	2.643	0.337			
LC0021	P_FETT_2	9	mg/kg	2.77	3.08	3.11	0.12	0.04	2.987	0.188	0.263	2.82	
LC0022	P_FETT_1	9	mg/kg	3.08	3.11	3.18			3.123	0.051			
LC0022	P_FETT_2	9	mg/kg	3.42	3.70	3.73			3.617	0.171	0.226	3.37	
LC0026	P_FETT_1	9	mg/kg	5.25	4.98	4.81			5.013	0.222			
LC0026	P_FETT_2	9	mg/kg	4.68	4.23	4.45			4.453	0.225	0.292	4.73	
LC0027	P_FETT_1	9	mg/kg	2.87	2.92	3.01			2.933	0.071			
LC0027	P_FETT_2	9	mg/kg	3.41	3.45	4.01			3.623	0.335	0.344	3.28	
LC0030	P_FETT_1	9	mg/kg	4.29	3.94	3.53			3.920	0.380			
LC0030	P_FETT_2	9	mg/kg	3.49	3.53	3.20			3.407	0.180	0.321	3.66	
LC0032	P_FETT_1	9	mg/kg	1.37	1.40	1.69	0.09	0.05	1.487	0.177			
LC0032	P_FETT_2	9	mg/kg	1.82	1.57	1.70			1.697	0.125	0.152	1.59	
LC0034	P_FETT_1	9	mg/kg	3.91	4.00	3.82	0.2	0.1	3.910	0.090			
LC0034	P_FETT_2	9	mg/kg	4.05	4.00	3.97	0.2	0.1	4.007	0.040	0.069	3.96	
LC0035	P_FETT_1	9	mg/kg	3.32	3.40	3.16			3.293	0.122			
LC0035	P_FETT_2	9	mg/kg	3.38	3.43	3.53			3.447	0.076	0.104	3.37	

Table 25: Individual results obtained for Vegetable Fat Sample "P_Fett" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
LC0038	P_FETT_1	9	mg/kg	5.21	4.40	3.91	0.15	0.075	4.507	0.657			
LC0038	P_FETT_2	9	mg/kg	4.77	3.88	3.55	0.15	0.075	4.067	0.631	0.556	4.29	
LC0039	P_FETT_1	9	mg/kg	3.19	2.78	3.45	0.2		3.140	0.338			
LC0039	P_FETT_2	9	mg/kg	3.39	3.81	3.01	0.2		3.403	0.400	0.321	3.27	
LC0040	P_FETT_1	9	mg/kg	3.80	3.77	3.56			3.710	0.131			
LC0040	P_FETT_2	9	mg/kg	3.15	3.01	3.15			3.103	0.081	0.263	3.41	
LC0101	P_FETT_1	9	mg/kg	3.90	3.57	4.85		0.1	4.107	0.665			
LC0101	P_FETT_2	9	mg/kg	3.99	3.70	4.21		0.1	3.967	0.256	0.415	4.04	
LC0202	**P_FETT_1**	**9**	**mg/kg**	**3.97**	**4.32**	**2.76**			**3.683**	**0.819**			
LC0202	**P_FETT_2**	**9**	**mg/kg**	**3.85**	**2.64**	**2.77**			**3.087**	**0.664**	**0.656**	**3.39**	**C (M9, PT)**
LC0211	P_FETT_1	9	mg/kg	3.61	3.34	3.36			3.437	0.150			
LC0211	P_FETT_2	9	mg/kg	3.05	2.86	3.08			2.997	0.119	0.211	3.22	
LC0213	P_FETT_1	9	mg/kg	2.87	3.05	3.02	0.28		2.980	0.096			
LC0213	P_FETT_2	9	mg/kg	3.30	3.24	3.14	0.28		3.227	0.081	0.124	3.10	
LC0225	P_FETT_1	9	mg/kg	2.57	2.82	2.81	0.15	0.05	2.733	0.142			
LC0225	P_FETT_2	9	mg/kg	3.05	4.09	3.77	0.15	0.05	3.637	0.533	0.487	3.19	
LC0008	P_FETT_1	10	mg/kg	2.69	2.92	2.91			2.840	0.130			
LC0008	P_FETT_2	10	mg/kg	2.60	2.62	2.87			2.697	0.150	0.129	2.77	
LC0015	P_FETT_1	10	mg/kg	4.78			0.34	0.23	4.780				
LC0015	P_FETT_2	10	mg/kg		3.58	4.42	0.34	0.23	4.000	0.594	0.586	4.39	
LC0031	P_FETT_1	10	mg/kg	3.84	3.70	3.75	0.15	0.1	3.763	0.071			
LC0031	P_FETT_2	10	mg/kg	3.66	3.55	3.40	0.15	0.1	3.537	0.131	0.126	3.65	
LC0302	**P_FETT_1**	**10**	**mg/kg**	**3.38**					**3.167**	**0.187**			
LC0302	**P_FETT_2**	**10**	**mg/kg**	**1.91**					**2.943**	**1.066**	**0.632**	**3.06**	**C (PT)**
LC0311	P_FETT_1	10	mg/kg	2.81					2.873	0.110			
LC0311	P_FETT_2	10	mg/kg	3.14					3.100	0.087	0.123	2.99	
LC0325	P_FETT_1	10	mg/kg	3.28	3.37	3.03	3.09	0.05	3.373	0.095			
LC0325	P_FETT_2	10	mg/kg	3.20	4.13	4.04	2.88	0.05	3.820	0.537	0.364	3.60	

Table 26: Individual results obtained for Grape Seed Oil Sample "T_Oel"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0002	T_OEL_1	IM	mg/kg	3.66	3.38	3.82			3.620	0.223			
LC0002	T_OEL_2	IM	mg/kg	4.03	3.85	3.53			3.803	0.253	0.209	3.71	
LC0003	T_OEL_1	IM	mg/kg	4.00	3.81	3.86	0.15	0.11	3.890	0.098			
LC0003	T_OEL_2	IM	mg/kg	3.89	3.85	3.74	0.15	0.11	3.827	0.078	0.077	3.86	
LC0011	T_OEL_1	IM	mg/kg	3.59	3.62	3.66			3.623	0.035			
LC0011	T_OEL_2	IM	mg/kg	3.54	3.41	3.81			3.587	0.204	0.120	3.61	
LC0013	T_OEL_1	IM	mg/kg	4.04	4.04	3.92	0.28		4.000	0.069			
LC0013	T_OEL_2	IM	mg/kg	3.48	3.61	3.42	0.28		3.503	0.097	0.214	3.75	
LC0024	T_OEL_1	IM	mg/kg	3.83	3.86	4.22	0.1	0.05	3.970	0.217			
LC0024	T_OEL_2	IM	mg/kg	4.21	3.66	3.57	0.1	0.05	3.813	0.346	0.245	3.89	
LC0025	T_OEL_1	IM	mg/kg	4.83	4.64		0.15	0.05	4.735	0.134			
LC0025	T_OEL_2	IM	mg/kg	4.60	4.61		0.15	0.05	4.605	0.007	0.094	4.67	
LC0033	T_OEL_1	IM	mg/kg	3.32	3.36	3.54			3.407	0.117			
LC0033	T_OEL_2	IM	mg/kg	3.19	3.17	4.01			3.457	0.479	0.286	3.43	
LC0203	T_OEL_1	IM	mg/kg	3.80	3.74	3.88	0.15	0.05	3.807	0.070			
LC0203	T_OEL_2	IM	mg/kg	3.97	3.79	3.82	0.15	0.05	3.860	0.096	0.072	3.83	
LC0432	T_OEL_1	IM	mg/kg	4.44	4.83	5.02			4.763	0.296			
LC0432	T_OEL_2	IM	mg/kg	3.97	4.27	3.32			3.853	0.486	0.496	4.31	
LC0001	T_OEL_1	8	mg/kg	4.10	3.32	2.96		0.1	3.460	0.583			
LC0001	T_OEL_2	8	mg/kg	3.74	3.10	2.97		0.1	3.270	0.412	0.419	3.37	
LC0019	T_OEL_1	8	mg/kg	3.54	3.66	3.75			3.650	0.105			
LC0019	T_OEL_2	8	mg/kg	3.62	3.54	3.78			3.647	0.122	0.093	3.65	
LC0028	T_OEL_1	8	mg/kg	3.21	3.43	3.83			3.490	0.314			
LC0028	T_OEL_2	8	mg/kg	3.36	3.58	3.58			3.507	0.127	0.196	3.50	
LC0029	T_OEL_1	8	mg/kg	2.92	2.80	3.23	0.3	0.1	2.983	0.222			
LC0029	T_OEL_2	8	mg/kg	3.21	3.05	4.16	0.3	0.1	3.473	0.600	0.420	3.23	
LC0102	T_OEL_1	8	mg/kg	4.29	3.82	4.01			4.040	0.236			
LC0102	T_OEL_2	8	mg/kg	3.87	3.50	3.73			3.700	0.187	0.223	3.87	
LC0111	T_OEL_1	8	mg/kg	3.57	3.69	3.53			3.597	0.083			
LC0111	T_OEL_2	8	mg/kg	3.23	3.38	3.22			3.277	0.090	0.149	3.44	
LC0004	T_OEL_1	9	mg/kg	3.60	3.43	3.52	0.25	0.1	3.517	0.085			
LC0004	T_OEL_2	9	mg/kg	3.34	3.18	3.23	0.25	0.1	3.250	0.082	0.128	3.38	
LC0005	T_OEL_1	9	mg/kg	3.11	4.24	4.21	0.8	0.4	3.853	0.644			
LC0005	T_OEL_2	9	mg/kg	4.81	4.18	5.38	0.8	0.4	4.790	0.600	0.636	4.32	
LC0006	T_OEL_1	9	mg/kg	4.32	4.53	4.18			4.343	0.176			

Table 26: Individual results obtained for Grape Seed Oil Sample "T_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0006	T_OEL_2	9	mg/kg	4.21	4.72	4.36			4.430	0.262	0.186	4.39	
LC0007	T_OEL_1	9	mg/kg	4.59	3.81	4.29	0.19	0.05	4.230	0.393			
LC0007	T_OEL_2	9	mg/kg	3.96	3.72	3.62	0.19	0.05	3.767	0.175	0.312	4.00	
LC0009	T_OEL_1	9	mg/kg	4.02	4.17	4.35	0.17	0.05	4.180	0.165			
LC0009	T_OEL_2	9	mg/kg	4.09	4.63	4.51	0.17	0.05	4.410	0.284	0.211	4.30	
LC0010	T_OEL_1	9	mg/kg	4.90	4.99	4.98			4.957	0.049			
LC0010	T_OEL_2	9	mg/kg	4.49	4.54	4.55			4.527	0.032	0.179	4.74	
LC0012	T_OEL_1	9	mg/kg	3.95	3.99	3.94			3.960	0.026			
LC0012	T_OEL_2	9	mg/kg	3.75	3.68	3.67			3.700	0.044	0.110	3.83	
LC0016	T_OEL_1	9	mg/kg	3.82	3.37	3.49	0.24		3.560	0.233			
LC0016	T_OEL_2	9	mg/kg	3.73	4.11	4.04	0.24		3.960	0.202	0.242	3.76	
LC0017	T_OEL_1	9	mg/kg	3.84	3.81	3.78			3.810	0.030			
LC0017	T_OEL_2	9	mg/kg	3.78	3.97	3.76			3.837	0.116	0.070	3.82	
LC0018	T_OEL_1	9	mg/kg	3.09	3.01	2.92			3.007	0.085			
LC0018	T_OEL_2	9	mg/kg	3.17	3.26	3.21			3.213	0.045	0.101	3.11	
LC0020	T_OEL_1	9	mg/kg	3.39	3.72	4.13	0.15	0.05	3.747	0.371			
LC0020	T_OEL_2	9	mg/kg	4.12	3.99	4.07	0.15	0.05	4.060	0.066	0.252	3.90	
LC0021	T_OEL_1	9	mg/kg	3.10	3.15	2.63			2.960	0.287			
LC0021	T_OEL_2	9	mg/kg	2.88	3.33	3.82			3.343	0.470	0.354	3.15	
LC0022	T_OEL_1	9	mg/kg	4.06	3.96	3.81			3.943	0.126			
LC0022	T_OEL_2	9	mg/kg	3.99	4.05	4.16			4.067	0.086	0.101	4.01	
LC0026	T_OEL_1	9	mg/kg	5.60	5.60	5.80			5.667	0.115			
LC0026	T_OEL_2	9	mg/kg	5.70	5.94	5.36			5.667	0.291	0.222	5.67	
LC0027	T_OEL_1	9	mg/kg	3.77	3.83	3.47			3.690	0.193			
LC0027	T_OEL_2	9	mg/kg	4.41	4.31	4.28			4.333	0.068	0.288	4.01	
LC0030	T_OEL_1	9	mg/kg	3.90	3.58	3.96			3.813	0.204			
LC0030	T_OEL_2	9	mg/kg	4.00	3.78	3.97			3.917	0.119	0.143	3.87	
LC0032	T_OEL_1	9	mg/kg	1.98	1.87	1.60	0.09	0.05	1.817	0.196			
LC0032	T_OEL_2	9	mg/kg	1.63	1.79	1.80			1.740	0.095	0.129	1.78	
LC0034	T_OEL_1	9	mg/kg	4.78	4.72	4.75	0.2	0.1	4.750	0.030			
LC0034	T_OEL_2	9	mg/kg	4.75	4.84	4.99	0.2	0.1	4.860	0.121	0.085	4.81	
LC0035	T_OEL_1	9	mg/kg	3.92	3.84	3.66			3.807	0.133			
LC0035	T_OEL_2	9	mg/kg	4.51	4.24	4.47			4.407	0.146	0.270	4.11	
LC0038	**T_OEL_1**	**9**	**mg/kg**	**3.68**	**4.19**	**6.51**	0.15	0.075	**4.793**	**1.508**			
LC0038	**T_OEL_2**	**9**	**mg/kg**	**5.16**	**3.54**	**4.10**	0.15	0.075	**4.267**	**0.823**	**1.015**	**4.53**	**C (M9, PT)**

Table 26: Individual results obtained for Grape Seed Oil Sample "T_Oel" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0039	T_OEL_1	9	mg/kg	3.73	3.73	3.80	0.2		3.753	0.040			
LC0039	**T_OEL_2**	**9**	**mg/kg**	3.85	3.82	**4.85**	0.2		**4.173 (3.835**	**0.586 (0.021)**	**0.380 (0.046)**	**3.96 (3.79)**	**A (M9, PT)**
LC0040	T_OEL_1	9	mg/kg	4.22	4.41	4.60			4.410	0.190			
LC0040	T_OEL_2	9	mg/kg	4.14	5.36	4.28			4.593	0.668	0.408	4.50	
LC0101	T_OEL_1	9	mg/kg	3.99	4.37	4.81		0.1	4.390	0.410			
LC0101	T_OEL_2	9	mg/kg	4.08	3.87	4.51		0.1	4.153	0.326	0.318	4.27	
LC0202	T_OEL_1	9	mg/kg	4.88	4.48	3.69			4.350	0.606			
LC0202	T_OEL_2	9	mg/kg	3.98	2.74	3.03			3.250	0.649	0.681	3.80	
LC0211	T_OEL_1	9	mg/kg	3.83	3.98	3.86			3.890	0.079			
LC0211	T_OEL_2	9	mg/kg	3.75	3.72	3.85			3.773	0.068	0.077	3.83	
LC0213	T_OEL_1	9	mg/kg	3.65	3.57	3.47	0.28		3.563	0.090			
LC0213	T_OEL_2	9	mg/kg	3.91	3.95	3.79	0.28		3.883	0.083	0.149	3.72	
LC0225	T_OEL_1	9	mg/kg	3.85	3.98	3.61	0.15	0.05	3.813	0.188			
LC0225	T_OEL_2	9	mg/kg	4.45	3.96	3.67	0.15	0.05	4.027	0.394	0.267	3.92	
LC0008	T_OEL_1	10	mg/kg	3.31	3.80	3.27			3.460	0.295			
LC0008	T_OEL_2	10	mg/kg	3.32	3.38	3.08			3.260	0.159	0.210	3.36	
LC0015	**T_OEL_1**	**10**	**mg/kg**	**5.78**	**6.70**	**6.21**	0.34	0.23	**6.230**	**0.460**			
LC0015	**T_OEL_2**	**10**	**mg/kg**	**4.82**	**4.36**	**4.31**	0.34	0.23	**4.497**	**0.281**	**0.773**	**5.36**	**C (PT)**
LC0031	T_OEL_1	10	mg/kg	4.00	4.10	4.18	0.15	0.1	4.093	0.090			
LC0031	T_OEL_2	10	mg/kg	4.06	3.80	3.88	0.15	0.1	3.913	0.133	0.118	4.00	
LC0302	**T_OEL_1**	**10**	**mg/kg**	**3.62**	**4.43**	**3.50**			**3.850**	**0.506**			
LC0302	**T_OEL_2**	**10**	**mg/kg**	**2.07**	**4.71**	**2.58**			**3.120**	**1.400**	**0.910**	**3.49**	**C (PT)**
LC0311	T_OEL_1	10	mg/kg	3.47	3.54	3.47			3.493	0.040			
LC0311	T_OEL_2	10	mg/kg	3.49	3.50	3.53			3.507	0.021	0.027	3.50	
LC0325	T_OEL_1	10	mg/kg	3.79	3.64	3.50	0.15	0.05	3.643	0.145			
LC0325	T_OEL_2	10	mg/kg	4.08	4.17	3.76	0.15	0.05	4.003	0.215	0.210	3.82	

Table 27: Individual results obtained for control sample "CONT"

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0002	CONT_20	IM	mg/kg	2.76	2.59	2.88			2.743	0.146			
LC0002	CONT_21	IM	mg/kg	3.05	2.88	2.93			2.953	0.087	0.130	2.85	
LC0003	CONT_20	IM	mg/kg	3.13	3.11	2.97	0.15	0.11	3.070	0.087			
LC0003	CONT_21	IM	mg/kg	3.01	3.13	2.99	0.15	0.11	3.043	0.076	0.068	3.06	
LC0011	CONT_20	IM	mg/kg	2.50	2.50				2.500	0.000			
LC0011	CONT_21	IM	mg/kg	2.59	2.55				2.570	0.028	0.038	2.54	
LC0013	CONT_20	IM	mg/kg	2.80	2.88	2.85	0.28		2.843	0.040			
LC0013	CONT_21	IM	mg/kg	2.46	2.44	2.49	0.28		2.463	0.025	0.158	2.65	
LC0024	CONT_20	IM	mg/kg	2.61			0.1	0.05	2.610				
LC0024	CONT_21	IM	mg/kg	2.78			0.1	0.05	2.780		0.120	2.70	
LC0025	CONT_20	IM	mg/kg	3.46	3.39		0.15	0.05	3.425	0.049			
LC0025	CONT_21	IM	mg/kg				0.15	0.05			0.049	3.43	
LC0033	CONT_20	IM	mg/kg	2.98	2.95	2.78			2.903	0.108			
LC0033	CONT_21	IM	mg/kg								0.108	2.90	
LC0203	CONT_20	IM	mg/kg	3.19	2.84	3.00	0.15	0.05	3.010	0.175			
LC0203	CONT_21	IM	mg/kg	3.00	3.10	2.94	0.15	0.05	3.013	0.081	0.111	3.01	
LC0432	CONT_20	IM	mg/kg		3.40	3.31			3.355	0.064			
LC0432	CONT_21	IM	mg/kg								0.064	3.36	
LC0001	**CONT_20**	**8**	**mg/kg**	**4.07**	**1.91**	**2.80**		0.1	**2.927**	**1.086**			
LC0001	**CONT_21**	**8**	**mg/kg**	**2.54**	**2.88**	**3.47**		0.1	**2.963**	**0.471**	**0.683**	**2.95**	**C (PT)**
LC0019	CONT_20	8	mg/kg	2.53	2.58				2.555	0.035			
LC0019	CONT_21	8	mg/kg	2.54	2.63				2.585	0.064	0.039	2.57	
LC0028	CONT_20	8	mg/kg	3.39	2.54	2.69			2.873	0.454			
LC0028	CONT_21	8	mg/kg	2.59	2.56	2.76			2.637	0.108	0.286	2.76	
LC0029	CONT_20	8	mg/kg	1.98	2.43	2.63	0.3	0.1	2.347	0.333			
LC0029	CONT_21	8	mg/kg	3.08	2.47	2.51	0.3	0.1	2.687	0.341	0.308	2.52	
LC0102	CONT_20	8	mg/kg	3.21	2.58	2.42			2.737	0.418			
LC0102	CONT_21	8	mg/kg	2.71	2.52				2.615	0.134	0.280	2.69	
LC0111	CONT_20	8	mg/kg	2.68	2.66				2.670	0.014			
LC0111	CONT_21	8	mg/kg	2.77	2.93				2.850	0.113	0.107	2.76	
LC0004	CONT_20	9	mg/kg	2.71			0.25	0.1	2.710				
LC0004	CONT_21	9	mg/kg	2.51			0.25	0.1	2.510		0.141	2.61	
LC0005	CONT_20	9	mg/kg	2.83	3.06		0.8	0.4	2.945	0.163			
LC0005	CONT_21	9	mg/kg	3.22	3.03		0.8	0.4	3.125	0.134	0.139	3.04	
LC0006	CONT_20	9	mg/kg	3.01	2.95	3.06			3.007	0.055			

Table 27: Individual results obtained for control sample "CONT" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
C0006	CONT_21	9	mg/kg								0.055	3.01	
LC0007	CONT_20	9	mg/kg	2.68	3.06	2.98	0.19	0.05	2.907	0.200			
LC0007	CONT_21	9	mg/kg				0.19	0.05			0.200	2.91	
LC0009	CONT_20	9	mg/kg	2.76	2.81	2.97	0.17	0.05	2.847	0.110			
LC0009	CONT_21	9	mg/kg	2.78	2.83	2.99	0.17	0.05	2.867	0.110	0.090	2.86	
LC0010	CONT_20	9	mg/kg	3.65	3.56	3.50			3.570	0.075			
LC0010	CONT_21	9	mg/kg	3.44	3.35	3.26			3.350	0.090	0.113	3.46	
LC0012	CONT_20	9	mg/kg	2.99	2.97	3.02			2.993	0.025			
LC0012	CONT_21	9	mg/kg	2.71	3.00	2.88			2.863	0.146	0.101	2.93	
LC0016	CONT_20	9	mg/kg	3.72	2.81	3.65	0.24		3.393	0.506			
LC0016	CONT_21	9	mg/kg	2.64	2.50	2.53	0.24		2.557	0.074	0.452	2.98	
LC0017	CONT_20	9	mg/kg	2.76					2.760				
LC0017	CONT_21	9	mg/kg	2.76					2.760		0.000	2.76	
LC0018	CONT_20	9	mg/kg	2.37					2.370				
LC0018	CONT_21	9	mg/kg	2.32					2.320		0.035	2.35	
LC0020	CONT_20	9	mg/kg	2.60	2.60	2.76	0.15	0.05	2.653	0.092			
LC0020	CONT_21	9	mg/kg	3.04	3.04	2.94	0.15	0.05	3.007	0.058	0.157	2.83	
LC0021	**CONT_20**	**9**	**mg/kg**	**3.97**	**2.64**	**2.22**	0.12	0.04	**2.943**	**0.914**			
LC0021	**CONT_21**	**9**	**mg/kg**				0.12	0.04			**0.914**	**2.94**	**C (M9, PT)**
LC0022	CONT_20	9	mg/kg	2.78					2.780				
LC0022	CONT_21	9	mg/kg	2.89					2.890		0.078	2.84	
LC0026	CONT_20	9	mg/kg	3.48	3.21	3.44			3.377	0.146			
LC0026	CONT_21	9	mg/kg	3.23	2.97	3.19			3.130	0.140	0.154	3.25	
LC0027	CONT_20	9	mg/kg	2.96	2.93				2.945	0.021			
LC0027	CONT_21	9	mg/kg								0.021	2.95	
LC0030	CONT_20	9	mg/kg	2.86	2.72	2.74			2.773	0.076			
LC0030	CONT_21	9	mg/kg	2.69	2.78	2.94			2.803	0.127	0.086	2.79	
LC0032	CONT_20	9	mg/kg	0.98	1.13	1.08	0.09	0.05	1.063	0.076			
LC0032	CONT_21	9	mg/kg								0.076	1.06	
LC0034	CONT_20	9	mg/kg	3.30	3.28	3.27	0.2	0.1	3.283	0.015			
LC0034	CONT_21	9	mg/kg	3.29	3.44	3.15	0.2	0.1	3.293	0.145	0.084	3.29	
LC0035	CONT_20	9	mg/kg	3.02	2.89	2.97			2.960	0.066			
LC0035	CONT_21	9	mg/kg	3.00	2.92	3.10			3.007	0.090	0.067	2.98	
LC0038	CONT_20	9	mg/kg	3.57	3.50	2.76	0.15	0.075	3.277	0.449			
LC0038	CONT_21	9	mg/kg				0.15	0.075			0.449	3.28	

Table 27: Individual results obtained for control sample "CONT" (cont.)

LCode	Sample aliquot	AM	Measuring unit	Single determinations			LOQ	LOD	MV	SD	SD sample	MV sample	Type of outlier
				1	2	3							
LC0039	CONT_20	9	mg/kg	2.88			0.2		2.880				
LC0039	CONT_21	9	mg/kg	2.75			0.2		2.750		0.092	2.82	
LC0040	CONT_20	9	mg/kg	2.96	2.95	3.10			3.003	0.084			
LC0040	CONT_21	9	mg/kg								0.084	3.00	
LC0101	**CONT_20**	**9**	**mg/kg**	**4.69**	**2.72**	**2.22**		0.1	**3.210**	**1.306**			
LC0101	**CONT_21**	**9**	**mg/kg**	**3.25**	**2.94**	**3.74**		0.1	**3.310**	**0.403**	**0.790**	**3.26**	**C (M9, PT)**
LC0202	**CONT_20**	**9**	**mg/kg**	**3.87**	**3.74**	**2.67**			**3.427**	**0.659**			
LC0202	**CONT_21**	**9**	**mg/kg**	**2.79**	**2.39**	**2.39**			**2.523**	**0.231**	**0.546**	**2.98**	**C (M9, PT)**
LC0211	CONT_20	9	mg/kg	2.98	3.39				3.185	0.290			
LC0211	CONT_21	9	mg/kg	2.99	2.88				2.935	0.078	0.195	3.06	
LC0213	CONT_20	9	mg/kg	2.39	2.49	2.40	0.28		2.427	0.055			
LC0213	CONT_21	9	mg/kg	3.02	2.94	3.07	0.28		3.010	0.066	0.243	2.72	
LC0225	CONT_20	9	mg/kg	2.43	2.55	2.74	0.15	0.05	2.573	0.156			
LC0226	CONT_20	9	mg/kg				0.15	0.05			0.156	2.57	
LC0008	CONT_20	10	mg/kg	2.61	2.39	2.49			2.497	0.110			
LC0008	CONT_21	10	mg/kg								0.110	2.50	
LC0015	CONT_20	10	mg/kg	3.22			0.34	0.23	3.220				
LC0015	CONT_21	10	mg/kg	3.81			0.34	0.23	3.810		0.417	3.52	
LC0031	CONT_20	10	mg/kg	2.85	3.20	3.05	0.15	0.1	3.033	0.176			
LC0031	CONT_21	10	mg/kg				0.15	0.1			0.176	3.03	
LC0302	CONT_20	10	mg/kg	2.20	3.41	2.60			2.737	0.616			
LC0302	CONT_21	10	mg/kg	2.03	2.36				2.195	0.233	0.475	2.52	
LC0311	CONT_20	10	mg/kg	2.13	2.31				2.220	0.127			
LC0311	CONT_21	10	mg/kg	2.55	2.60				2.575	0.035	0.189	2.40	
LC0325	CONT_20	10	mg/kg	2.83	2.96	2.89	0.15	0.05	2.893	0.065			
LC0325	CONT_20	10	mg/kg				0.15	0.05			0.065	2.89	

Table 28: z-Scores of the laboratories for materials L_Oel, B_Fett, F_Oel, P_Fett, T_Oel

Laboratory-code	AM	B_Fett			L_Oel			F_Oel			P_Fett			T_Oel		
		SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score
LC0002	IM	0.133	0.92	0.27	0.093	0.42	0.74	0.173	1.78	0.30	0.124	3.34	-0.06	0.209	3.71	-0.34
LC0003	IM	0.034	0.85	-0.10	0.006	0.20	-0.62	0.017	1.64	-0.19	0.101	3.66	0.35	0.077	3.86	-0.07
LC0011	IM	0.021	0.76	-0.57	0.016	0.18	-0.70	0.065	1.54	-0.53	0.187	2.99	-0.53	0.120	3.61	-0.53
LC0013	IM	0.074	0.74	-0.64				0.078	1.50	-0.66	0.203	3.13	-0.34	0.214	3.75	-0.26
LC0024	IM	0.057	0.85	-0.09	0.020	0.25	-0.32	0.057	1.78	0.30	0.173	2.94	-0.59	0.245	3.89	-0.01
LC0025	IM	0.068	0.83	-0.22	0.004	0.17	-0.76	0.183	1.79	0.33	0.154	3.65	0.33	0.094	4.67	1.37
LC0033	IM	0.107	1.18	1.59	0.071	0.52	1.30	0.188	2.08	1.35	0.249	3.73	0.44	0.286	3.43	-0.84
LC0203	IM	0.053	0.82	-0.27	0.009	0.19	-0.65	0.047	1.63	-0.21	0.302	3.52	0.16	0.072	3.83	-0.12
LC0432	IM	0.090	0.79	-0.42	1.228	1.14	5.00	0.790	2.10	1.44	0.339	3.58	0.25	0.496	4.31	0.73
LC0001	8	0.117	0.90	0.13				0.157	1.34	-1.24	0.142	3.11	-0.37	0.419	3.37	-0.95
LC0019	8	0.042	0.84	-0.15	0.010	0.14	-0.96	0.241	1.74	0.16	0.133	3.09	-0.39	0.093	3.65	-0.45
LC0028	8	0.157	1.15	1.40	0.028	0.40	0.60	0.060	1.64	-0.17	0.229	3.03	-0.47	0.196	3.50	-0.72
LC0029	8	0.100	0.58	-1.48				0.189	1.23	-1.60	0.532	3.02	-0.49	0.420	3.23	-1.20
LC0102	8	0.169	0.78	-0.45	0.050	0.28	-0.15	0.192	1.62	-0.26	0.349	3.23	-0.20	0.223	3.87	-0.05
LC0111	8	0.026	0.71	-0.80	0.022	0.21	-0.53	0.095	1.47	-0.78	0.148	3.00	-0.51	0.149	3.44	-0.83
LC0004	9	0.015	0.69	-0.91				0.037	1.45	-0.84	0.072	2.96	-0.56	0.128	3.38	-0.92
LC0005	9	0.288	1.07	1.03				0.210	1.91	0.78	0.137	3.30	-0.11	0.636	4.32	0.75
LC0006	9	0.059	0.99	0.61	0.035	0.21	-0.52	0.115	1.87	0.62	0.195	3.75	0.47	0.186	4.39	0.87
LC0007	9	0.120	0.91	0.19	0.145	0.74	2.60	0.354	2.01	1.13	0.144	3.73	0.45	0.312	4.00	0.18
LC0009	9	0.067	0.78	-0.47	0.012	0.19	-0.69	0.150	1.73	0.13	0.216	3.34	-0.07	0.211	4.30	0.70
LC0010	9	0.150	1.21	1.74	0.197	0.75	2.70	0.074	2.13	1.54	0.227	4.03	0.84	0.179	4.74	1.50
LC0012	9	0.038	0.84	-0.18	0.006	0.22	-0.46	0.051	1.68	-0.03	0.113	3.36	-0.05	0.110	3.83	-0.12
LC0016	9	0.033	0.79	-0.40		0.27	-0.18	0.055	1.62	-0.26	0.173	3.23	-0.21	0.242	3.76	-0.25
LC0017	9	0.022	0.78	-0.46	0.032	0.20	-0.63	0.137	1.85	0.57	0.078	3.19	-0.26	0.070	3.82	-0.14
LC0018	9	0.077	0.73	-0.70	0.104	0.35	0.27	0.234	1.22	-1.65	0.105	2.74	-0.84	0.101	3.11	-1.41
LC0020	9	0.185	1.42	2.80	0.261	0.76	2.78	0.085	1.95	0.92	0.218	3.12	-0.35	0.252	3.90	0.01
LC0021	9	0.365	0.85	-0.10				0.306	1.38	-1.08	0.263	2.82	-0.75	0.354	3.15	-1.34
LC0022	9	0.083	0.97	0.50	0.009	0.28	-0.14	0.168	1.76	0.23	0.226	3.37	-0.03	0.101	4.01	0.19
LC0026	9	0.057	1.07	1.00	0.025	0.31	0.05	0.183	2.22	1.83	0.292	4.73	1.75	0.222	5.67	3.15
LC0027	9	0.066	0.80	-0.38	0.033	0.18	-0.75	0.100	1.74	0.18	0.344	3.28	-0.15	0.288	4.01	0.20
LC0030	9	0.128	0.98	0.53	0.059	0.43	0.76	0.100	1.52	-0.60	0.321	3.66	0.36	0.143	3.87	-0.06
LC0032	9	0.090	0.43	-2.25	0.028	0.11	-1.14	0.064	0.81	-3.08	0.152	1.59	-2.35	0.129	1.78	-3.79
LC0034	9	0.067	0.96	0.47	0.009	0.21	-0.53	0.075	2.00	1.09	0.069	3.96	0.74	0.085	4.81	1.61
LC0035	9	0.038	0.91	0.20	0.033	0.37	0.44	0.156	1.85	0.56	0.104	3.37	-0.03	0.270	4.11	0.37

Table 28: z-Scores of the laboratories for materials L_Oel, B_Fett, F_Oel, P_Fett, T_Oel (cont.)

Laboratory-code	AM	B_Fett			L_Oel			F_Oel			P_Fett			T_Oel		
		SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score	SD sample	3-MCPD (mg/kg)	z-Score
LC0038	9	0.142	0.87	-0.01		0.16	-0.84	0.247	1.84	0.53	0.556	4.29	1.17	1.015	4.53	1.12
Table 28: z-Scores of the laboratories for materials L_Oel, B_Fett, F_Oel, P_Fett, T_Oel (cont.)																
LC0039	9	0.067	0.84	-0.14	0.045	0.25	-0.31	0.065	1.56	-0.47	0.321	3.27	-0.15	0.380	3.96	0.11
LC0040	9	0.083	1.04	0.88	0.148	0.57	1.62	0.069	1.78	0.33	0.263	3.41	0.02	0.408	4.50	1.07
LC0101	9	0.203	1.26	1.99				0.102	1.73	0.12	0.415	4.04	0.84	0.318	4.27	0.66
LC0202	9	0.140	0.92	0.24	0.042	0.28	-0.14	0.197	1.67	-0.09	0.656	3.39	-0.01	0.681	3.80	-0.18
LC0211	9	0.050	0.81	-0.33	0.004	0.25	-0.32	0.094	1.58	-0.37	0.211	3.22	-0.23	0.077	3.83	-0.12
LC0213	9	0.041	0.77	-0.50				0.098	1.60	-0.33	0.124	3.10	-0.37	0.149	3.72	-0.32
LC0225	9	0.037	0.73	-0.71	0.037	0.27	-0.20	0.170	1.48	-0.74	0.487	3.19	-0.27	0.267	3.92	0.04
LC0008	10	0.014	0.76	-0.54	0.010	0.27	-0.17	0.029	1.44	-0.89	0.129	2.77	-0.81	0.210	3.36	-0.96
LC0015	10	0.368	1.20	1.66		0.37	0.42	0.386	2.20	1.78	0.586	4.39	1.31	0.773	5.36	2.61
LC0031	10	0.118	0.83	-0.20	0.042	0.23	-0.40	0.053	1.62	-0.26	0.126	3.65	0.34	0.118	4.00	0.18
LC0302	10	0.172	0.67	-1.04	0.363	0.41	0.68	0.632	1.61	-0.28	0.632	3.06	-0.44	0.910	3.49	-0.74
LC0311	10	0.047	0.73	-0.70	0.026	0.21	-0.55	0.082	1.50	-0.65	0.123	2.99	-0.53	0.027	3.50	-0.71
LC0325	10	0.125	0.88	0.05	0.086	0.35	0.32	0.180	1.60	-0.31	0.364	3.60	0.27	0.210	3.82	-0.14

Outliers (LC0032 was not included in the calculation of statistical values since systematic errors during analysis were observed.)

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List of Abbreviations for Table 22 to Table 28

AM		Analytical method
	IM	Own in-house method of the laboratory
	8	BfR Method 8
	9	BfR Method 9
	10	BfR Method 10
LOQ		Limit of quantification
MV		Mean value of individual determinations in the respective subsample
LOD		Limit of detection
MV Sample		Mean concentration in the overall sample (composed by subsample A and B) analyzed on day 1 and day 2
SD		Standard deviation of each sample aliquot
SD Sample		Standard deviation of the laboratory
Type of outlier A		Grubbs' outliers of type A (within laboratory)
	(a)	The values put in brackets are found when Grubbs' outliers of type A are taken into account. They were included in the calculation of the statistical values.
	C	Cochran outliers
		(M8) outliers identified by calculation for BfR Method 8
		(M9) outliers identified by calculation for BfR Method 9
		(PT) outliers identified by calculation for the proficiency test

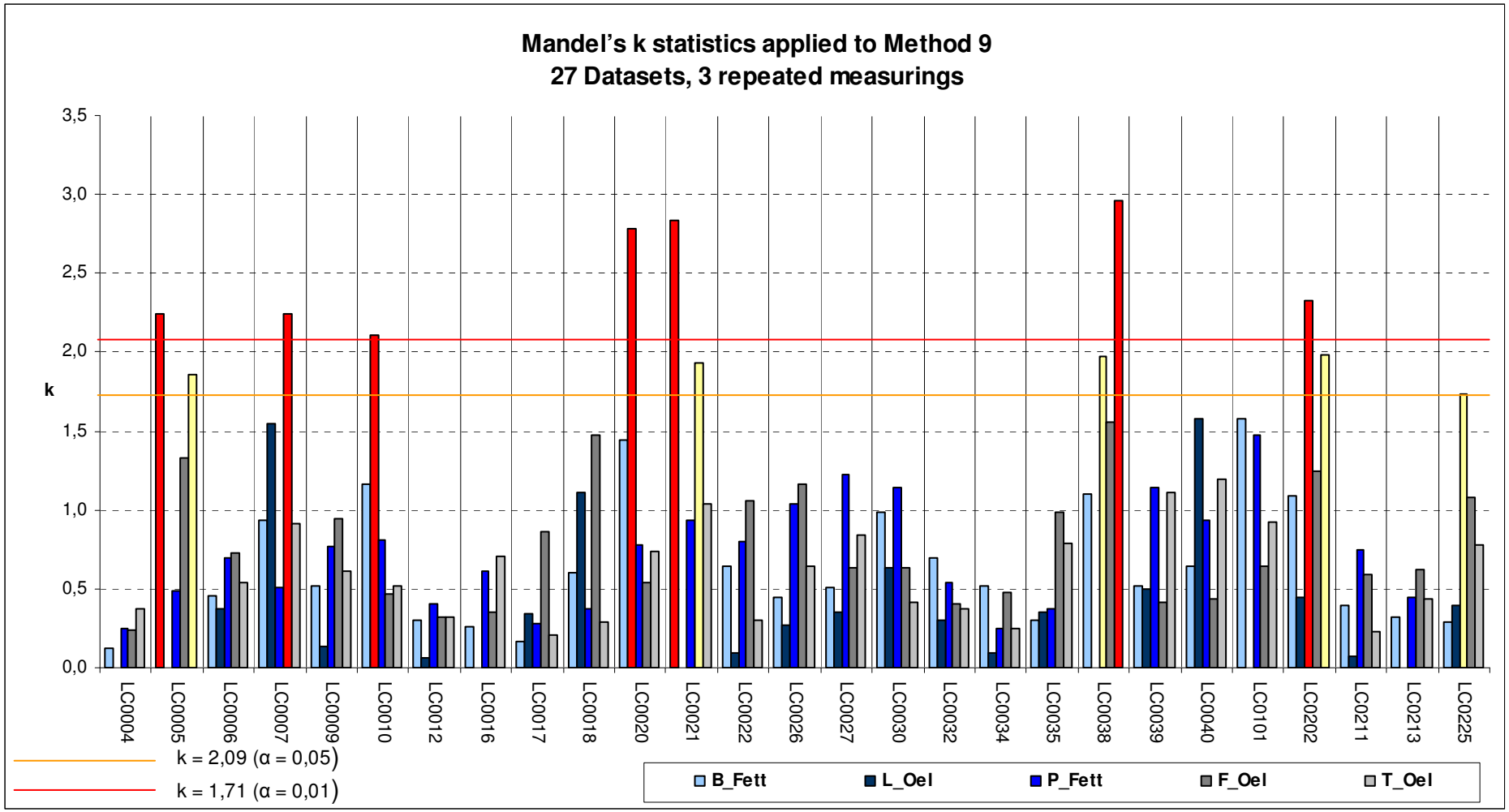

Figure 7: Representation of the intra-laboratory variance in the individual laboratories when using Method 9, by means of the within-laboratory consistency statistics *k* (Mandel's *k* statistics)

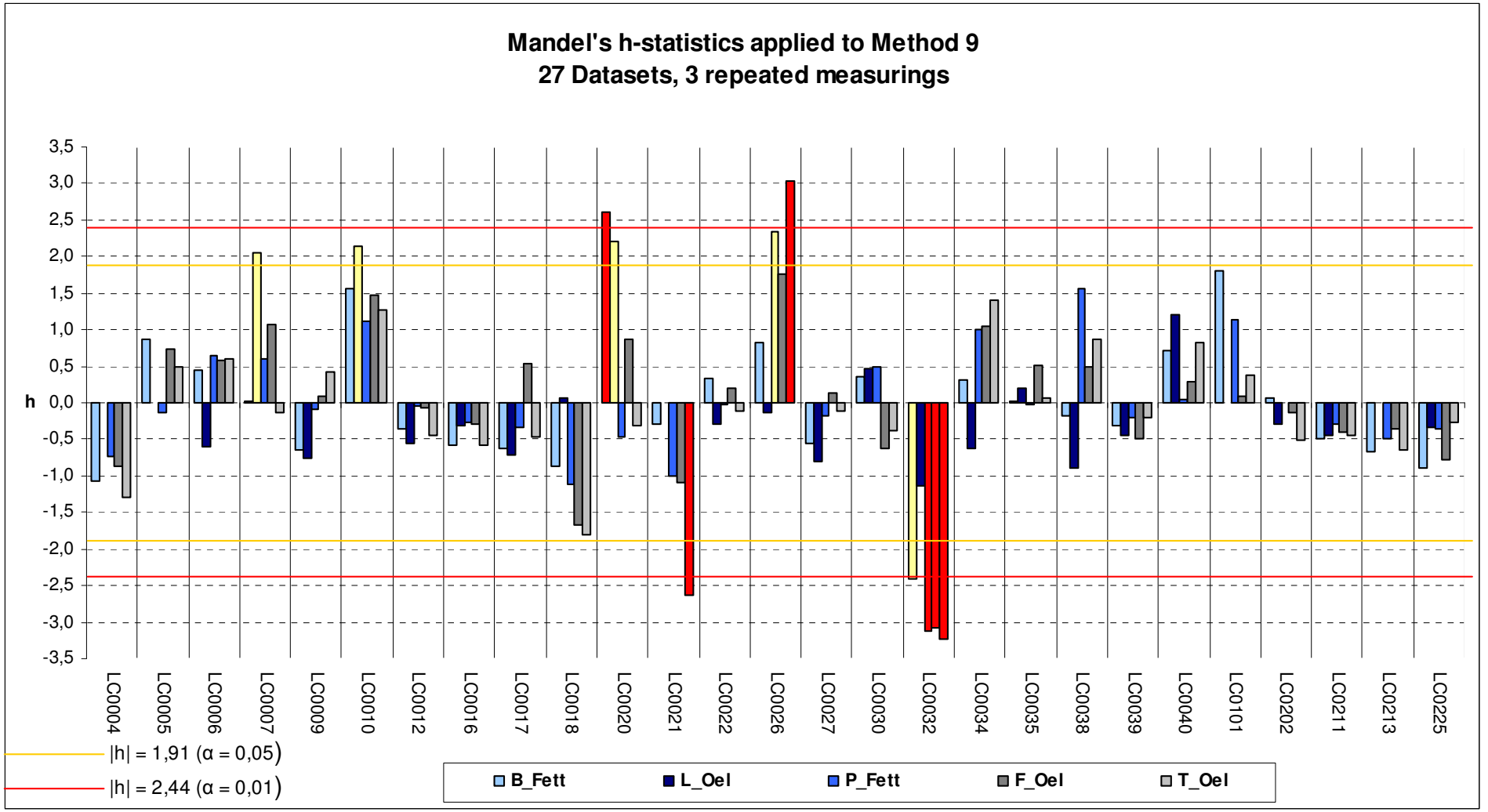

Figure 8: Representation of the deviation of the mean values of the individual laboratories from the total mean value when using Method 9, by means of the between-laboratory consistency statistics h (Mandel's h statistics)

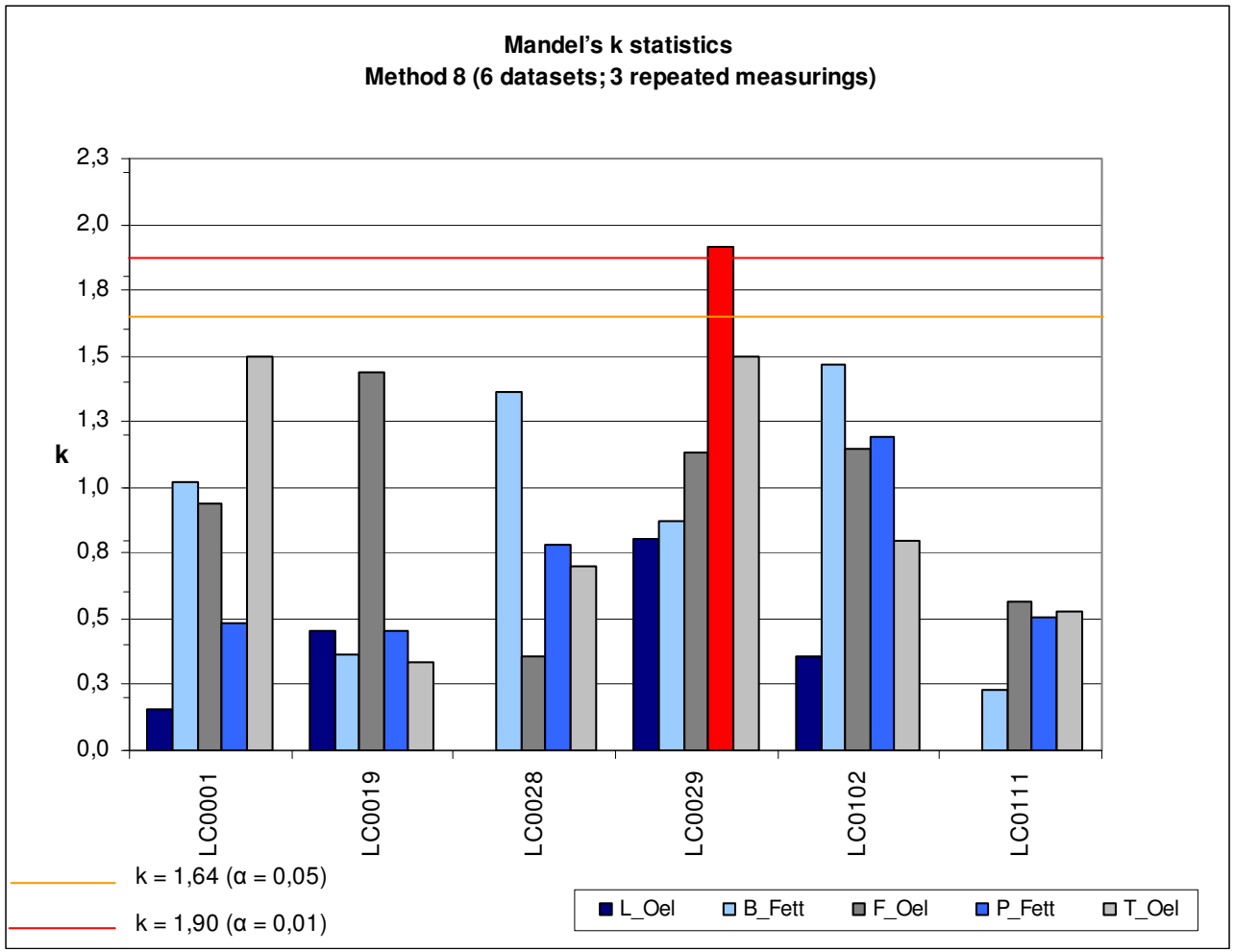

Figure 9: Presentation of the intra-laboratory variance in the individual laboratories when using Method 8, by means of the within-laboratory consistency statistics k (Mandel's k statistics)

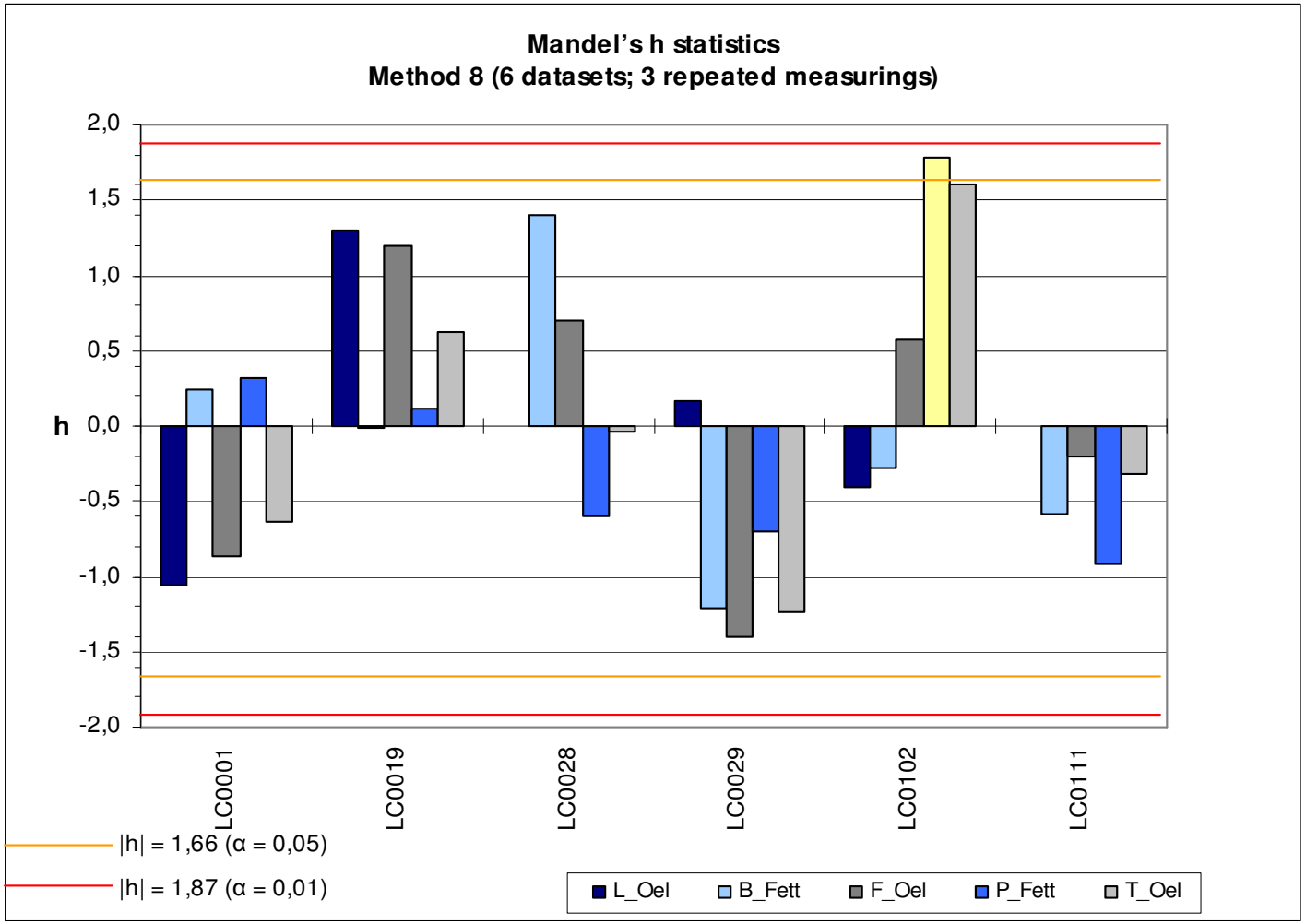

Figure 10: Representation of the deviation of the mean values of the individual laboratories from the total mean value when using Method 8, by means of the between-laboratory consistency statistics *h* (Mandel's *h* statistics)

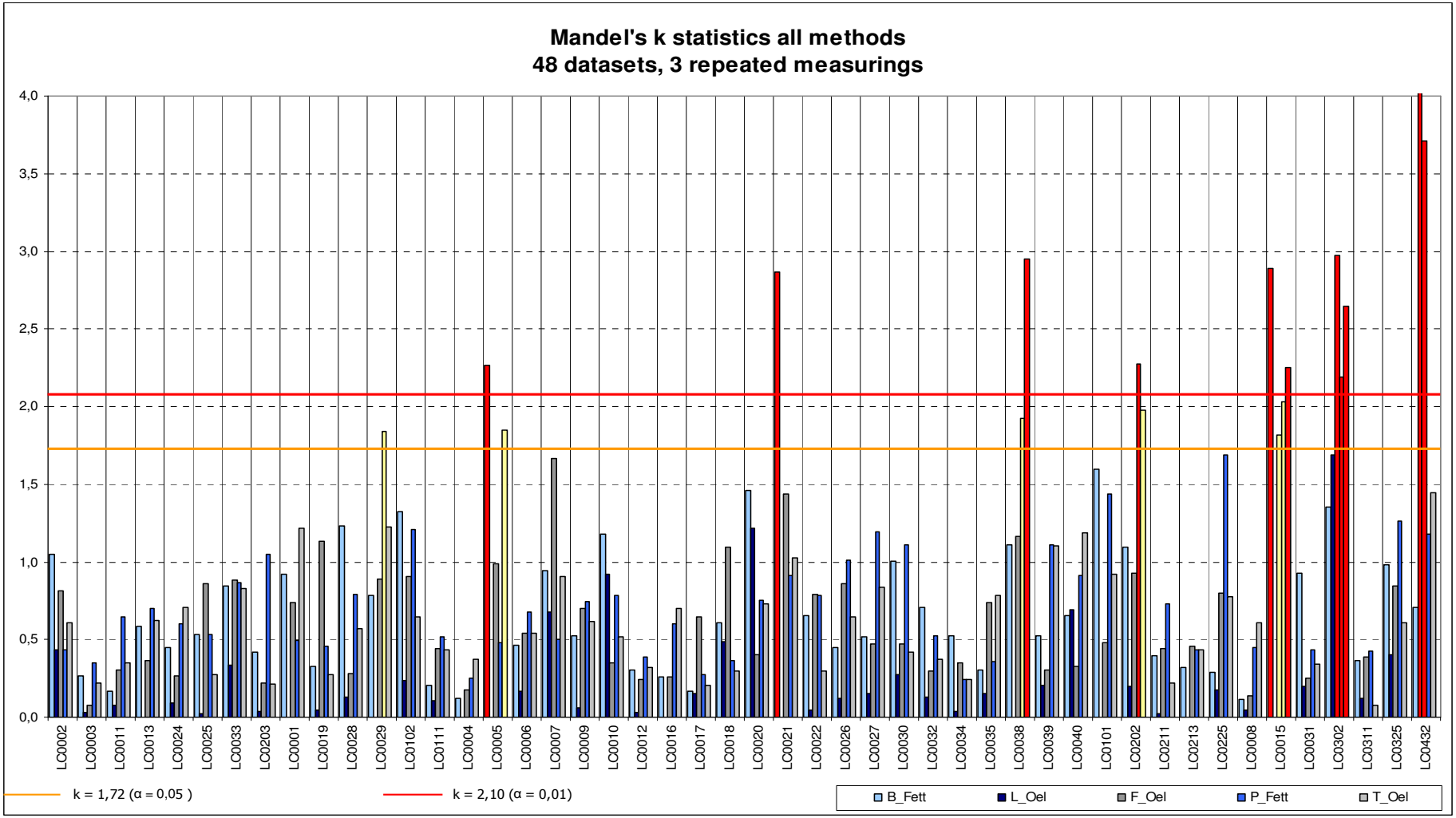

Figure 11: Representation of the intra-laboratory variance in the individual laboratories within the proficiency test, by means of the within-laboratory consistency statistics *k* (Mandel's *k* statistics)

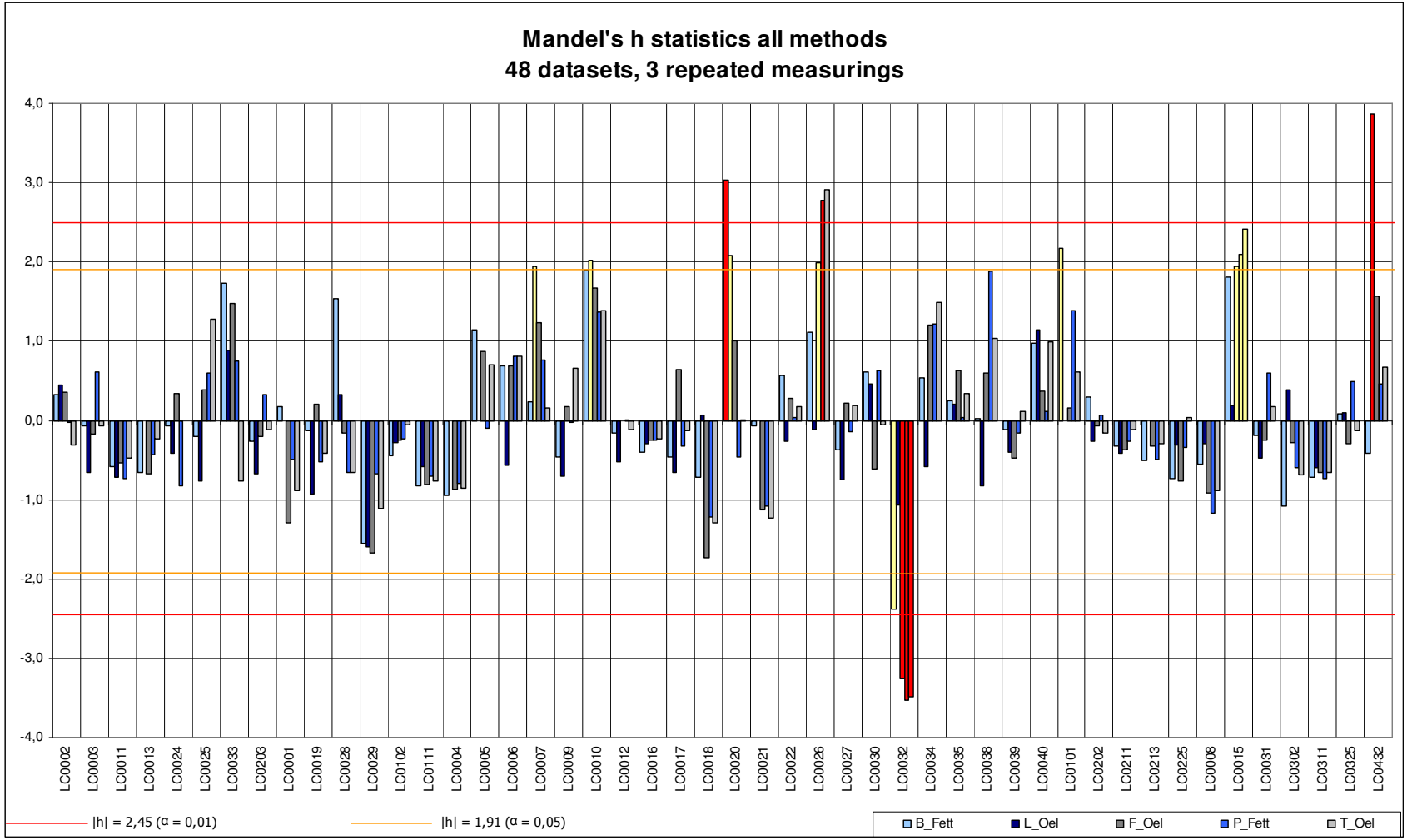

Figure 12: Representation of the deviation of the mean values of the individual laboratories from the total mean value within the proficiency test, by means of the between-laboratory consistency statistics *h* (Mandel's *h* statisics

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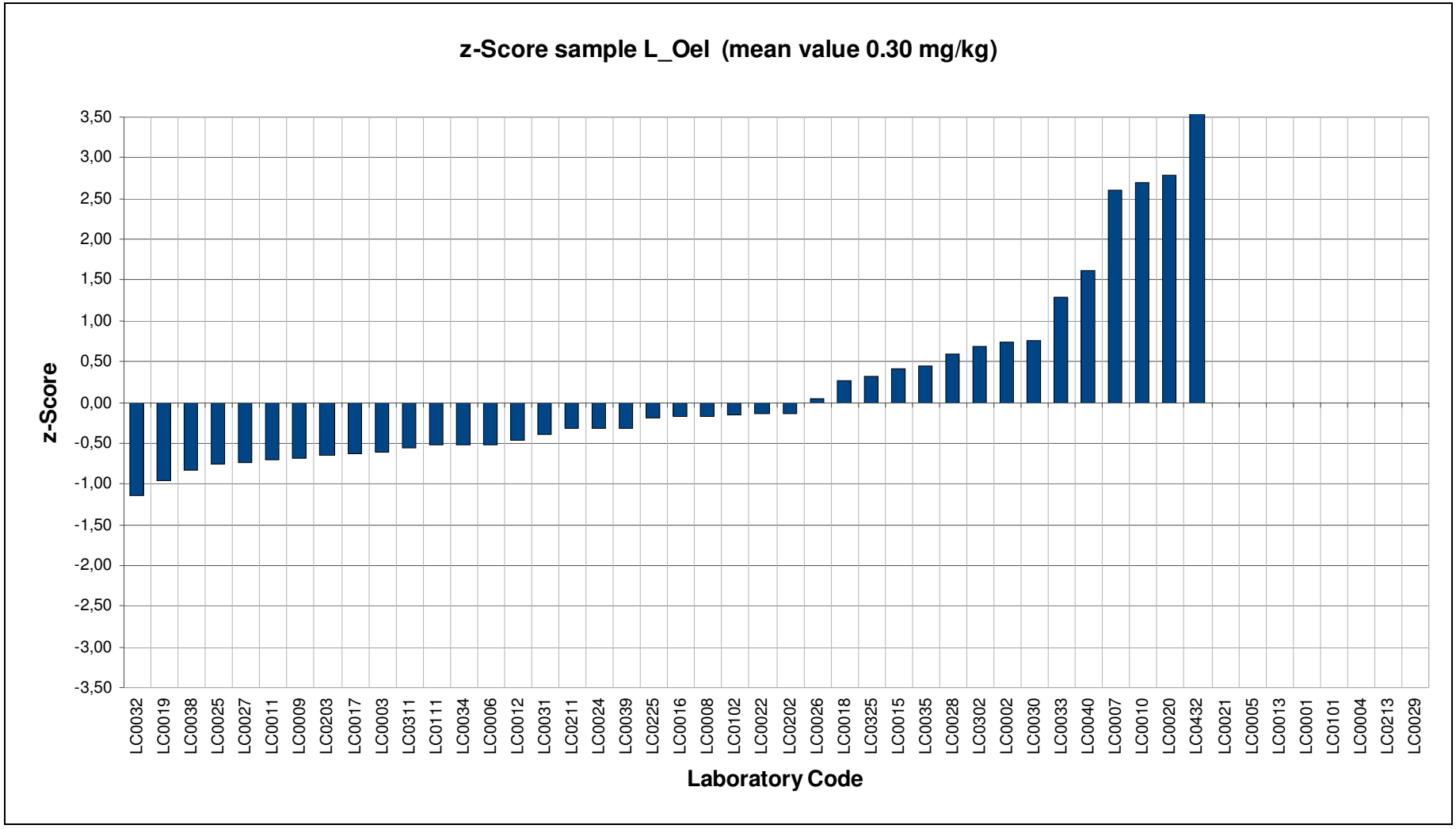


Figure 13: z-Scores obtained by participants for sample L_Oel (arranged in ascending order)

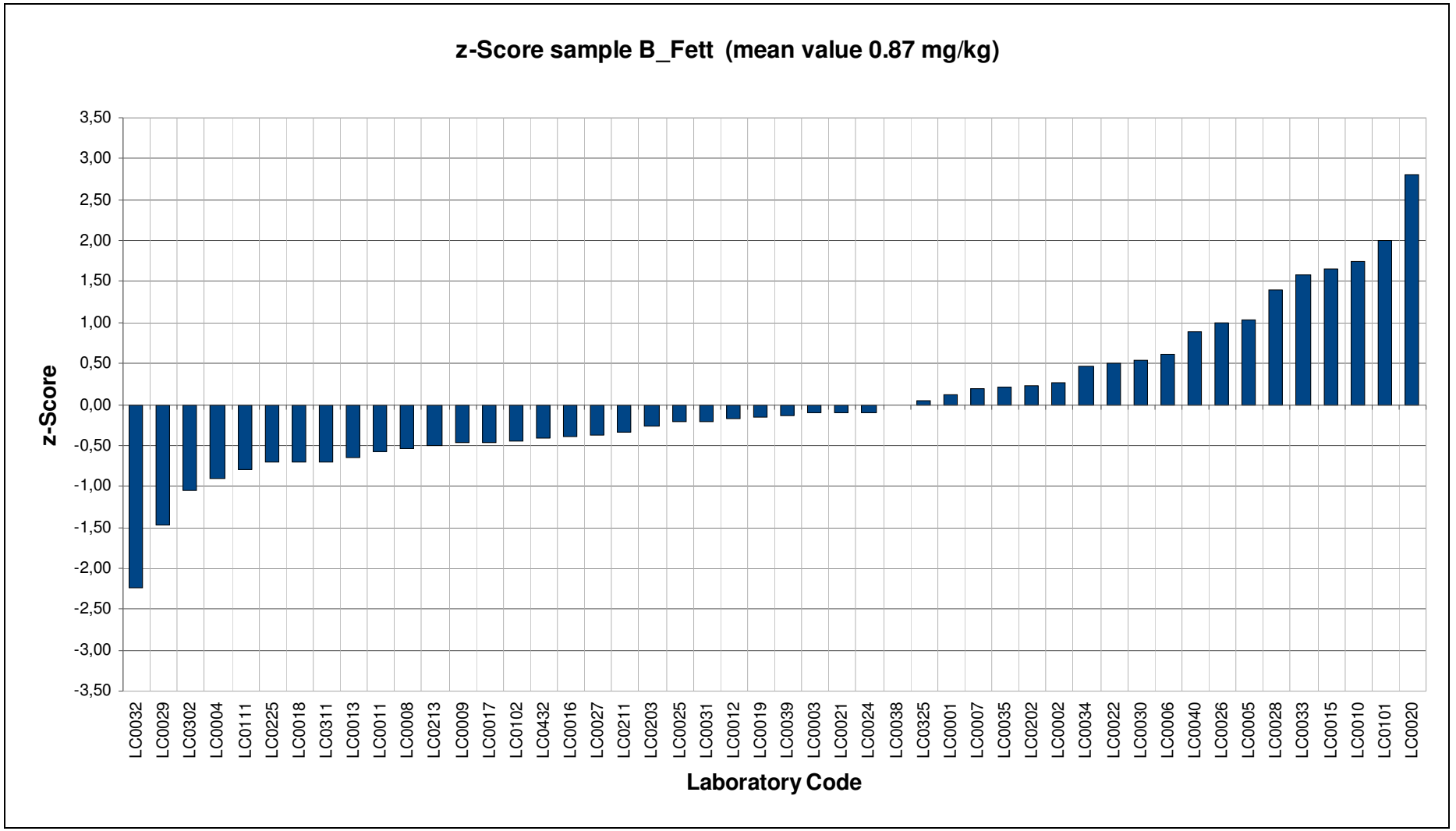


Figure 14: z-Scores obtained by participants for sample B_Fett (arranged in ascending order)

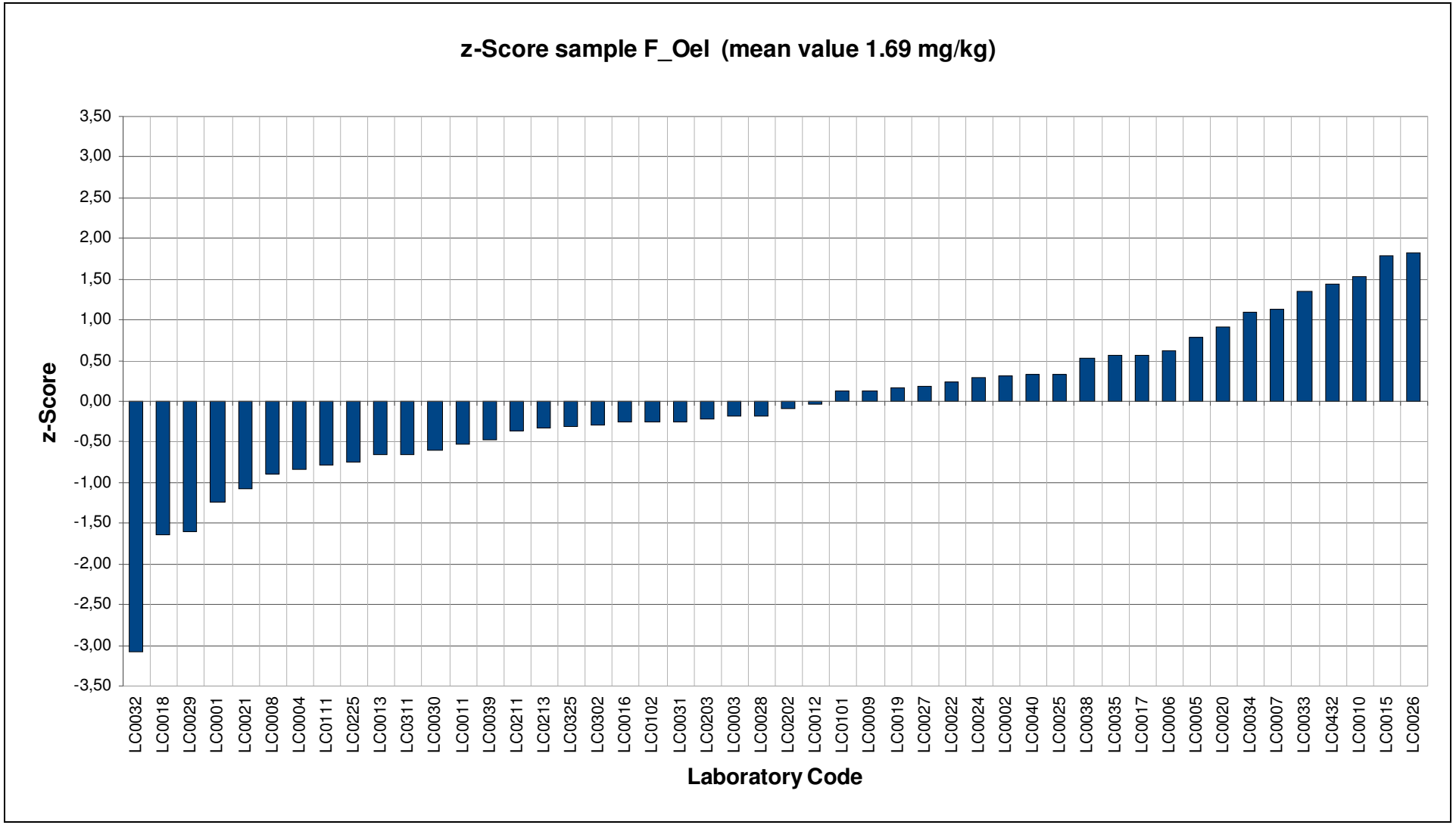


Figure 15: z-Scores obtained by participants for sample F_Oel (arranged in ascending order)

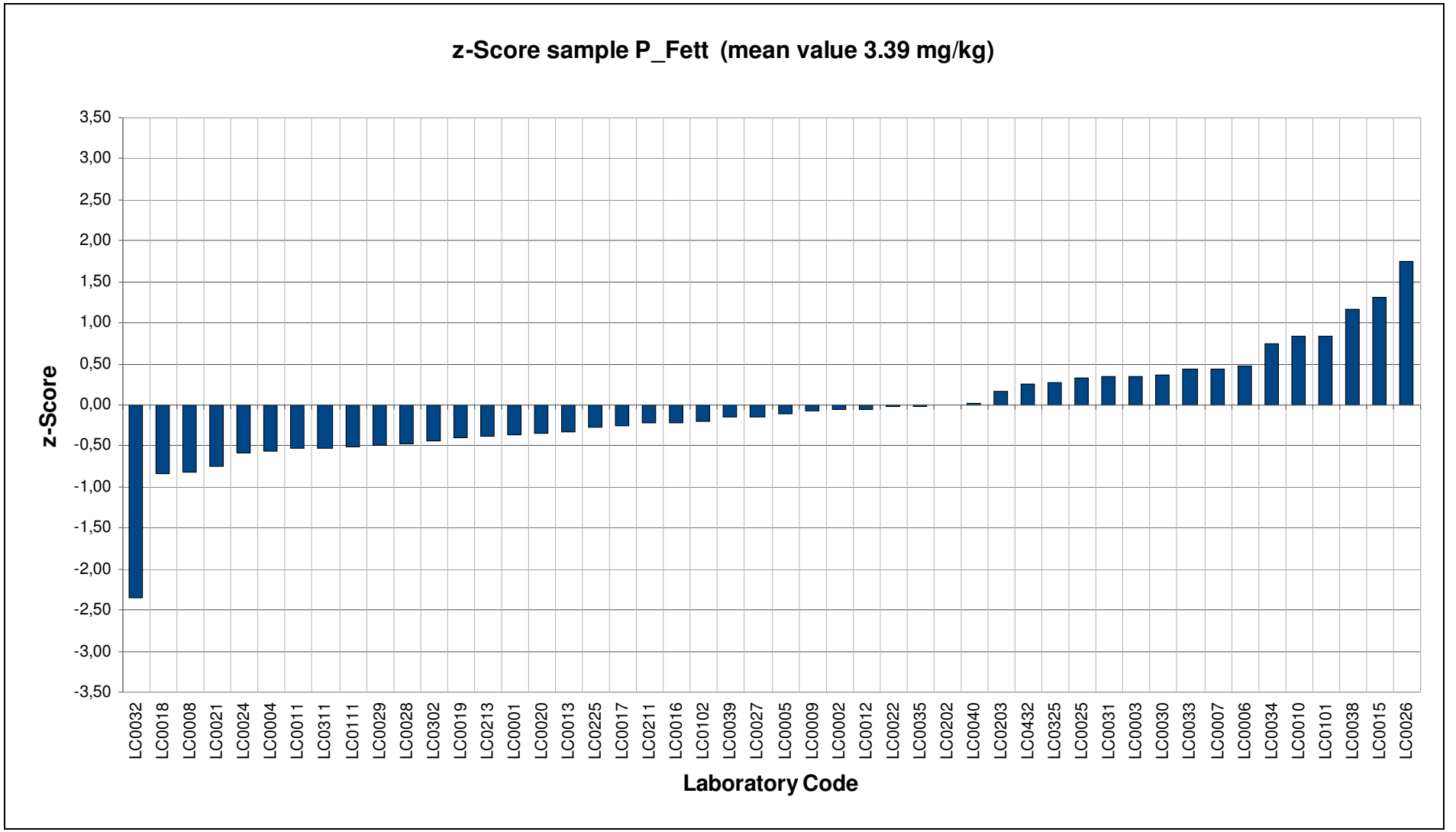


Figure 16: z-Scores obtained by participants for sample P_Fett (arranged in ascending order)

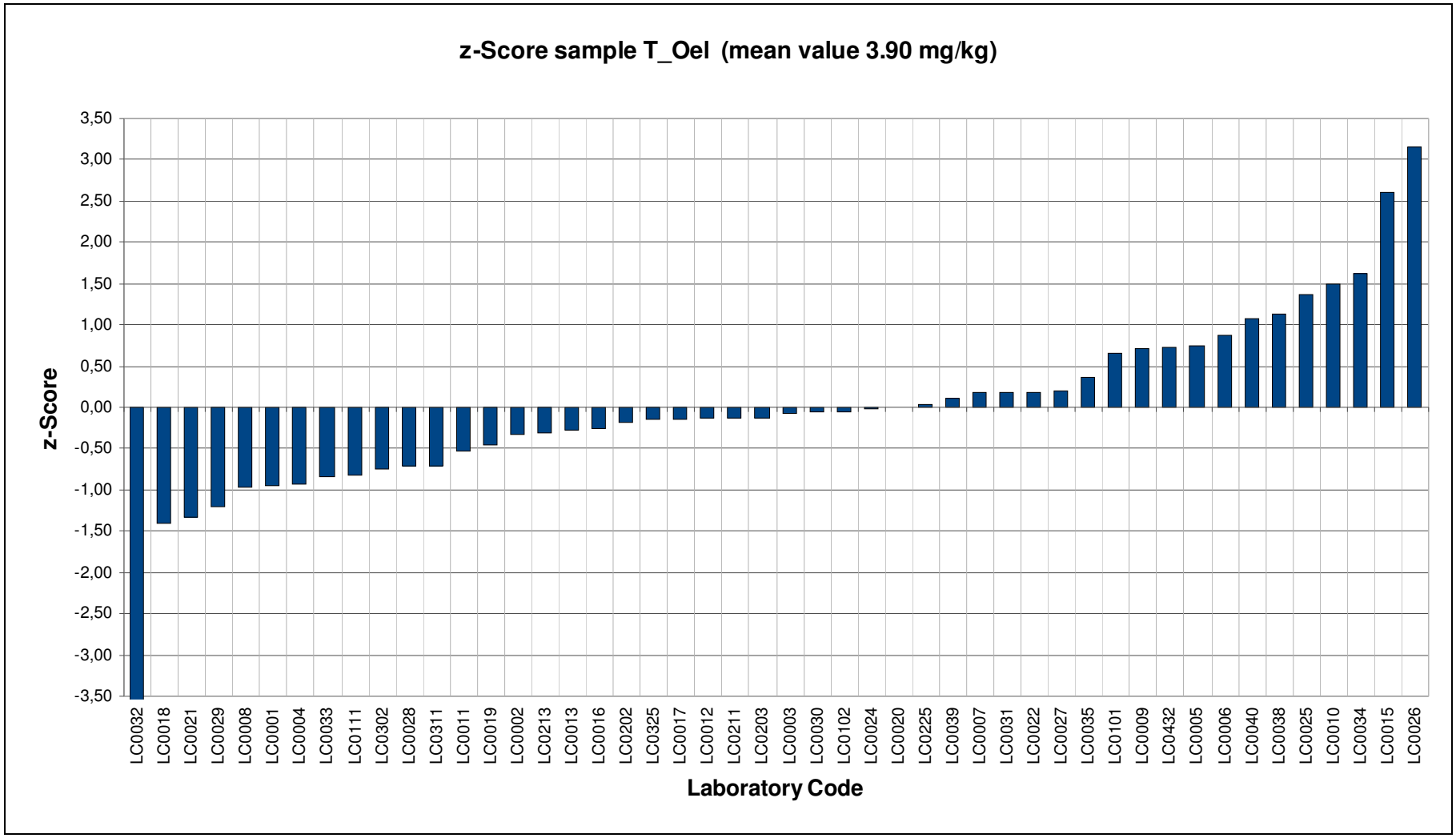


Figure 17: z-Scores obtained by participants for sample T_Oel (arranged in ascending order)

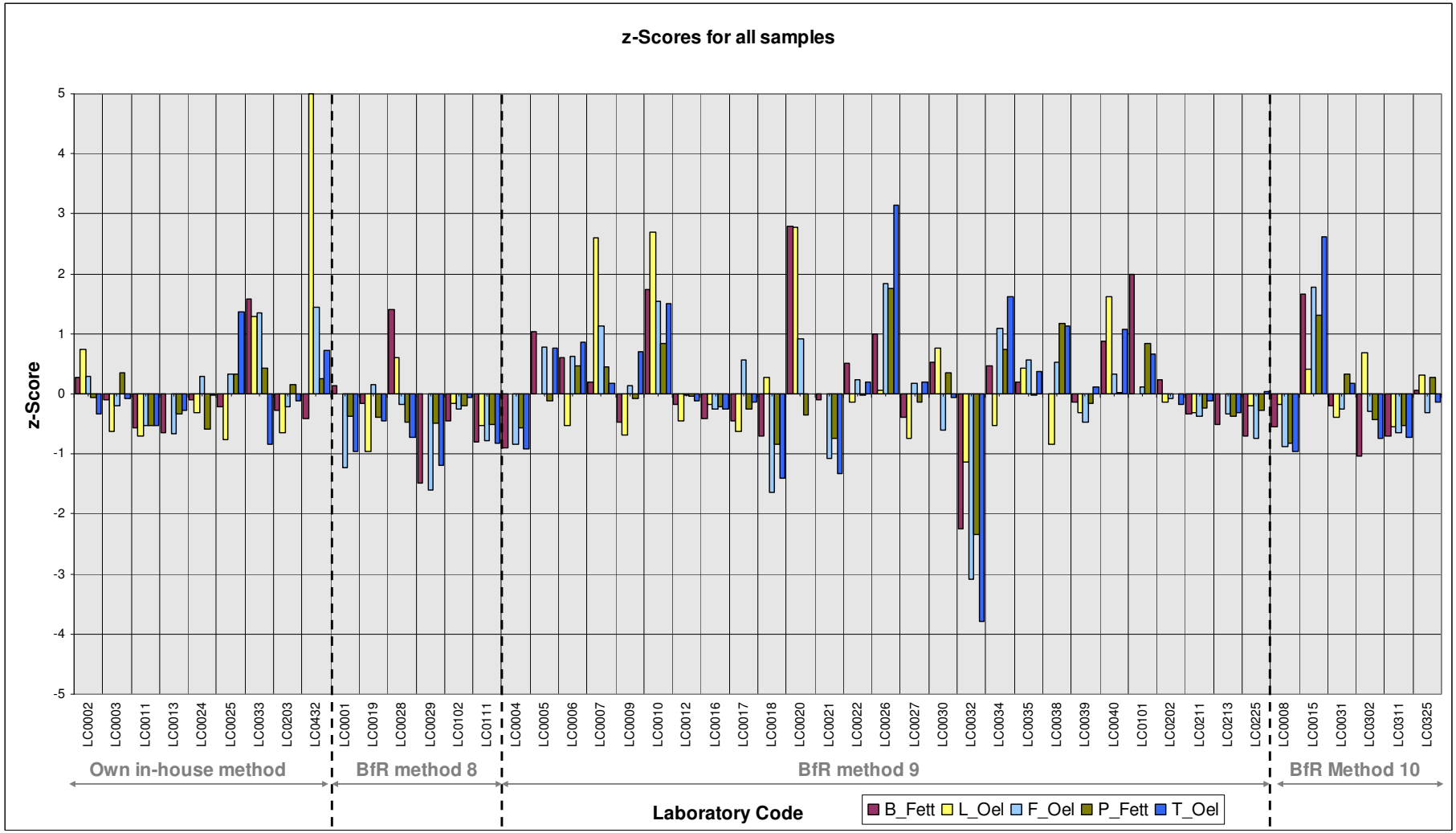


Figure 18: Overview of z-scores for all samples for each laboratory

7.5 Accompanying Documents

Covering Letter

«Labor»
«Titel» «Vorname» «Name»
«Straße»
«PLZ» «Ort»

82-2248-03-5282468 2355 27.07.2009 82.25 Wöhrlin

3-MCPD-Esters – 2nd Collaborative Study Concerning the Determination of 3-MCPD Esters in Edible Fats and Oils- Part I

Dear «Anrede» «Titel»«Name»,

Please find enclosed the test materials that shall be used in the collaborative study for determination of 3-MCPD esters in edible oils and fats. For detailed information on the procedure, please refer to the protocol attached.

Thank you very much for communication of your experiences made so far with the analytical methods. No principle modifications of the methods are necessary, but some explanatory notes relating to your annotations and questions:

- If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.
- If only a non-warmed evaporator is available, evaporation of solvents can take place at room temperature.
- To improve the transfer of the upper phase, the use of test tubes with a small cross section dimension (1 cm) has proven themselves in practice.

Annotations to the analytical method procedures in particular:

Method_82_FC-008-01 and Method_82_FC-009-01

- During the extraction with ethyl acetate take care that no water passed into the organic extracts. To avoid this, it is better to transfer the upper organic phase incompletely; in this case an additional extraction with ethyl acetate may be performed.
- Cyclohexane or isooctane may be used instead of acetone for the dissolution of the phenylboronic acid derivatives.

Method_82_FC-010-01

- Before performance of the derivatization with HFBA, sample extracts and standards have to be evaporated to complete dryness.
- Item 9.1.1 …determine the areas of the quantifying ions of the **HFBA** derivatives…

For reporting your data, we will send you data form templates by e-mail in the next few days (**RINGDAT3.exe, «Kurzbez».LAB, «Kurzbez».LA2**). Additionally, you will receive a data form template for giving particulars about the analytical parameters you used (**Conditions_BfR_3MCPD_E_XXX.xls**).

Please insert the details and save the file adding your lab code (replacing XXX) to the data file name.

In the case the files cannot be opened by you for technical reasons, we will provide them on CD-Rom.
Please return the files with your inserted data (**«Kurzbez».LAB, «Kurzbez».LA2, Conditions_BfR_3MCPD_E_XXX.xls**) not later than **September 18, 2009** to the following e-mail address: Friederike.woehrlin@bfr.bund.de

We wish you success analysing the samples!

Sincerely yours,

by order
Dr. Wolfgang Mathar

4 annexes

Protocol

1. Aim of the Study

Aim of the study is the validation of at least one method for the determination of 3-MCPD fatty acid esters in edible fats and oils. 3-MCPD forming substances are not determined.

Three analytical methods were provided by BfR. Apart from these methods, you may use your own in-house methods. Data of these results will be used for the evaluation of a proficiency-test.

2. Lab Code: «Laborcode»

3. Samples

You receive 6 samples of edible oil and 4 samples of edible fat. The samples must be analysed by way of two separate series (Day 1 and 2) on two different days. For the sample distribution, please refer to the table below:

Day 1	
Edible Fat	(**BFR_0000**)
Edible Oil	(**BFR_0000**)
Edible Oil	(**BFR_0000**)
Edible Fat	(**BFR_0000**)
Edible Oil	(**BFR_0000**)
Fat of 1.collaborative trial	(**BFR_0000**)

Day 2	
Edible Fat	(**BFR_0000**)
Edible Oil	(**BFR_0000**)
Edible Oil	(**BFR_0000**
Edible Fat	(**BFR_0000**)
Edible Oil	(**BFR_0000**)

Samples were purchased at local retail, homogenized, portioned and tested for homogeneity. All samples were proved to be homogenous. 3-MCPD was analysed by using Method_82_FC-009-01 and Method_82_FC-010-01.

Stability tests will be carried out under appropriate storing conditions in our laboratory over the entire period of this study.

4. Quality Control Samples

Each laboratory already received one of our samples of solid fat with declared 3-MCPD content that was already subject to analysis during the first collaborative trial. Enclosed herewith, you receive another aliquot (**«CONT_20»**). This sample is subject to quality control for each series. Please also report these results. For additional quality control a reagent blank is suggested.

5. Acknowledgement of Receipt of Samples

Please acknowledge receipt and report the conditions of the test material by returning the reply form (**Annex 2**) by fax to:

+49 30 8412 3457

If necessary, you will receive a replacement sample.

6. Sample Handling

In order to ensure comparability of measurements, you are required to store the samples immediately after receipt in a refrigerator at +6 (± 4) °C until analysis. Please find general recommendations for the handling of the samples in **Annex 4**.

7. Performance

For the method validation study only participants can be considered who exactly adhere to the analytical methods distributed by BfR. For validation of a method at least eight laboratories must report results. The results of our survey in June 2009 indicate that Method_82_FC-009-01 will presumably achieve this criterion. But also both other methods will be used by a couple of laboratories. A final decision which method(s) will be validated can first be made after receipt of the results.

You may choose more then one analytical method to participate in this collaborative study. Please specify the analytical parameters you have used by filling in the excel data sheet. (**Conditions_BfR_3-MCPD_E_«Labor».xls**). Macros have to be activated while opening the data file.

Three replicate determinations of each sample, respectively, following the entire analysis scheme are mandatory! Altogether both series contain at least 16 samples. The series must be processed and measured separately from each other on two different days.

8. Weighing of Samples

The weights of the samples should be documented as well as the analytical results by filling in the data file **«Labor».LAB**. Please record the weights with an accuracy of one digit after the decimal point. Relating to the analytical methods, the sample amount may be raised up to 200 mg.

9. Results shall be stated in mg/kg with an accuracy of two digits after the decimal point. In case of values below the limit of quantification or the limit of detection, please specify these particular limits.

10. Please specify the analytical parameters you have used by filling in the excel data sheet (**Conditions_BfR_3-MCPD_E_XXX.xls**). We wish to point out that your reporting on the particulars for each method is most profitable and valuable for the outcome of the study. In the same context you are also required to meticulously report any irregularities or disturbance that may occur during the measurement. Equally must be mentioned any change of operator, as the case may be, reasons for missing results.

11. Please send a chromatogram of a sample of your choice that comprises all relevant ion traces and a total ion chromatogram (TIC).

Please clearly indicate your Lab Code («Laborcode») on the chromatogram.

12. You will find more information for reporting your data in **Annex 3**. Please return the completed data form templates (**«Labor».LAB**, **«Labor».LA2, Conditions_BfR_3-MCPD_E_XXX.xls**) by e-mail before **18.09. 2009 to friederike.woehrlin@bfr.bund.de.**

Additionally, please make a print-out of the result tables and, after you have signed it, send it to the following address within the period specified above:

Federal Institute for Risk Assessment
FG 82
Friederike Wöhrlin
Thielallee 88-92
14195 Berlin
Fax: +49 30 8412 3457

13. Queries regarding the analysis should be addressed to friederike.woehrlin@bfr.bund.de

2nd Collaborative Study Determination of 3-MCPD Esters in Edible Fats and Oils- Part I

Acknowledgement of Receipt of Samples

Name	«Labor»		
Institute	«Bezeichnung»		
Lab Code	«Laborcode»	**Date of Sample Arrival**	

Test Material	**Sample Code**		**Condition at Arrival**
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Fat of 1.collaborative trial	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐
Edible Oil/Fat	(BfR_0000)	o.k. ☐	damaged ☐

Date **Signature**

After receipt of the samples, please return this form to:

Federal Institute for Risk Assessment
FG 82
Friederike Wöhrlin
Thielallee 88-92
14195 Berlin
Fax: +49 30 8412 3457

User Guide for Data Reporting – Electronic Recording

You will receive the following templates (four files), they should be used to enter and return your data:

RINGDAT3_intern.exe
English_Version03072008.xml
«Kurzbez».LAB (Result tables)
«Kurzbez».LA2 (configuration file, includes sample information and legend)

To report your data, please proceed as follows:

1. Copy the provided data files on a selected directory on your hard disk.

2. If you chose more then one analytical method please change the name of the data file **«Kurzbez».LAB** in **«Kurzbez»_1.LAB**, **«Kurzbez»_2.LAB,** etc. before opening the data file.

3. First open the file **RINGDAT3_intern.exe** (double-click): The window "**Entry of the test result**"(Entry lab dates) appears.

4. Click "Open" on the menu bar. A menu window will appear where you can find the file **«Kurzbez».LAB** in the corresponding directory. Open that file now, and the table "**2nd Collaborative Study/PT 3-MCPD-FA-Ester in Oil/fat**" will appear.

5. Please enter the respective sample **amount** (stated **in mg**) by filling in column "**WSX** "(X = 1;2;3) with an accuracy of one digit after the decimal point. In this program you have to use a comma instead of a decimal point

6. Then enter the respective **analytical result** (stated **in mg/kg**) into column "**ValueX**" (X = 1;2;3) with an accuracy of two digits after the comma (decimal point).

7. In case of values below the quantification limit, please enter "**< (LOQ)**" (LOQ = Value Limit of quantification, e.g. "< 0,1").

8. In case contents are lower than the limit of detection, please enter nothing into the respective column "ValueX". Enter the limit of detection (LOD) into the column "LOD" e.g. "0,10".

9. Save your files by using the command "Save" when you have entered your data.

10. Reduce every column to a minimum size, still allowing for legibility though, by positioning the cursor on top of the column and drawing the width to a smaller scale. Not till then, when you will have changed the display format, will you be able to make a print-out of the result tables (protocol) by clicking on “Protocol”. Otherwise an error message will be displayed.

11. Please send the result tables (protocol) with your signature to the address given below within the period specified above:

Bundesinstitut für Risikobewertung
5Z Friederike Wöhrlin
Thielallee 88-92
14195 Berlin
Fax: +49 30 8412 3685

Please return all templates after inserting your data

«Kurzbez».LAB, **«Kurzbez».LA2**,
Conditions_BfR_3-MCPD_E_XXX

by e-mail before **18 September, 2009** to friederike.woehrlin@bfr.bund.de

Please send the datafiles xxx.LAB and xxx.LA2 back to
friederike.woehrlin@bfr.bund.de

2nd Collaborative Study/PT 3-MCPD-FS-Esters in edible fats and oils

Test results

Lab.-code: LC0023

Sample code	Measurand	Description	Unit	Analytical	WS1	Value 1	WS2	Value 2	WS3	Value 3	LOQ	LOD
BFR_0110	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0114	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0124	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0175	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0191	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0285	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0287	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0338	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0348	3MCPD_E	3-MCPD-Ester	mg/kg									
BFR_0375	3MCPD_E	3-MCPD-Ester	mg/kg									
CONT_20	3MCPD_E	3-MCPD-Ester	mg/kg									
CONT_21	3MCPD_E	3-MCPD-Ester	mg/kg									

....
Place and date Manager of laboratory (in block letters)

.....
Signature

Printout Excel-sheet « Conditions_BfR_3-MCPD_XXX « for BfR Method 9

Method description

Method_82_FC-009-01

Testing Laboratory
Contact person
Lab Code

Date of sample preparation Day 1 ☐ same operator
Date of sample preparation Day 2 ☐ second operator

Determination was: carried out according exactly to the analytical method:

modification(s) took place in the following analysis step(s):

- ☐ 7.1 Sample preparation / weighing of samples
- ☐ 7.2 Ester cleavage / defatting
- ☐ 7.3 Extraction of 3-MCPD
- ☐ 7.4 Calibration standards
- ☐ 7.5 Derivatization
- ☐ 7.6 Subsquent processing of samples and standards

please specify any modifications:

GC/MS-Analysis

Date of sample measurement
Day 1
Day 2 same apparaus Day 1 and 2: ☐ yes ☐ no

please specify special features of GC/MS-Analysis (backflush-unit, CIS...)

Quantifying ion 3-MCPD ☐ m/z 196
☐ m/z 147
3-MCPD-d_5 ☐ m/z 201
☐ m/z 150

Comments:

Limit of detection and quantifcation

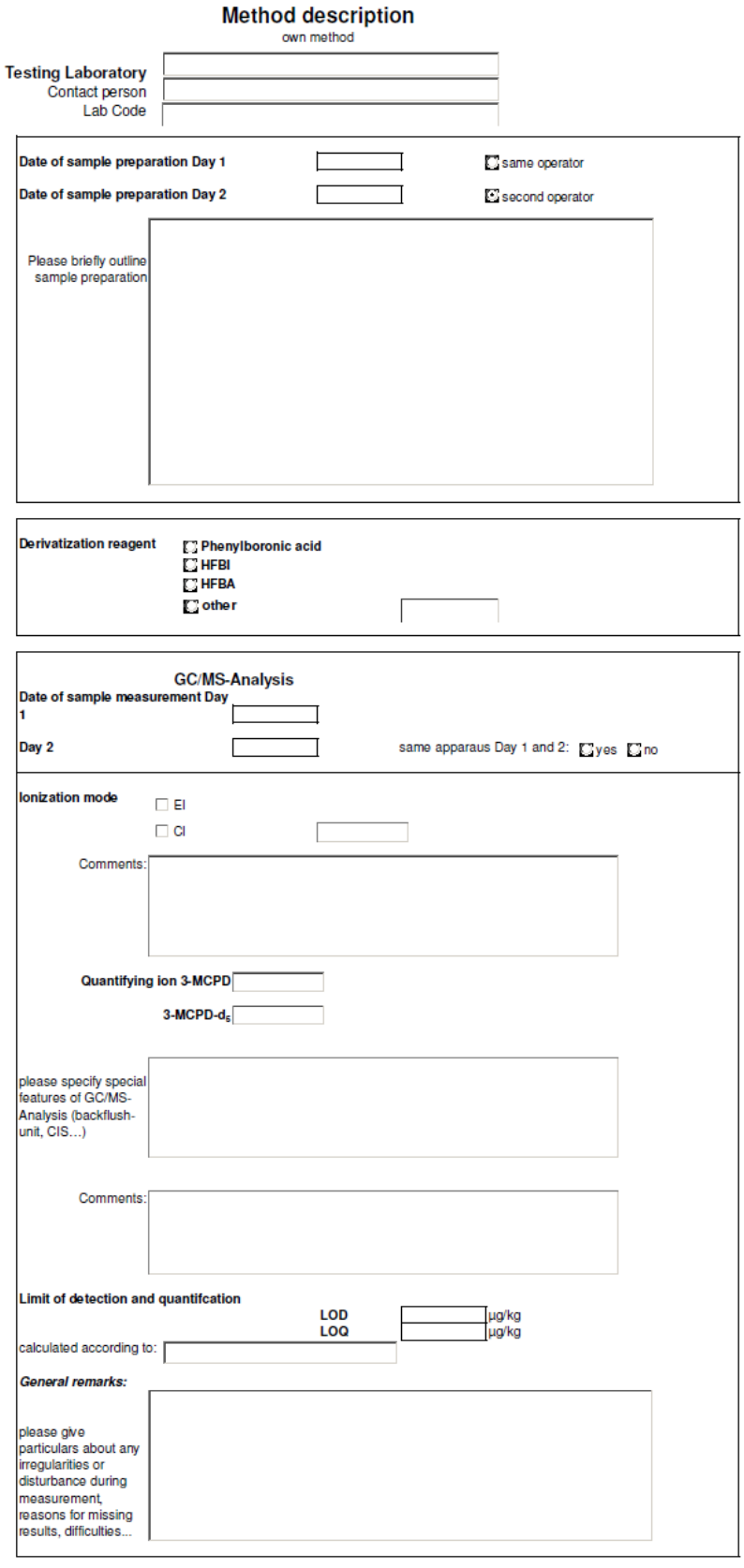
LOD µg/kg
LOQ µg/kg

calculated according to:

General remarks:

please give particulars about any irregularities or disturbance during measurement, reasons for missing results, difficulties...

Printout Excel-sheet « Conditions_BfR_3-MCPD_XXX « für own in-house methods



Method description

own method

Testing Laboratory
Contact person
Lab Code

Date of sample preparation Day 1 ☐ same operator
Date of sample preparation Day 2 ☐ second operator

Please briefly outline sample preparation

Derivatization reagent
☐ **Phenylboronic acid**
☐ **HFBI**
☐ **HFBA**
☐ **other**

GC/MS-Analysis

Date of sample measurement Day 1
Day 2 same apparaus Day 1 and 2: ☐ yes ☐ no

Ionization mode
☐ EI
☐ CI

Comments:

Quantifying ion 3-MCPD
3-MCPD-d_5

please specify special features of GC/MS-Analysis (backflush-unit, CIS…)

Comments:

Limit of detection and quantifcation
LOD µg/kg
LOQ µg/kg
calculated according to:

General remarks:

please give particulars about any irregularities or disturbance during measurement, reasons for missing results, difficulties...

Recommendations for Handling the Test Material You Received

To ensure comparability of measurements, please store samples immediately after receipt in a refrigerator at +6 (± 4) °C until analysis.

Standards, edible oils and fats must be tempered to room temperature before analysis.

Solid fats must be tempered to 60-70°C in closed test tubes before analysis. You will find it easier to dissolve the fat in the solvent if you re-warm the test tubes e.g. in a hot water bath after weighing of samples. Our own experiences demonstrate that samples necessarily have to be dissolved completely in t-BME before continuing analysis.

7.6 List of figures

7.7 List of tables

7.8 List of Abbreviations

3-MCPD	3-chloropropane-1,2-diol
ANOVA	Analysis of variance
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment)
BMELV	Bundesministerium für Ernährung Landwirtschaft und Verbraucherschutz (Federal Ministry of Food, Agriculture and Consumer Protection)
°C	Degree Celcius
CV	Coefficient of variation
CVUA	Chemisches Landes- und Staatliches Veterinäruntersuchungsamt (Official food control and animal health laboratory of the German Federal Land Baden-Wuerttemberg)
DGF	Deutsche Gesellschaft für Fettwissenschaft (German scientific association concerned with lipids, fats and oils)
et al.	et alii (Latin: “and others“)
GC-MS	Gas chromatography / mass spectrometry
HFBA	Heptafluorobutyric anhydride
kg	Kilogramme
LCode	Laboratory code
lM	Own in-house method
LOD	Limit of detection
LOQ	Limit of quantification
mg	Milligramme
min	Minute(s)
mL	Millilitre
MV	Mean value
µg	Microgram
NaCl	Sodium chloride
SCF	Scientific Committee on Food of the European Commission
SD	Standard deviation
SD Teilpr	Standard deviation of subsamples 1 and 2
SD Laboratory	Standard deviation of the laboratory
t-BME	Methyl tert-butyl ether
TDI	Tolerable daily intake
WHO	World Health Organizat

7.9 "BfR Method 8"

Method_82_FC-008-02

Determination of 3-MCPD Fatty Acid Esters in Edible Oils and Solid Fats by GC-MS
An Indirect Determination by Detection of free 3-MCPD released from 3-MCPD-Esters by Acid Hydrolysis and by Derivatization with Phenylboronic Acid

1 Scope of Application

This method describes the determination of ester-bound 3-chloropropane-1,2-diol (3-MCPD fatty acid esters) in edible fats and oils by means of gas chromatography-mass spectrometry.

2 Principle of Method

The fat sample (100 - 200 mg) is dissolved in t-BME and an internal standard (d_5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by acid hydrolysis with methanol and sulphuric acid; as a result fatty acids and free 3-MCPD are formed. The reaction is stopped with a saturated sodium hydrogen carbonate solution. The sample is defatted with isohexane and subsequently the released 3-MCPD is derivatized with phenylboronic acid. After extraction of the derivatives with cyclohexane, the sample is evaporated to complete dryness, and then dissolved in isooctane, and finally the analysis of an aliquot is carried out by means of GC-MS.

Warning and Safety Precautions/ Important Notes:

- When handling acids, bases, organic solvents and standard substances (pure substances and solutions) gloves must be used. Use solvents in places provided with an fume hood.
- Attention is drawn to the information contained in the Safety Data Sheet (SDS) and the regulations which specify the handling of reagents and solvents.
- All crucial steps in this method are marked by arrowheads:➔

3 Reagents and Products

Note: The names of manufacturers have been mentioned for information purposes only.

3.1 Reference Substances

3.1.1 3-MCPD 3-chloropropane-1,2-diol (e.g. *FLUKA*)

3.1.2 d_5-3-MCPD fivefold deuterated 3-chloropropane-1,2-diol (e.g. *CIL*)

All stock solutions are prepared in ethanol and stored at +6 (±4) °C in the dark.

The 3-MCPD working solutions are prepared by diluting the stock solutions with ethyl acetate and are stored in the same way.
The d_5-3-MCPD working solutions are prepared by diluting the stock solutions with t-BME and are also stored in the same way.

3.1.3 Stock solutions

3.1.3.1 3-MCPD stock solution (S0-solution):
Weigh 10 (±0.1) mg standard substance (3.1.1) into a 10 mL volumetric flask (4.1) and make up to the mark by adding ethanol (3.2.1) (c= 1 mg/mL).

3.1.3.2 d_5-3-MCPD stock solution (SV 0-solution):

Weigh 100 (±0.1) mg standard substance (3.1.2) into a 50 mL volumetric flask (4.1) and fill up to the mark by adding ethanol (3.2.1) (c=2 mg/mL)

3.1.4 3-MCPD-standard solutions for the calibration function

3.1.4.1 S2-3-MCPD standard solution:
Pipet 100 µL stock solution S0 (3.1.3.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.3) (c=10 µg/mL).

3.1.4.2 S3-3-MCPD standard solution:
Pipet 1 mL S2-standard solution (3.1.4.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.3) (c=1 µg/mL).

3.1.5 d_5-3-MCPD working solution as internal standard solution

Pipet 1 mL of the stock solution SV 0 (3.1.3) into a100 mL volumetric flask (4.1) and fill up to the mark by adding t-BME (3.2.8) (c=20 µg/mL).

3.2 Reagents

If not otherwise specified, all reagents shall have at least p.a. quality.

3.2.1 Ethanol absolute (e.g. Merck 100983)

3.2.2 Methanol, p.a (e.g. Merck 106035)

3.2.3 Ethyl acetate, p.a. (e.g. Promochem SO1191)

3.2.4 Sulphuric acid, p.a. (e.g. 100 %, for conductivity measurements: Merck 112223, or 98 %: Merck 112080)

3.2.5 Sodium hydrogen carbonate, p.a. (e.g. Merck 106329)

3.2.6 Isohexane, for residue analysis (e.g. Promochem SO1251)

3.2.7 Cyclohexane, for GC (e.g. Merck 102817)

3.2.8 Methyl tert-butyl ether (t-BME), for GC (e.g. Merck 101995)

3.2.9 Acetone, for Residue Analysis (e.g. Merck 100012)

3.2.10 Phenylboronic acid > 95 %, for residue analysis (e.g. Fluka 78181)

3.2.11 Ammonium sulphate, p.a. (e.g. Merck 101217)

3.2.12 Distilled water

3.2.13 Isooctane, for GC (e.g. Merck 115440)

3.3 Solutions

3.3.1 Hydrolysis reagent:
Dissolve 1.8 mL sulphuric acid (3.2.4) in 100 mL methanol (3.2.2)

3.3.2 Stop reagent:
Sodium hydrogen carbonate (3.2.5) saturated in distilled water (3.2.12)
(ca. 4.8 g $NaHCO_3$ in 50 mL water); use only the supernatant.

3.3.3 Derivatisation reagent:
Dissolve 5 (±0.1) g phenylboronic acid (3.2.10) in a mixture of 19 mL acetone (3.2.9) and 1 mL distilled water (3.2.12).

3.3.4 Ammonium sulphate solution:
Dissolve 20 (±1) g ammonium sulphate (3.2.11) in 50 mL distilled water (3.2.12).

Note: *For preparation of the solutions (3.3.2 to 3.3.4), the use of an ultrasonic bath is recommended to facilitate dissolution (4.10).*

3.4 Gases

3.4.1 Helium (5.0) (e.g. Air Liquide)

3.4.2 Nitrogen (5.0) (e.g. Air Liquide)

4 Apparatus

Note: The names of manufacturers have been mentioned for information purposes only.

4.1 Calibrated volumetric flasks in various volume ranges

4.2 Pasteur pipettes

4.3 Displacement or single channel pipettes

4.4 Micro tips

4.5 Centrifuge with cooler (e.g. ThermoScientific, RT7 and Rotor RTH 750)

4.6 Analytical balance

4.7 Vortex test tubes shaker (e.g. Scientific Industries)

4.8 Overhead shaker (e.g Heidolph, Reax-2)

4.9 Thick-walled test tubes (ca. 5 mL, e.g. Hecht Assistent 75x12 mm, No. 2775/6)

4.10 Ultrasonic bath

4.11 Drying oven

4.12 Evaporator (e.g. Barkey)

4.13 2 mL crimp vials, wide opening, clear glass (e.g. Agilent 5181-3375), with inserts (0.1 mL)

4.14 11 mm crimp caps with PTFE/silicone/PTFE septa (e.g. Agilent 5181-1211)

4.15 Manual crimper for 11 mm crimp caps (e.g. Chromacol CR-11)

5 GC-MS system

5.1 Capillary gas chromatograph with an integrated programmable column oven providing a temperature up to at least 300°C (e.g. Agilent – 6890 / 7890A)

5.2 Split/split less injector (e.g. Agilent) or

5.3 PTV system (e.g. Gerstel: CIS 4)

5.4 Automatic Sampler

5.5 Capillary column, low bleeding (e.g. Agilent, DB-5MS, 30 m x 0.25 mm, 0.25 µm)

5.6 Pre-column (Fused Silica, deactivated, 5 m x 0.32 mm)

5.7 Liner, single taper, 4 mm ID, QW (e.g. Agilent 19251-60540) or

5.8 Glass liners CIS 4 filled with glass wool, deactivated (e.g. Gerstel 010850)

5.9 Mass selective detector (e.g. Agilent MSD 5973 or 5975C) with ion source for electron-impact ionization

6 Samples and Sampling

6.1 Lab samples

6.1.1 Sufficient sample amount should be provided to allow at least for triple determination.

6.1.2 Unequivocal identification of samples must be ensured throughout the process of sampling and sample packaging.

6.1.3 Please provide for proper packaging, preservation and transport of samples in order to ensure the good condition of the samples so that the analytical results are not affected. Store the oil and fat samples at +6 (±4) °C in the dark.

6.1.4 Sufficient sample amount must be provided in order to ensure homogeneity.

6.2 Test samples

6.2.1 Blank sample

6.2.2 Reference sample

7 Procedure

7.1 Sample preparation and weighing of samples

7.1.1 Prior to weighing, bring the edible oils to room temperature. Heat solid fats in a drying oven until the melting point is reached (4.11) and weigh in immediately. The temperature depends on the melting point of the solid fat.

7.1.2 Weighing of samples:

Weigh 100 (± 5) mg sample by means of an analytical balance (4.6) into a test tube (4.9) and record the weight.

➔ Note: The sample amount may be raised up to 200 mg, if the 3-MCPD concentrations are expected to be very low (< 0.5 mg /kg). There were no tests with higher sample amounts.

7.1.3 Dissolution of samples:

Dissolve the sample in 0.5 mL t-BME (3.2.8). The use of a Vortex (4.7) for 20 s proved to be suitable for this purpose. In case the sample has already solidified by then, dip the test tube into a hot water bath for a moment and re-dissolve. The temperature depends on the melting point of the solid fat.

7.1.4 Add 20 µL of the internal standard solution (d_5-3-MCPD working solution 3.1.5)

7.2 Ester cleavage by means of acid hydrolysis and subsequent defatting

After addition of 1.8 mL hydrolysis reagent (3.3.1), shake 10 s using a Vortex (4.7).

Then shake the sample in an overhead shaker (4.8) for two hours.

➔ Note: Since the two phases segregate immediately, this step is necessary in order to avoid under-rating results.

After this, incubate the sample **at 40°C** for at least **16 hours** in the drying cabinet (4.11) (possible up to 20 h). Close the centrifuge tubes tightly (by using glass or plastic plugs for example).

After the incubation period, stop the reaction by adding 0.5 mL stop reagent (3.3.2) and by shaking carefully at low rotation speed for 20 s (Vortex 4.7).

➔ Note: Attention, formation of foam; add slowly with the necessary caution.

Defat the sample by adding 1 mL isohexane (3.2.6) and shaking for 10 s (Vortex 4.7). To improve the phase separation, centrifuge the sample (4.5) at 207 x g for 2 min at **room temperature** and then discard the upper phase.

Repeat defatting by adding again 1 mL isohexane (3.2.6) to the aqueous phase, shaking for 10 s (Vortex 4.7), then centrifuging (4.5) at 207 x g for 2 min at **room temperature** and discarding the upper phase.

7.3 3-MCPD calibration standards

7.3.1 Preparation of the calibration standards:

Preparation of the calibration standards in test tubes (4.9):
In succession pipet 20 µL, respectively, of d_5-3-MCPD working solution (3.1.5) into a test tube and add in the same way the respective volumes of the 3-MCPD-standard solutions S2 (3.1.4.1) and S3 (3.1.4.2) (see Table 1).

Then add 1.8 mL ammonium sulphate solution (3.3.4), respectively.

Table 1 Pipetting Scheme for Calibration Standards

Std-Nr.	IS d_5-3-MCPD (3.1.5)	3-MCPD in EtoAc (3.1.4.1) and (3.1.4.2)	$(NH_4)_2SO_4$-LSG (3.3.4)	IS d_5-3-MCPD	3-MCPD absolute (µg) in the derivatization preparation	3-MCPD (mg/kg) per 100 mg sample amount
Std_1	20 µL	25 µL S3	1.8 mL	0.4 µg absolute in the derivatization preparation or 4 mg/kg sample	0.025	0.25
Std_2		50 µL S3			0.05	0.5
Std_3		100 µL S3			0.1	1.0
Std_4		20 µL S2			0.2	2.0
Std_5		30 µL S2			0.3	3.0
Std_6		40 µL S2			0.4	4.0
Std_7		50 µL S2			0.5	5.0
Std_8		60 µL S2			0.6	6.0

7.4 Derivatization

Note: *The derivatization of the pre-prepared standards (Table 1) and the samples (7.2) has to be performed simultaneously.*

7.4.1 Add 250 µL, respectively, of the derivatization reagent (3.3.3) to the samples and standards.

7.4.2 The derivatization reaction takes place in the ultrasonic bath (4.10) at room temperature for 2-3 min.

7.5 Extraction of the phenylboronic acid derivatives of the standards

The phenylboronic acid derivatives of the calibration standards are extracted with 500 µL isooctane (3.2.13) by shaking for 10 to 20 s on a test tube shaker (Vortex 4.7) and subsequently centrifuging at 207 x g for 2 min at **10 °C**.

Note: To achieve higher signal intensity, the extraction volume can be reduced to 200 µL isooctane.

If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.

The extracts can be stored at room temperature for 3 to 5 days prior to GC/MS-analysis.

7.6 Subsequent processing of the samples

To remove matrix components that could interfere with GC/MS analysis, the sample is extracted into an organic phase which has to be dried carefully and finally the analytes are redissolved into a more polar solvent.

7.6.1 Extraction of the derivatives into organic phase

After adding 1 mL cyclohexane (3.2.7) to the sample with a pipette, shake the mixture for 10 s (Vortex 4.7) and subsequently centrifuge (4.5) at 207 x g for 2 min at 10 °C. Transfer the upper phase to a test tube. Repeat this extraction twice.

Note: If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.

During the extraction with cyclohexane take care that no water passed into the organic extracts. To avoid this, it is better to transfer the upper organic phase incompletely; in this case an additional extraction with ethyl acetate may be performed.

7.6.2 Drying of the merged extracts

Dry the merged extracts in an evaporator (4.12) at 40 °C using nitrogen. During the drying process a white precipitate forms at the rim of the glass tube. The sample must be evaporated to complete dryness before further treatment.

Note: If only a non-warmed evaporator is available, centrifugation can take place at room temperature.

7.6.3 Dissolution of the residue

Mix the residue with 500 µL isooctane (3.2.13) for 10 s (Vortex 4.7). Note that the white precipitate at the rim of the glass tube remains there. Centrifuge the samples (4.5) at 207 x g for 2 min at 10 °C. Transfer an aliquot into a GC crimp vial with a glass insert (4.13) and close with a crimp cap (4.14) by means of a manual crimper (4.15).

Note: Cyclohexane or isooctane may be used instead of acetone for the dissolution of the phenylboronic acid derivatives.

To achieve higher signal intensity the volume can be reduced to 200 µL isooctane.

If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.

8 GC-MS Analysis

The following specifications (8.1.1) are given by way of example.

The GC-MS analysis is based on electron-impact ionization operated in SIM mode with the following parameters:

8.1.1 GC-MS and injector conditions

8.1.1.1 Injector conditions with split/split less injector or PTV injector system

Split/split less injector (mode: pulsed split less)	
Injector temperature	180 °C
Insert liner	Liner, single taper with glass wool (5.7)
Cooled injection (mode: split less)	
CIS programme	60°C (keep constant for 0.2 min); 10°C / min up to 80°C (kept constant for 0.8 min); 10°C / min up to 280°C (kept constant for 5 min)
Liner for CIS 4	Liners with glass wool, deactivated
Injection volume	2.0 µL

8.1.1.2 GC and MS conditions

GC-column	DB-5MS (5.5)
Pre-column	Fused silica, deactivated (5.6)
Flow	1.2 mL/min (constant)
Carrier gas	Helium (3.4.1)
GC-oven-programme	60°C (kept constant for 1 min); 6°C/min up to 190°C; 30°C/min up to 280°C (kept constant for 10 min)
Temp. transfer line	280°C
Temp. ion source	230°C
Temp. quadrupole	150°C

8.1.1.3 Parameters for SIM-mode

	Ions (m/z)	Dwell (ms)
3-MCPD-derivative	196	80
	147	80
	Ions (m/z)	Dwell
d_5-3-MCPD-derivative	201	80
	150	80

9 Evaluation

9.1 GC-MS Evaluation

9.1.1 Response ratios and calibration function

9.1.1.1 Ion 196 and Ion 147 shall be used as quantifying ions for 3-MCPD [1].Based on the calibration standards, determine the areas of the quantifying ions of the phenylboronic acid derivatives of the 3-MCPD as well as d_5-3-MCPD internal standard, and form the response ratios of analyte/internal standard.

$$R = \frac{A \quad (m/z \quad 196)}{A \quad (m/z \quad 201)} \qquad \text{or} \qquad R = \frac{A \quad (m/z \quad 147)}{A \quad (m/z \quad 150)}$$

R	Response ratio of standard/internal standard
A	Response area

To set up the calibration function, plot the response ratio of the 3-MCPD standard and the d_5-3-MCPD internal standard against the concentration of the 3-MCPD standard (µg). Calculate the calibration function by means of linear regression.

$$R = a * m_{3-MCPD} + b$$

R	Response ratio
a	Slope of the regression line
b	Intercept of the regression line
$m_{3\text{-MCPD}}$	Absolute amount of 3-MCPD (µg) in the derivatization preparation of the standard

[1] Interference due to matrix and/or GC-MS properties (such as conditions of the column or of the ion source, altered by age or other) may cause faulty ion traces of the 3-MCPD phenylboronic acid derivatives. Therefore it is recommended to determine the response ratio of the ion traces 147 and 196. The response ratios should range between 4.5 and 5.8. Any outlying ratio suggests a disturbance.

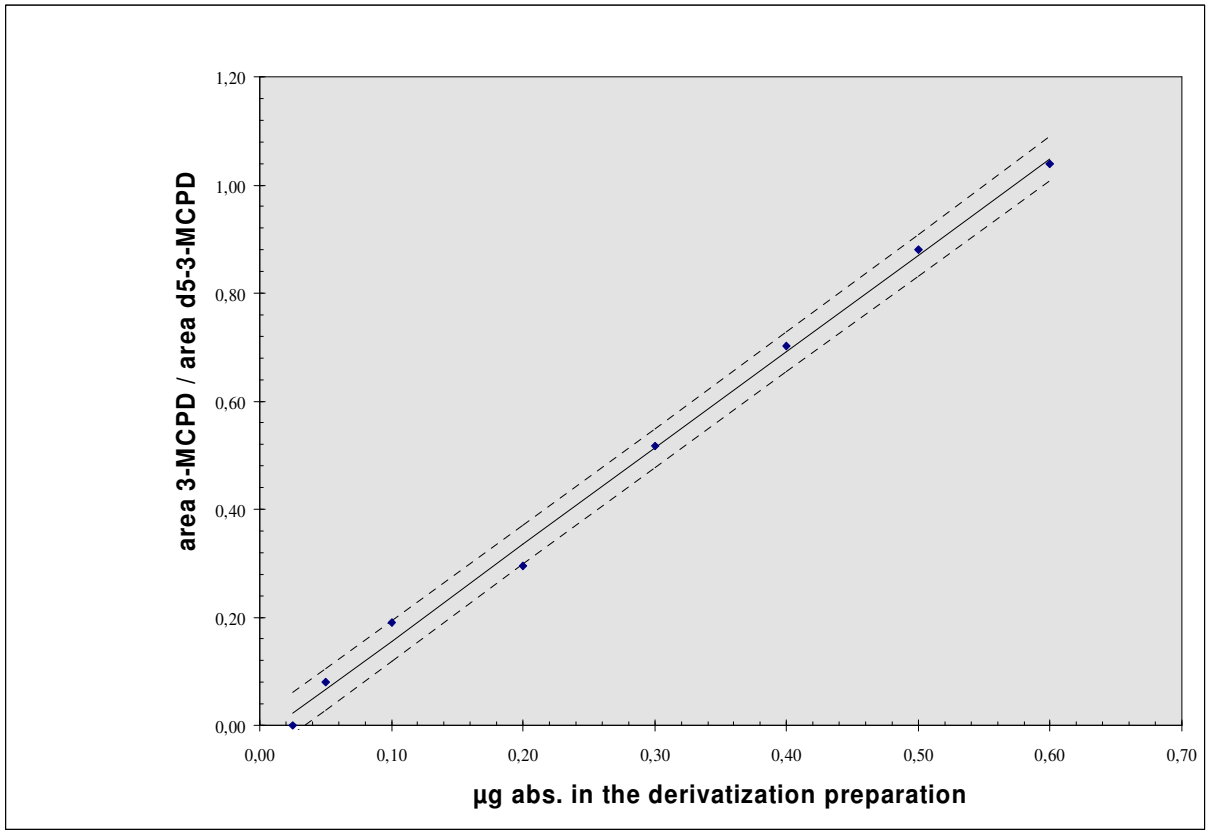


Figure 1Model for a calibration line

9.1.2 Calculation of the 3-MCPD concentrations in the derivatization preparation of the samples

The 3-MCPD concentration of the sample extracts is stated in µg as absolute amount of 3-MCPD in the derivatization preparation.

$$m_{3MCPD} = \frac{(R_{\text{Probe}} - b)}{a}$$

$m_{\text{3-MCPD}}$	Absolute amount (µg) 3-MCPD in the sample extract
R_{Sample}	Response ratio of analyte/internal standard determined in the sample extract

9.1.3 Calculation of the 3-MCPD concentrations in the sample (mg/kg)

$$\omega = \frac{m_{3-MCPD}}{m}$$

ω	3-MCPD concentration stated in mg/kg
m	Sample amount stated in g

The concentration should be given with an accuracy of one significant digit.

10 Selected Chromatograms

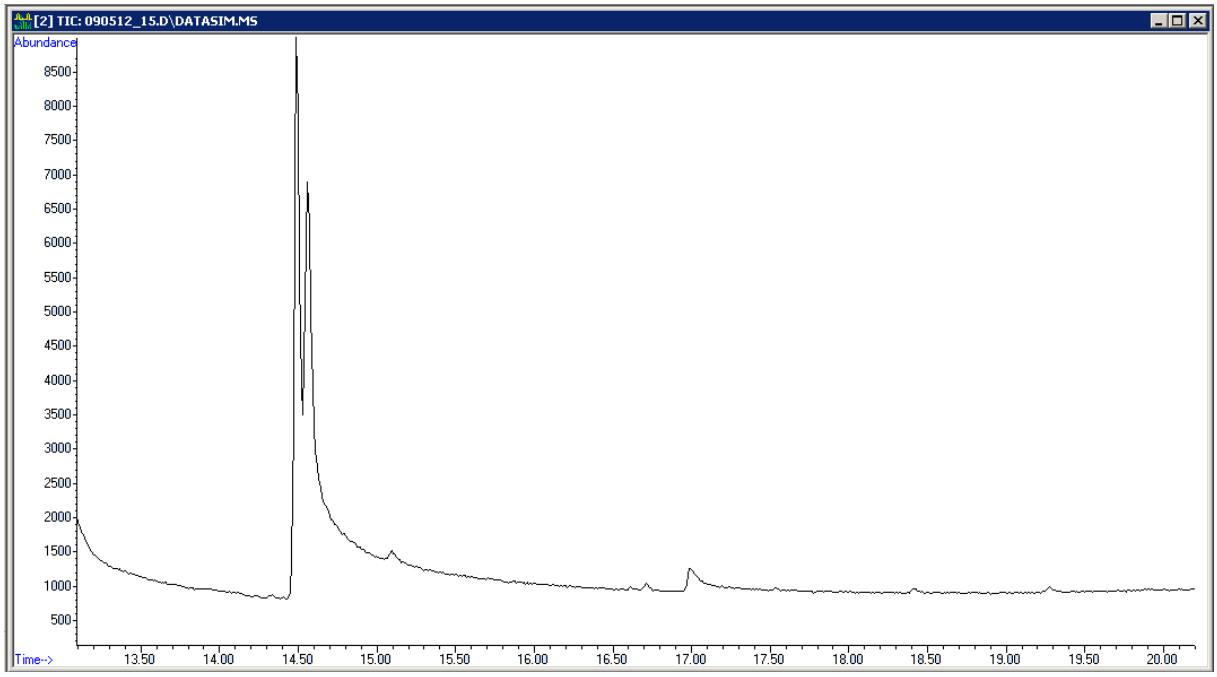


Figure 2 **TIC of a standard solution with 0.4 µg 3-MCPD und 0.4 µg of d_5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d_5-3-MCPD)**

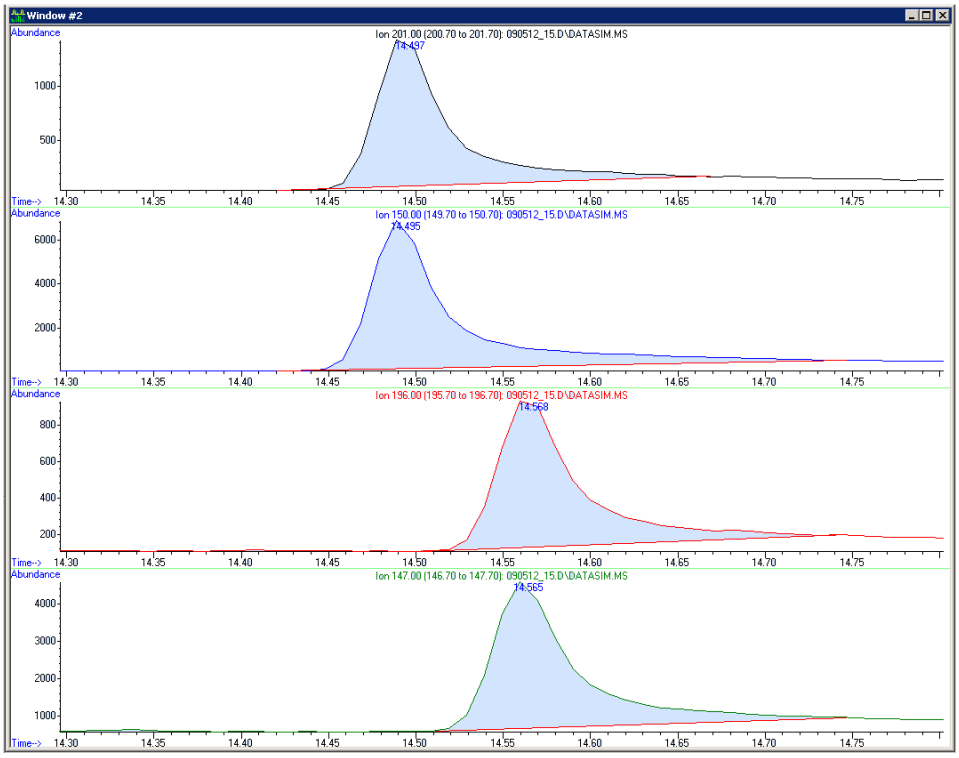


Figure 3 **Ion traces of a standard solution with 0.4 µg 3-MCPD and 0.4 µg of d5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d5-3-MCPD)**

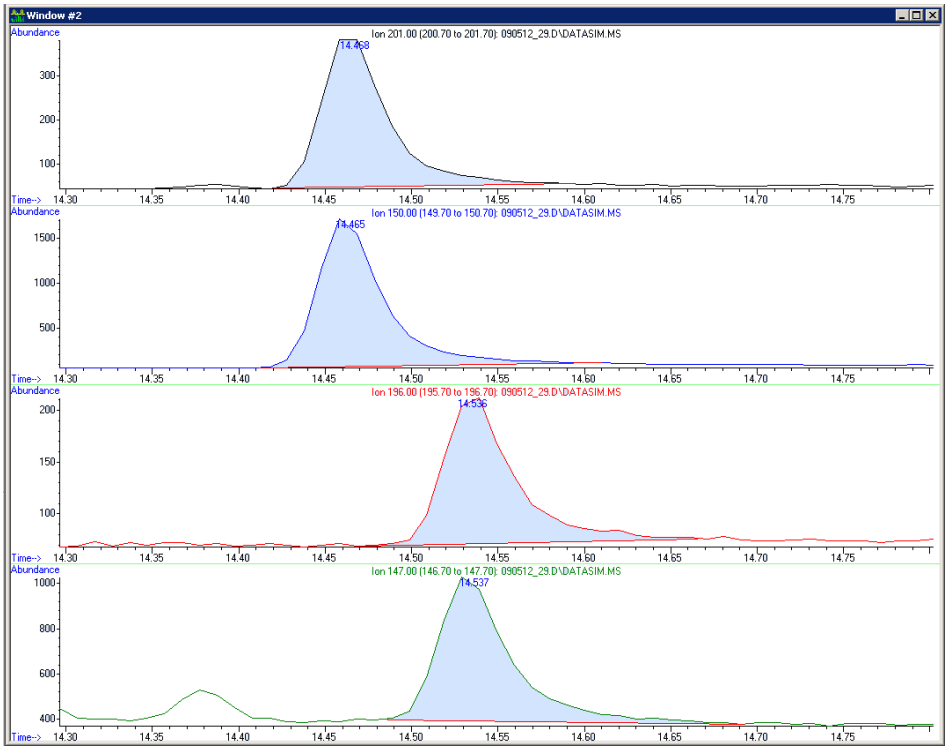


Figure 4 **Ion traces of a sample of solid fat (ca. 3 mg/kg 3-MCPD; spiked with 4 mg/kg d5-3-MCPD)**

11 Validation

11.1 Detection limit and quantification limit

The detection limit and the quantification limit were determined according to DIN 32 645 by means of a calibration function.

Characteristic data were obtained for both by spiking a blank sample with 3-MCPD: Oil samples devoid of analyte were spiked with 3-MCPD calibration solutions (3.1.4.2) which had a low 3-MCPD concentration (ranging between 0.1 and 0.6 mg/kg; equidistant intervals).

The determination of the detection limit was based on a significance level of $\alpha = 0.05$ and the determination of the quantification limit was based on a relative probability of error of 33 % (k=3). Evaluation was performed by means of the ion traces m/z 147 (3-MCPD) and m/z 150 (d_5-3-MCPD).

Table 2 presents the determined detection and quantification limit as well as the characteristic values of the linear regression.

Table 2 Detection limit and quantification limit according to DIN 32 645

Characteristic values/data	
Slope	**1.1374**
y-intercept	**0.0006**
Coefficient of determination (r)	**0.9934**
Detection limit (mg/kg); (α=0.05)	**0.09**
Quantification limit (mg/kg); (probability of error 20 %)	**0.23**

11.2 Determination of the recovery rate

In order to determine the recovery rate, an oil sample devoid of analyte (a blank matrix was repeatedly confirmed as containing no 3-MCPD) was spiked with free 3-MCPD (3.1.1) as well as with 3-MCPD ester (1,2-bis-palmitol-3-chloropropane-1,2-diol; TRC) in three different concentrations, respectively.
Evaluation was performed by means of the ion traces m/z 147 (3-MCPD) and m/z 150 (d_5-3-MCPD). Table 3 shows the mean value of the recovery rate relating to the three concentrations. The recovery rate was determined as a ratio based on the relation between the concentration actually found and the concentration that should have been expected.
After complete cleavage, 5.4 mg 3-MCPD ester correspond to 1 mg free 3-MCPD.

Table 3: Recovery rate

Added analyte concentration	**3-MCPD**	**Mean value of recovery**	Number of samples
0.2 mg/kg 3-MCPD	0.2 mg/kg	**110.5 %**	4
1.0 mg/kg 3-MCPD	1.0 mg/kg	**110.1 %**	5
3.0 mg/kg 3-MCPD	3.0 mg/kg	**85.4 %**	5
1.06 mg/kg 3-MCPD-ester	0.2 mg/kg	**100.7 %**	5
5.40 mg/kg 3-MCPD-ester	1.0 mg/kg	**98.7 %**	5
16.2 mg/kg 3-MCPD-ester	3.0 mg/kg	**95.9 %**	5

11.3 Laboratory precision

In order to determine the laboratory precision with regard to repeatability conditions, three fat samples with different 3-MCPD concentrations, respectively were subject to manifold analysis (performed by identical laboratory assistant with identical sample and identical laboratory apparatus (see Table 4).

Table 4: Laboratory precision related to repeatability conditions

Sample	**Number of determinations**	**Mean value 3-MCPD (mg/kg)**	**Standard deviation (mg/kg)**	**Coefficient of variation (%)**
Sample 1 (rape oil)	8	0.12	0.005	4.18
Sample 2 (safflower oil)	8	1.34	0.061	4.47
Sample 3 (solid fat)	8	2.88	0.138	4.79

11.4 Results of the Method Validation Study

The statistical values for five sample materials are the result of an interlaboratory study which was organised by BfR in September 2009. (Report: Collaborative Study for the Determination of 3-MCPD-Fatty Acid Esters in Edible Fats and Oils, Second Collaborative Study – Part I; BfR 2010)

Table 5: Statistical values for all sample materials (BfR Method 8)

	L_Oel	B_Fett	F_Oel	P_Fett	T_Oel	Cont
Mean value of 3-MCPD [mg/kg]	**(0.26)**	**0.83**	**1.49**	**3.06**	**3.51**	**2.71***
Rel. SD according to Horwitz (rel. sH) [%]	(19.63)	16.46	15.07	13.52	13.24	13.77
Reproducibility SD (sR) [mg/kg]	(0.12)	0.23	0.23	0.19	0.37	0.46
Rel. reproducibility SD (rel s_R) [%]	(45.04)	27.43	15.25	6.23	10.52	17.11
Repeatability SD (s_r) [mg/kg]	(0.03)	0.12	0.15	0.17	0.31	0.46
Rel. repeatability SD (rel. s_r) [%]	(11.11)	14.74	9.97	5.54	8.88	17.11
Intermediate SD (s_Z) [mg/kg]	(0.04)	0.14	0.17	0.19	0.33	0.46
Rel. intermediate SD (rel. s_Z) [%]	(16.73)	17.04	11.36	6.23	9.28	17.11
Number of datasets (after elimination of outliers)	(4)	6	6	5	6	6
Total of datasets	(6**)	6	6	6	6	6
Number of outliers				1		
Ratio sr/sR	(0.25)	0.54	0.65	0.89	0.84	1.00
HorRat	(2.3)	1.7	1.0	0.5	0.8	1.2

* The concentration of the control sample was previously specified to be 3.0 ± 0.5 mg/kg
**Two laboratories stated values as “<LOD“ or “<LOQ“

7.10 „BfR Method 9“

Method_82_FC-009-01

Determination of 3-MCPD Fatty Acid Esters in Edible Oils and Solid Fats
by GC-MS

An Indirect Determination by Detection of free 3-MCPD released from 3-MCPD-Esters by Alkaline Hydrolysis and by Derivatization with Phenylboronic Acid

1 Scope of Application

This method describes the determination of ester-bound 3-chloropropane-1,2-diol (3-MCPD fatty acid esters) in edible fats and oils by means of gas chromatography-mass spectrometry.

2 Principle of Method

The fat sample (100 - 200 mg) is dissolved in t-BME and an internal standard (d_5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by alkaline hydrolysis with a sodium methylate solution; as a result fatty acid methyl esters and free 3-MCPD are formed. The reaction is stopped with a solution of ammonium sulphate and sulphuric acid. The sample is defatted with isohexane and subsequently the released 3-MCPD is extracted with ethyl acetate, derivatized with phenylboronic acid and dried. The residue is dissolved in acetone and an aliquot is taken for analysis by GC-MS.

Warning and Safety Precautions/
Important Notes:

- When handling acids, bases, organic solvents and standard substances (pure substances and solutions) gloves must be used. Use solvents in places provided with an extractor hood.
- Attention is drawn to the information contained in the Safety Data Sheet (SDS) and the regulations which specify the handling of reagents and solvents.
- All crucial steps in this method are marked by arrowheads: ➔

3 Reagents and Products

Note: The names of manufacturers have been mentioned for information purposes only.

3.1 Reference Substances

3.1.1 3-MCPD 3-chloropropane-1,2-diol (e.g. *FLUKA*)

3.1.2 d_5-3-MCPD fivefold deuterated 3-chloropropane-1,2-diol (e.g. *CIL*)

All stock solutions are prepared in ethanol and stored at +6 (±4) °C in the dark.

The 3-MCPD working solutions are prepared by diluting the stock solutions with ethyl acetate and are stored in the same way.
The d_5-3-MCPD working solutions are prepared by diluting the stock solutions with t-BME and are also stored in the same way.

3.1.3 Stock solutions

3.1.3.1 3-MCPD stock solution (S0-solution):
Weigh 10 (±0.1) mg standard substance (3.1.1) into a 10 mL volumetric flask (4.1) and make up to the mark by adding ethanol (3.2.2) (c= 1 mg/mL).

3.1.3.2 d_5-3-MCPD stock solution (SV 0-solution):

Weigh 100 (±0.1) mg standard substance (3.1.2) into a 50 mL volumetric flask (4.1) and fill up to the mark by adding ethanol (3.2.2) (c=2 mg/mL)

3.1.4 3-MCPD-standard solutions for the calibration function

3.1.4.1 S2-3-MCPD standard solution:
Pipet 100 µL stock solution S0 (3.1.3.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.5) (c=10 µg/mL).

3.1.4.2 S3-3-MCPD standard solution:
Pipet 1 mL S2-standard solution (3.1.4.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.5) (c=1 µg/mL).

3.1.5 d_5-3-MCPD working solution as internal standard solution

Pipet 1 mL of the stock solution SV 0 (3.1.3.2) into a100 mL volumetric flask (4.1) and fill up to the mark by adding t-BME (3.2.1)

3.2 Reagents

If not otherwise specified, all reagents shall have at least p.a. quality.

3.2.1 Methyl tert-butyl ether (t-BME), for GC (e.g. Merck 101995)

3.2.2 Ethanol absolute (e.g. Merck 100983)

3.2.3 Sodium methylate ≥ 97 % (z.B. Fluka 71750)

3.2.4 Methanol, p.a (e.g. Merck 106035)

3.2.5 Ethyl acetate, p.a. (e.g. Promochem SO1191)

3.2.6 Ammonium sulphate, p.a. (e.g. Merck 101217)

3.2.7 Sulphuric acid, p.a. (e.g. 100 % for conductivity measurements, e.g. Merck 112223, or 98 %, e.g. Merck 112080)

3.2.8 Isohexane, for residue analysis (e.g. Promochem SO1251)

3.2.9 Acetone, for residue analysis (e.g. Merck 100012)

3.2.10 Phenylboronic acid > 95 %, for residue analysis (e.g. Fluka 78181)

3.2.11 Diethylether, p.a. (e.g. Promochem SO1187)

3.2.12 Distilled water

3.3 Solutions

3.3.1 Hydrolysis reagent (sodium methylate solution):
➔ Dissolve 0.27 (± 0.01) g $NaOCH_3$ (3.2.3) in 10 mL methanol (3.2.4) (c=0.5 mol/L)

3.3.2 Stop reagent (solution of ammonium sulphate and sulphuric acid; 50+3; v/v):

3.3.2.1 Ammonium sulphate solution:
Dissolve 10 (± 0.1) g ammonium sulphate (3.2.6) in 25 mL water

3.3.2.2 25 % H_2SO_4 (v/v):

Dilute sulphuric acid (3.2.7) with distilled water (3.2.12)

Mix 25 mL ammonium sulphate solution (3.3.2.1) and 1.5 mL 25 % sulphuric acid (3.3.2.2)

3.3.3 Derivatization reagent:
Phenylboronic acid (3.2.10) saturated in diethylether (3.2.11)

Ca. 0.4 g phenylboronic acid is necessary for 10 mL diethylether.

Note: For preparation of the solutions (3.3.1 to 3.3.3), the use of an ultrasonic bath is recommended to facilitate dissolution (4.9).

3.4 Gases

3.4.1 Helium (5.0) (e.g. Air Liquide)

3.4.2 Nitrogen (5.0) (e.g. Air Liquide)

4 Apparatus

Note: The names of manufacturers have been mentioned for information purposes only.

4.1 Calibrated volumetric flasks in various volume ranges

4.2 Pasteur pipettes

4.3 Displacement or single channel pipettes

4.4 Micro tips

4.5 Centrifuge with cooler (e.g. ThermoScientific, RT7 and Rotor RTH 750)

4.6 Analytical balance

4.7 Vortex test tubes shaker (e.g. Scientific Industries)

4.8 Thick-walled test tubes (ca. 5 mL, e.g. Hecht Assistent 75x12 mm, No. 2775/6)

4.9 Ultrasonic bath

4.10 Drying oven

4.11 Evaporator (e.g. Barkey)

4.12 2 mL crimp vials, wide opening, clear glass (e.g. Agilent 5181-3375), with inserts (0.1 mL)

4.13 11 mm crimp caps with PTFE/silicone/PTFE septa (e.g. Agilent 5181-1211)

4.14 Manual crimper for 11 mm crimp caps (e.g. Chromacol CR-11)

5 GC-MS system

5.1 Capillary gas chromatograph with an integrated programmable column oven providing a temperature up to at least 300°C (e.g. Agilent – 6890 / 7890A)

5.2 Split/split less injector (e.g. Agilent) or

5.3 PTV system (e.g. Gerstel: CIS 4)

5.4 Automatic Sampler

5.5 Capillary column, low bleeding (e.g. Agilent, DB-5MS, 30 m x 0.25 mm, 0.25 µm)

5.6 Pre-column (e.g. Phenomenex, fused silica, deactivated, 5 m x 0.32 mm)

5.7 Liner, single taper, 4 mm ID, QW (e.g. Agilent 19251-60540) or

5.8 Glass liners CIS 4 filled with glass wool, deactivated (e.g. Gerstel 010850)

5.9 Mass selective detector (e.g. Agilent MSD 5973 or 5975C) with ion source for electron-impact ionization

6 Samples and Sampling

6.1 Lab samples

6.1.1 Sufficient sample amount should be provided to allow for triple determination at least.

6.1.2 Unequivocal identification of samples must be ensured throughout the process of sampling and sample packaging.

6.1.3 Please provide for proper packaging, preservation and transport of samples in order to ensure the good condition of the samples so that the analytical results are not affected. Store the oil and fat samples at +6 (±4) °C in the dark.

6.1.4 Sufficient sample amount must be provided in order to ensure homogeneity.

6.2 Test samples

6.2.1 Blank sample

6.2.2 Reference sample

7 Procedure

7.1 Sample preparation and weighing of samples

7.1.1 Prior to weighing, bring the edible oils to room temperature. Heat solid fats in a drying oven until the melting point is reached (4.10) and weigh in immediately. The temperature required depends on the melting point of the solid fat.

7.1.2 Weighing of samples:

Weigh 100 (± 5) mg sample by means of an analytical balance (4.6) into a test tube (4.8) and record the weight.

7.1.2.1 ➔ Note: The sample amount may be raised up to 200 mg, if the 3-MCPD concentrations are expected to be very low (< 0.5 mg /kg). There were no tests with higher sample amounts.

7.1.3 Dissolution of samples:

Dissolve the sample in 0.5 mL t-BME (3.2.1). The use of a Vortex (4.7) for 20 s proved to be suitable for this purpose. In case the sample has already solidified by then, dip the test tube into a hot water bath for a moment and re-dissolve. The water temperature required depends on the melting point of the solid fat.

7.1.4 Add 20 µL of the internal standard solution (d_5-3-MCPD working solution 3.1.5)

7.2 Ester cleavage by means of alkaline hydrolysis and subsequent defatting

After addition of 0.2 mL hydrolysis reagent (3.3.1), shake 10 s using a Vortex (4.7).

Then incubate the sample at room temperature for 9-10 min.

After the incubation period, stop the reaction by adding 0.6 mL stop reagent (3.3.2) and by shaking vigorously at high rotation speed for 20 s (Vortex 4.7).

Defat the sample by adding 1 mL isohexane (3.2.8) and shaking for 10 s (Vortex 4.7). To improve the phase separation, centrifuge the sample (4.5) at 207 x g for 2 min at **room temperature** and discard the upper phase.

➔ Note: Distinct phase separation takes place even though dependent on the fat sample it may not always be clearly visible.

Repeat defatting by adding again 1 mL isohexane (3.2.8) to the aqueous phase, shaking for 10 s (Vortex4.7), then centrifuging (4.5) at 207 x g for 2 min at **room temperature** and discarding the upper phase.

7.3 Extraction of the released 3-MCPD into organic phase

To remove matrix components that could interfere with GC-MS analysis, the sample is extracted into an organic phase which is derivatized and carefully dried. Then the analytes are re-dissolved into a more polar solvent.

7.3.1 Extraction of the released 3-MCPD into organic phase

After adding 0.6 mL ethyl acetate (3.2.5) to the sample with a pipette, shake the mixture for 10 s (Vortex 4.7) and subsequently centrifuge (4.5) at 207 x g for 2 min at 10°C. Transfer the upper phase to a test tube. Repeat this extraction one time.

Note: If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.

During the extraction with ethyl acetate take care that no water passed into the organic extracts. To avoid this, it is better to transfer the upper organic phase incompletely; in this case an additional extraction with ethyl acetate may be performed.

7.4 3-MCPD calibration standards

7.4.1 Preparation of the calibration standards:

Preparation of the calibration standards in test tubes (4.8):
In succession pipet 20 µL, respectively, of d_5-3-MCPD working solution (3.1.5) into a test tube and add in the same way the respective volumes of the 3-MCPD-standard solutions S2 (3.1.4.1) and S3 (3.1.4.2) (see Table).

Then add 1.2 mL ethyl acetate (3.2.5), respectively.

Table 1 Pipetting Scheme for Calibration Standards

Std-Nr.	IS d_5-3-MCPD (3.1.5)	3-MCPD in EtoAc (3.1.4.1) and (3.1.4.2)	Ethyl acetate (3.2.5)	IS d_5-3-MCPD	3-MCPD absolute (µg) in the derivatization preparation	3-MCPD (mg/kg) per 100 mg sample amount
Std_1	20 µL	25 µL S3	1.8 mL	0.4 µg absolute in the derivatization preparation or 4 mg/kg sample	0.025	0.25
Std_2		50 µL S3			0.05	0.5
Std_3		100 µL S3			0.1	1.0
Std_4		20 µL S2			0.2	2.0
Std_5		30 µL S2			0.3	3.0
Std_6		40 µL S2			0.4	4.0
Std_7		50 µL S2			0.5	5.0
Std_8		60 µL S2			0.6	6.0

7.5 Derivatization

Note: *The derivatization of the pre-prepared standards (7.4) and the samples (7.3) has to be performed simultaneously.*

7.5.1 Add 100 µL, respectively, of the derivatization reagent (3.3.3) to the samples and standards.

7.5.2 The derivatization reaction takes place in the ultrasonic bath (4.9) at room temperature for 2-3 min.

7.6 Subsequent processing of the samples

7.6.1 Drying of the phenylboronic acid derivatives

Evaporate the phenylboronic acid derivatives of the samples and standards in an evaporator (4.11) at 40°C with nitrogen to complete dryness. During the drying process a white precipitate forms at the rim of the glass tube.

Note: If only a non-warmed evaporator is available, evaporation of solvents can take place at room temperature.

7.6.2 Dissolution of the residue

Mix the residue with 500 µL acetone (3.2.9) for 10 s (Vortex 4.7). Note that the white precipitate at the rim of the glass tube remains there. Centrifuge the samples (4.5) at 207 x g for 2 min at 10°C. Transfer an aliquot of the residue into a GC crimp vial with a glass insert (4.12) and close with a crimp cap (4.13) by means of a manual crimper (4.14).

Note: To achieve higher signal intensity the volume can be reduced to 200 µL acetone.

Cyclohexane or isooctane may be used instead of acetone for the dissolution.

The extracts can be stored at room temperature for 3 to 5 days prior to GC-MS-analysis.

8 GC-MS Analysis

The following specifications (8.1.1) are given by way of example.

The GC-MS analysis is based on electron-impact ionization operated in SIM mode with the following parameters:

8.1.1 GC-MS and injector conditions

8.1.1.1 Injector conditions with split/splitless injector or PTV injector system

Split/splitless injector (mode: pulsed splitless)	
Injector temperature	180 °C
Insert liner	Liner, single taper with glass wool (5.7)
Cooled injection (mode: splitless)	
CIS programme	60°C (keep constant for 0.2 min); 10°C / min up to 80°C (kept constant for 0.8 min); 10°C / min up to 280°C (kept constant for 5 min)
Liner for CIS 4	Lines with glass wool, deactivated
Injection volume	2.0 µL

8.1.1.2 GC and MS conditions

GC-column	DB-5MS (5.5)
Pre-column	Fused silica, deactivated (5.6)
Flow	1.2 mL/min (constant)
Carrier gas	Helium (3.4.1)
GC-oven-programme	60°C (kept constant for 1 min); 6°C/min up to 190°C; 30°C/min up to 280°C (kept constant for 10 min)
Temp. transfer line	280°C
Temp. ion source	230°C
Temp. quadrupole	150°C

8.1.1.3 Parameters for SIM-mode

	Ions (m/z)	Dwell (ms)
3-MCPD-derivative	196	80
	147	80
	Ions (m/z)	Dwell
d_5-3-MCPD-derivative	201	80
	150	80

9 Evaluation

9.1 GC-MS Evaluation

9.1.1 Response ratios and calibration function

Ion 196 and Ion 147 shall be used as quantifying ions for 3-MCPD[2].
Based on the calibration standards, determine the areas of the quantifying ions of the phenylboronic acid derivatives of the 3-MCPD as well as d_5-3-MCPD internal standard, and form the response ratios of analyte/internal standard.

$$R = \frac{A \quad (m/z \quad 196)}{A \quad (m/z \quad 201)} \qquad \text{or} \qquad R = \frac{A \quad (m/z \quad 147)}{A \quad (m/z \quad 150)}$$

R	Response ratio of standard/internal standard
A	Response area

To set up the calibration function, plot the response ratio of the 3-MCPD standard and the d_5-3-MCPD internal standard against the concentration of the 3-MCPD standard (µg). Calculate the calibration function by means of linear regression.

$$R = a * m_{3-MCPD} + b$$

R	Response ratio
a	Slope of the regression line
b	Intercept of the regression line
m 3-MCPD	Absolute amount of 3-MCPD (µg) in the derivatization preparation of the standard

[2] Interference due to matrix and/or GC-MS properties (such as conditions of the column or of the ion source, altered by age or other) may cause faulty ion traces of the 3-MCPD phenylboronic acid derivatives. Therefore, it is recommended to determine the response ratio of the ion traces 147 and 196. The response ratios should range between 4.5 and 5.8. Any outlying ratio suggests a disturbance.

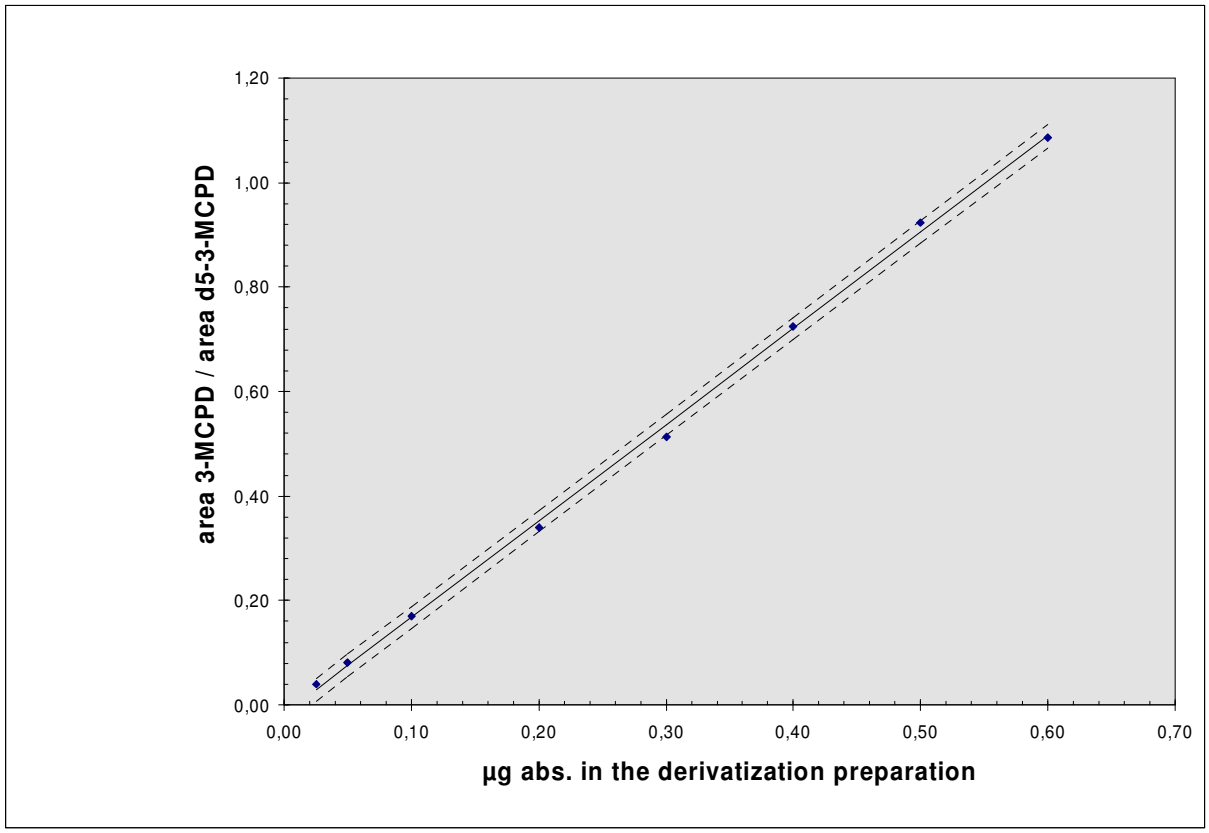


Figure 1 Model for a calibration line

9.1.2 Calculation of the 3-MCPD concentrations in the derivatization preparation of the samples

The 3-MCPD concentration of the sample is stated in µg as absolute amount of 3-MCPD in the derivatization solution.

$$m_{3MCPD} = \frac{(R_{\text{Probe}} - b)}{a}$$

$m_{\text{3-MCPD}}$ Absolute amount (µg) 3-MCPD in the sample extract
R_{Sample} Response ratio of analyte/internal standard determined in the sample extract

9.1.3 Calculation of the 3-MCPD concentrations in the sample (mg/kg)

$$\omega = \frac{m_{3-MCPD}}{m}$$

ω 3-MCPD concentration stated in mg/kg
m Sample amount stated in g

The concentration should be given with an accuracy of one significant digit.

10 Selected Chromatograms

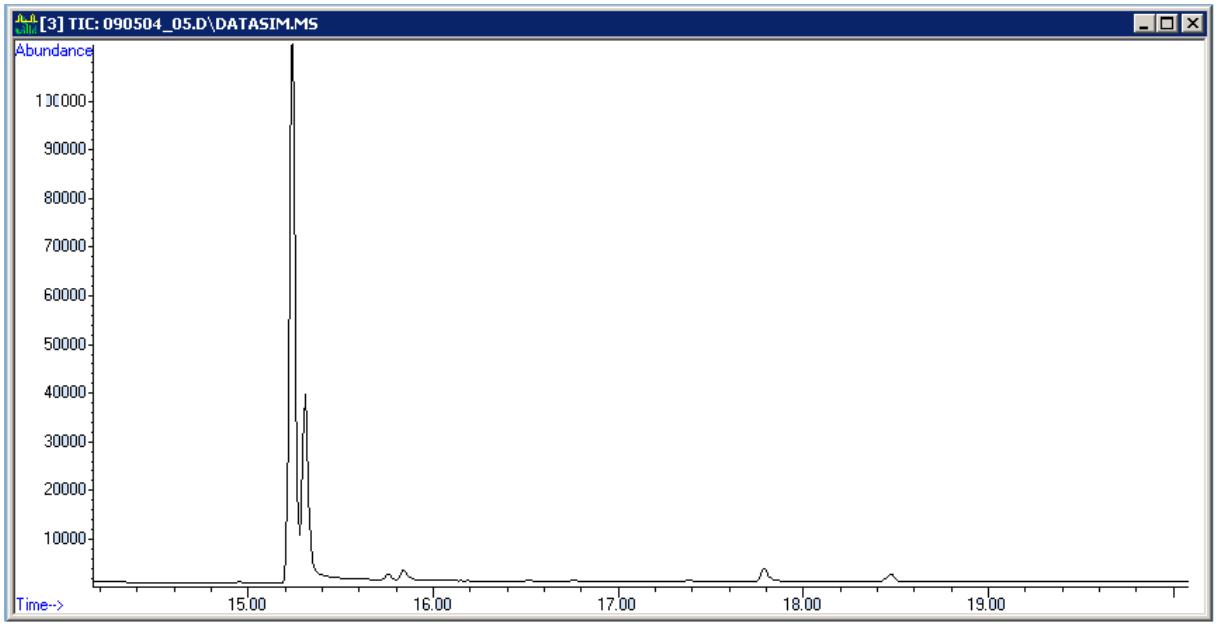


Figure 2 **TIC of a standard solution with 0.4 µg 3-MCPD und 0.4 µg of d_5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d_5-3-MCPD)**

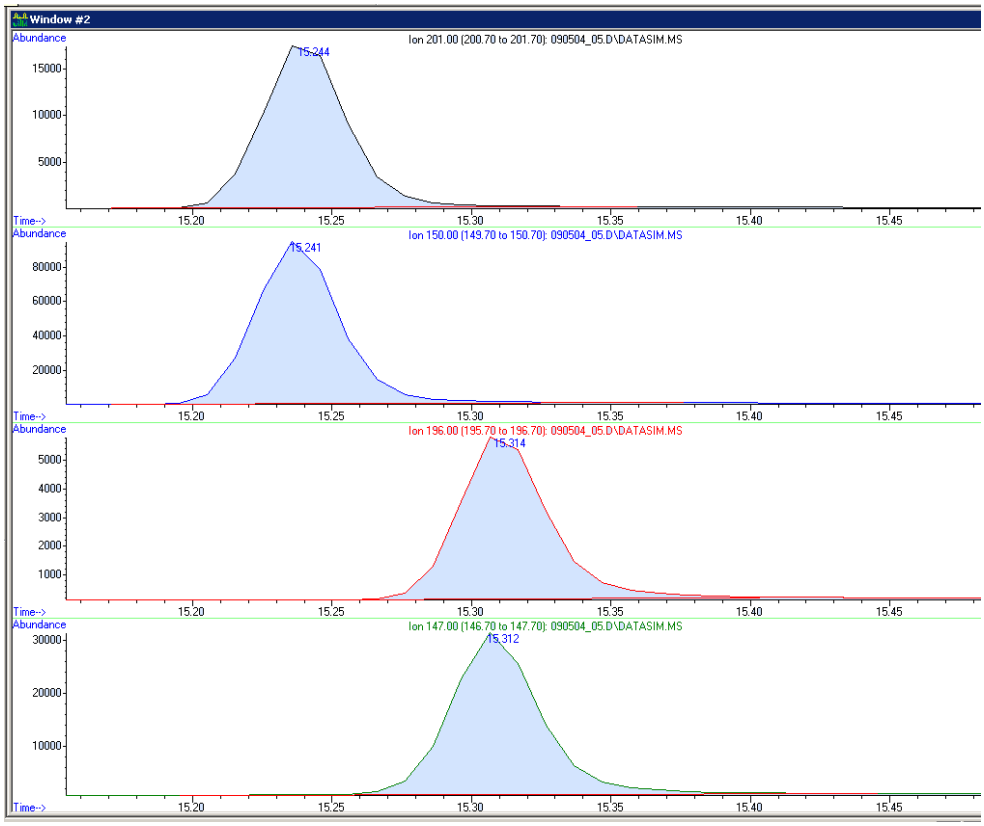


Figure 3 **Ion traces of a standard solution with 0.4 µg 3-MCPD and 0.4 µg of d_5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d_5-3-MCPD)**

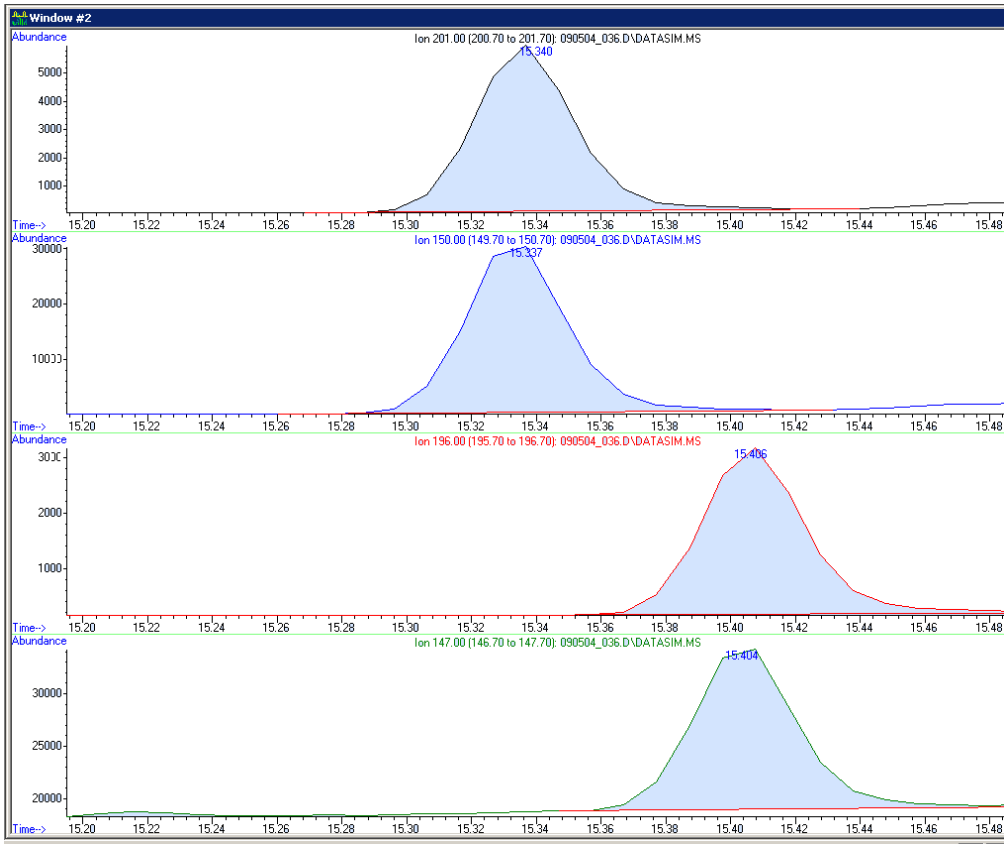


Figure 4 **Ion traces of a sample of solid fat (ca. 3 mg/kg 3-MCPD; spiked with 4 mg/kg d_5-3-MCPD)**

11 Validation

11.1 Detection limit and quantification limit

The detection limit and the quantification limit were determined according to DIN 32 645 by means of a calibration function.

Characteristic data were obtained for both by spiking a blank sample with 3-MCPD: Oil samples devoid of analyte were spiked with 3-MCPD calibration solutions (3.1.4.2) which had a low 3-MCPD concentration (ranging between 0.1 and 0.6 mg/kg; equidistant intervals).

The determination of the detection limit was based on a significance level of $\alpha = 0.05$ and the determination of the quantification limit was based on a relative probability of error of 33 % (k=3). Evaluation was performed by means of the ion traces m/z 196 (3-MCPD) and m/z 201 (d_5-3-MCPD).

Table presents the determined detection and quantification limit as well as the characteristic values of the linear regression.

Table 2 Detection limit and quantification limit according to DIN 32 645

Characteristic values/data	
Slope	**1.69**
y-intercept	**0.0009**
Coefficient of determination (r)	**0.9944**
Detection limit (mg/kg); (α=0.05)	**0.08**
Quantification limit (mg/kg); (probability of error 20 %)	**0.21**

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11.2 Determination of the recovery rate

In order to determine the recovery rate, an oil sample devoid of analyte (a blank matrix was repeatedly confirmed as containing no 3-MCPD) was spiked with free 3-MCPD (3.1.1) as well as with 3-MCPD ester (1,2-bis-palmitol-3-chloropropane-1,2-diol; TRC) in three different concentrations, respectively.
Evaluation was performed by means of the ion traces m/z 196 (3-MCPD) and m/z 201 (d_5-3-MCPD). Table shows the mean value of the recovery rate relating to the three concentrations. The recovery rate was determined (in %) as a ratio based on the relation between the concentration actually found and the concentration that should have been expected.
After complete cleavage, 5.4 mg 3-MCPD ester correspond to 1 mg free 3-MCPD.

Table 3 Recovery rate

Added analyte concentration	**3-MCPD**	**Mean Value of Recovery**	Number of Samples
0,20 mg/kg 3-MCPD	0,2 mg/kg	**106.8 %**	5
1,00 mg/kg 3-MCPD	1,0 mg/kg	**106.2 %**	5
3,00 mg/kg 3-MCPD	3,0 mg/kg	**97.6 %**	5
1,06 mg/kg 3-MCPD-ester	0,2 mg/kg	**93.8 %**	5
5,40 mg/kg 3-MCPD-ester	1,0 mg/kg	**114.5 %**	4
16,20 mg/kg 3-MCPD-ester	3,0 mg/kg	**94.8 %**	4

11.3 Laboratory precision

In order to determine the laboratory precision with regard to repeatability conditions, three fat samples with different 3-MCPD concentrations, respectively were subject to manifold analysis (performed by identical laboratory assistant with identical sample and identical laboratory apparatus (see Table 4).

Table 4 Laboratory precision related to repeatability conditions

Sample	**Number of determinations**	**Mean value 3-MCPD (mg/kg)**	**Standard deviation (mg/kg)**	**Coefficient of variation (%)**
Sample 1 (rape oil)	7	0.11	0.004	3.16
Sample 2 (safflower oil)	8	1.65	0.065	3.93
Sample 3 (solid fat)	8	3.00	0.095	3.16

11.4 Results of the Method Validation Study

The statistical values for five sample materials are the result of an interlaboratory study which was organised by BfR in September 2009. (Report: Collaborative Study for the Determination of 3-MCPD-Fatty Acid Esters in Edible Fats and Oils, Second Collaborative Study – Part I; BfR 2010)

Table 5 Statistical values for all sample materials (BfR Method 9)

	L_Oel	B_Fett	F_Oel	P_Fett	T_Oel	Cont
Mean value of 3-MCPD [mg/kg]	**0.30**	**0.91**	**1.72**	**3.46**	**4.04**	**2.96***
Rel. SD according to Horwitz (rel. sH) [%]	19.17	16.23	14.74	13.27	12.96	13.59
Reproducibility SD (sR) [mg/kg]	0.17	0.21	0.28	0.55	0.62	0.38
Rel. reproducibility SD (rel s_R) [%]	55.22	22.87	16.38	15.77	15.26	12.82
Repeatability SD (s_r) [mg/kg]	0.05	0.10	0.14	0.25	0.28	0.18
Rel. repeatability SD (rel. s_r) [%]	16.23	11.43	8.08	7.37	6.85	6.13
Intermediate SD (s_Z) [mg/kg]	0.09	0.11	0.20	0.34	0.37	0.38
Rel. intermediate SD (rel s_Z) [%]	31.52	12.52	11.52	9.82	9.11	12.82
Number of datasets (after elimination of outliers)	19	25	25	25	25	23
Total of datasets	27**	27	27	27	27	27
Number of outliers***	2	1	1	1	1	3
Ratio sr/sR	0.29	0.50	0.49	0.47	0.45	0.48
HorRat	2.9	1.4	1.1	1.2	1.2	0.9

* The concentration of the control sample was previously specified to be 3.0 ± 0.5 mg/kg
** Five laboratories stated values as "<LOD" or "<LOQ"
***One data set was completely excluded from evaluation due to the laboratory's systematic deviation from the method.

7.11 „BfR Method 10“

Method_82_FC-010-02

Determination of 3-MCPD Fatty Acid Esters in Edible Oils and Solid Fats
by GC-MS

An Indirect Determination of free 3-MCPD released from 3-MCPD-Esters
by Alkaline Hydrolysis
and by
Derivatization with Heptafluorobutyric Anhydride

1 Scope of Application

This method describes the determination of ester-bound 3-chloropropane-1,2-diol (3-MCPD fatty acid esters) in edible fats and oils by means of gas chromatography-mass spectrometry.

2 Principle of Method

The fat sample (100 - 200 mg) is dissolved in t-BME and an internal standard (d5-labeled 3-MCPD) is added. Cleavage of the ester bond is performed by alkaline hydrolysis with a sodium methylate solution; as a result fatty acid methyl esters and free 3-MCPD are formed. The reaction is stopped with a solution of ammonium sulphate and sulphuric acid. The sample is defatted with isohexane and subsequently the released 3-MCPD is extracted with ethyl acetate, then dried, and derivatized with heptafluorobutyric anhydride (HFBA). The derivatization reaction is stopped and an aliquot of the organic phase is analysed by GC-MS.

Warning and Safety Precautions/
Important Notes:

- When handling acids, bases, organic solvents and standard substances (pure substances and solutions) gloves must be used. Use solvents in places provided with an fume hood.
- Attention is drawn to the information contained in the Safety Data Sheet (SDS) and the regulations which specify the handling of reagents and solvents.
- All crucial steps in this method are marked by arrowheads:➔

3 Reagents and Products

Note: The names of manufacturers have been mentioned for information purposes only.

3.1 Reference Substances

3.1.1 3-MCPD 3-chloropropane-1,2-diol (e.g. *FLUKA*)

3.1.2 d_5-3-MCPD fivefold deuterated 3-chloropropane-1,2-diol (e.g. *CIL*)

All stock solutions are prepared in ethanol and stored at +6 (±4) °C in the dark.

The 3-MCPD working solutions are prepared by diluting the stock solutions with ethyl acetate and are stored in the same way.
The d_5-3-MCPD working solutions are prepared by diluting the stock solutions with t-BME and are also stored in the same way.

3.1.3 Stock solutions

3.1.3.1 3-MCPD stock solution (S0-solution):
Weigh 10 (±0.1) mg standard substance (3.1.1) into a 10 mL volumetric flask (4.1) and make up to the mark by adding ethanol (3.2.3) (c= 1 mg/mL).

3.1.3.2 d_5-3-MCPD stock solution (SV 0-solution):

3.1.3.3 Weigh 100 (±0.1) mg standard substance (3.1.2) into a 50 mL volumetric flask (4.1) and fill up to the mark by adding ethanol (3.2.3) (c=2 mg/mL)

3.1.4 3-MCPD-standard solutions for the calibration function

3.1.4.1 S2-3-MCPD standard solution:
Pipet 100 µL stock solution S0 (3.1.3.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.5) (c=10 µg/mL).

3.1.4.2 S3-3-MCPD standard solution:
Pipet 1 mL S2-standard solution (3.1.4.1) into a 10 mL volumetric flask (4.1) and fill up to the mark by adding ethyl acetate (3.2.6) (c=1 µg/mL).

3.1.5 d_5-3-MCPD working solution as internal standard solution

Pipet 1 mL of the stock solution SV 0 (3.1.3.2) into a100 mL volumetric flask (4.1) and fill up to the mark by adding t-BME (3.2.2) (c=20 µg/mL).

3.2 Reagents

3.2.1 If not otherwise specified, all reagents shall have at least p.a. quality.

3.2.2 Methyl tert-butyl ether (t-BME), for GC (e.g. Merck 101995)

3.2.3 Ethanol absolute (e.g. Merck 100983)

3.2.4 Sodium methylate ≥ 97 % (z.B. Fluka 71750)

3.2.5 Methanol, p.a (e.g. Merck 106035)

3.2.6 Ethyl acetate, p.a. (e.g. Promochem SO1191)

3.2.7 Ammonium sulphate, p.a. (e.g. Merck 101217)

3.2.8 Sulphuric acid, p.a. (e.g. 100 % for conductivity measurements, e.g. Merck 112223, or 98 %, e.g. Merck 112080)

3.2.9 Isohexane, for residue analysis (e.g. Promochem SO1251)

3.2.10 Isooctane, for GC (e.g. Merck 115440)

3.2.11 Heptafluorobutyric anhydride, derivatization grade (e.g. Sigma Aldrich 394912)

Note: The derivatization reagent is sensitive to water. Storage in a desiccator at room temperature proved to be suitable.

3.2.12 Distilled water

3.3 Solutions

3.3.1 Hydrolysis reagent (sodium methylate solution):
➔ Dissolve 0.27 (± 0.01) g $NaOCH_3$ (3.2.4) in 10 mL methanol (3.2.5) (c=0.5 mol/L)

3.3.2 Stop reagent (solution of ammonium sulphate and sulphuric acid; 50+3; v/v):

3.3.2.1 Ammonium sulphate solution:
Dissolve 10 (± 0.1) g ammonium sulphate (3.2.7) in 25 mL water

3.3.2.2 25 % H_2SO_4 (v/v):

Dilute sulphuric acid (3.2.8) with distilled water (3.2.12)

Mix 25 mL ammonium sulphate solution (3.3.2.1) and 1.5 mL 25 % sulphuric acid (3.3.2.2)

Note: For preparation of the solutions 3.3.1and 3.3.2.1 the use of an ultrasonic bath is recommended to facilitate dissolution (4.10).

3.4 Gases

3.4.1 Helium (5.0) (e.g. Air Liquide)

3.4.2 Nitrogen (5.0) (e.g. Air Liquide)

3.4.3 Methane (5.5) (e.g. Air Liquide)

4 Apparatus

Note: The names of manufacturers have been mentioned for information purposes only.

4.1 Calibrated volumetric flasks in various volume ranges

4.2 Pasteur pipettes

4.3 Displacement or single channel pipettes

4.4 Micro tips

4.5 Centrifuge with cooler (e.g. ThermoScientific, RT7 and Rotor RTH 750)

4.6 Analytical balance

4.7 Vortex test tubes shaker (e.g. Scientific Industries)

4.8 Centrifuge tubes with ground-glass stoppers (ca. 12 mL)

4.9 Thick-walled test tubes (ca. 5 mL, e.g. Hecht Assistent 75x12 mm, No. 2775/6)

4.10 Ultrasonic bath

4.11 Drying oven

4.12 Evaporator (e.g. Barkey)

4.13 2 mL crimp vials, wide opening, clear glass (e.g. Agilent 5181-3375), with glass inserts (0.1 mL)

4.14 11 mm crimp caps with PTFE/silicone/PTFE septa (e.g. Agilent 5181-1211)

4.15 Manual crimper for 11 mm crimp caps (e.g. Chromacol CR-11)

5 GC-MS System

5.1 Capillary gas chromatograph with an integrated programmable column oven providing a temperature up to at least 300°C (e.g. Agilent – 6890 / 7890A)

5.2 Split/split less injector (e.g. Agilent) or

5.3 Automatic Sampler

5.4 Capillary column, low bleeding
(e.g. Agilent, DB-5MS, 30 m x 0.25 mm, 0.25 µm)

5.5 Liner, single taper, 4 mm ID, QW (e.g. Agilent 19251-60540) or

5.6 Mass selective detector (e.g. Agilent MSD 5973 or 5975C) with ion source for chemical ionization or electron-impact ionization

6 Samples and Sampling

6.1 Lab samples

6.1.1 Sufficient sample amount should be provided to allow for triple determination at least.

6.1.2 Unequivocal identification of samples must be ensured throughout the process of sampling and sample packaging.

6.1.3 Please provide for proper packaging, preservation and transport of samples in order to ensure the good condition of the samples so that the analytical results are not affected. Store the oil and fat samples at +6 (±4) °C in the dark.

6.1.4 Sufficient sample amount must be provided in order to ensure homogeneity.

6.2 Test samples

6.2.1 Blank sample

6.2.2 Reference sample

7 Procedure

7.1 Sample preparation and weighing of samples

7.1.1 Prior to weighing, bring the edible oils to room temperature. Heat the solid fats in a drying oven (4.11) until the melting point is reached and weigh in immediately. The temperature required depends on the melting point of the solid fat.

7.1.2 Weighing of samples:

Weigh 100 (± 5) mg sample by means of an analytical balance (4.6) into a test tube (4.9) and record the weight.

➔ Note: The sample amount may be raised up to 200 mg, if the 3-MCPD concentrations are expected to be very low (< 0.5 mg /kg). There were no tests with higher sample amounts.

7.1.3 Dissolution of samples:

Dissolve the sample in 0.5 mL t-BME (3.2.2). The use of a Vortex (4.7) for 20 s proved to be suitable for this purpose. In case the sample has already solidified by then, dip the centrifuge tube into a hot water bath for a moment and re-dissolve completely. The water temperature required depends on the melting point of the solid fat.

7.1.4 Add 20 µL of the internal standard solution (d_5-3-MCPD working solution 3.1.5)

7.2 Ester cleavage by means of alkaline hydrolysis and subsequent defatting

After addition of 0.2 mL hydrolysis reagent (3.3.1), shake 10 s using a Vortex (4.7).

Then incubate the sample at room temperature for 9-10 min.

After the incubation period, stop the reaction by adding 0.6 mL stop reagent (3.3.2) and by shaking vigorously at high rotation speed for 20 s (Vortex 4.7).

Defat the sample by adding 1 mL isohexane (3.2.9) and shaking for 10 s (Vortex 4.7). To improve the phase separation, centrifuge the sample (4.5) at 207 x g for 2 min at **room temperature** and discard the upper phase.

➔ Note: Distinct phase separation takes place even though dependent on the fat sample it may not always be clearly visible.

Repeat defatting by adding again 1 mL isohexane (3.2.9) to the aqueous phase, shaking for 10 s (Vortex 4.7), and then centrifuging (4.5) at 207 x g for 2 min at **room temperature** and discarding the upper phase.

7.3 Extraction of the released 3-MCPD into organic phase

Since derivatization takes place in an organic medium, the sample is extracted with ethyl acetate, derivatized subsequently and carefully concentrated.

7.3.1 Extraction into organic phase

After adding 0.6 mL ethyl acetate (3.2.6) to the sample with a pipette, shake the mixture for 10 s (Vortex 4.7) and subsequently centrifuge (4.5) at 207 x g for 2 min at 10°C. Transfer the upper phase into a fresh centrifuge tube. Repeat this extraction one time.

➔ ***Note:*** ***Derivatzation with HFBA being sensitive to water, take care that no water from the lower phase gets in the organic extracts.***

If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.

7.4 3-MCPD calibration standards

7.4.1 Preparation of the calibration standards:

Preparation of the calibration standards in centrifuge tubes (4.8):
In succession pipet 20 µL of d_5-3-MCPD working solution (3.1.5) and 100 µL of ethyl acetate (3.2.6) respectively, into a centrifuge tube and add in the same way the respective volumes of the 3-MCPD-standard solutions S2 (3.1.4.1) and S3 (3.1.4.2) (see Table).

Table 1 Pipetting Scheme for Calibration Standards

Std-Nr.	IS d_5-3-MCPD (3.1.5)	EtoAc (3.2.6)	3-MCPD in EtoAc (3.1.4.1) and (3.1.4.2)	IS d_5-3-MCPD	3-MCPD absolut (µg) in the derivatization preparation	3-MCPD (mg/kg) per 100 mg sample amount
Std_1	20 µL	100 µL	25 µL S3	0.4 µg absolute in the derivatization preparation or 4 mg/kg sample	0.025	0.25
Std_2			50 µL S3		0.05	0.5
Std_3			100 µL S3		0.1	1.0
Std_4			20 µL S2		0.2	2.0
Std_5			30 µL S2		0.3	3.0
Std_6			40 µL S2		0.4	4.0
Std_7			50 µL S2		0.5	5.0
Std_8			60 µL S2		0.6	6.0

7.5 Subsequent processing of the samples and standards

7.5.1 Derivatization and concentrating

The merged sample extracts and the standards shall be evaporated to complete dryness in an evaporator (4.12) at 40°C with nitrogen.

➔ *Note*: *It is recommended to add isooctane immediately after drying (see step 7.6.1).*

If only a non-warmed evaporator is available, evaporation of solvents can take place at room temperature

7.6 Derivatization

Note: *The derivatization of the pre-prepared standards (see 7.4.1) and the samples (7.5.1) has to be performed simultaneously.*

7.6.1 Dissolve the dried samples and standards with 250 µL isooctane (3.2.10), respectively, by shaking for 10 s (Vortex 4.7).

7.6.2 After adding 50 µL of the derivatization reagent (3.2.11), shake the mixture for 10 s (Vortex 4.7).
The derivatization reaction takes place in the drying oven (4.11) at 70°C for 20 min.

7.6.3 Stop the reaction by adding 250 mL distilled water (3.2.11) and by shaking at high rotation speed for 20 s (Vortex 4.7).

7.6.4 Centrifuging

Centrifuge (4.5) the samples and standards at 828 x g for 2 min at 10°C. Transfer an aliquot of the upper organic phase into a GC crimp vial with a glass insert (4.13) and close with a crimp cap (4.14) by means of a manual crimper (4.15).

➔ *Note*: *If only a non refrigerated centrifuge is available, centrifugation can take place at room temperature.*

The extracts can be stored at room temperature for 2 to 3 days prior to GC-MS analysis.

8 GC-MS Analysis

GC-MS analysis of the HFBA derivatives was performed by means of chemical ionisation. The following specifications (GC-MS parameters, see 8.1and 8.2) are given by way of example.

➔Note: **Carry out measurements without pre-column.**

8.1 Chemical ionisation in SIM mode

GC-MS analysis with chemical ionisation in SIM mode was performed with the following parameters:

8.1.1 GC - MS and injector conditions

8.1.1.1 Injector conditions with split/split-less injector

Split/Split-less-injector, mode: split-less	
Injector temperature	270 °C
Purge time	1 min
Insert liner	Liner single taper with glass wool (5.5)
Injection volume	2.0 µL

8.1.1.2 GC and MS conditions

GC-column	DB-5MS (5.4)
Pre-column	None
Flow	1.6 mL/min. (constant)
Carrier gas	Helium (3.4.1)
Reactant gas	Methane (3.4.3)
GC-oven-programme	50°C kept constant for 2 min; 2°C / up to 78°C; 1°C / up to 81°C; 40°C / min up to 250°C; kept constant for 3 min; 40°C / min up to 280°C; kept constant for 5 min
Temp. transfer line	280 °C
Temp. Ion source	150 °C
Temp. quadrupole	106 °C

8.1.1.3 Parameters for SIM mode CI

	Ions (m/z)	Dwell (m s)
3-MCPD derivative	482	50
	446	50
	484	50
	502	50
	Ions (m/z)	Dwell
d_5-3-MCPD derivative	486	50
	507	50

8.2 Electron-impact-ionisation in SIM mode

GC-MS-analysis by electron-impact-ionisation in SIM mode is also possible. The following parameters have proved to be suitable for this purpose:

8.2.1 GC - MS and injector conditions

8.2.1.1 Injector conditions with split/split-less injector

Split/split-less injector, mode: pulsed split-less	
Injector temperature	270 °C
Insert liner	Liner, single taper with glass wool (5.5)

8.2.1.2 GC- MS conditions

GC-column	DB-5MS (5.4)
Pre-column	None
Flow	1 mL/min. (constant)
Carrier gas	Helium (3.4.1)
GC-oven-programme	50 °C ; kept constant for 1 min; 2 °C / min up to 84 °C; 35 °C / min up to 270 °C; ; kept constant for 5 min; 30 °C / min up to 300 °C; ; kept constant for 2 min
Temp. transfer line	280 °C
Temp. ion source	230 °C
Temp. quadrupole	150 °C

8.2.1.3 Parameters for SIM mode EI

	Ions (m/z)	Dwell (m s)
3-MCPD derivative	253	80
	289	80
	275	80
	453	80
d_5-3-MCPD derivative	Ions (m/z)	Dwell
	257	80

9 Evaluation

The following evaluation relates to GC-MS measurement with chemical ionization and is given by way of example. It is necessary to exchange the respective ions in the equation when measurement is performed by electron-impact-ionisation.

9.1 GC-MS Evaluation

9.1.1 Response ratios and calibration function

Ion 482 and Ion 502 shall be used as quantifying ions for 3-MCPD with chemical ionization.

Based on the calibration standards, determine the areas of the quantifying ions of the HFBA derivatives of the 3-MCPD and the d_5-3-MCPD internal standard, and form the response ratios of analyte/internal standard.

$$R = \frac{A\ (m/z\ 482)}{A\ (m/z\ 486)} \quad \text{or} \quad R = \frac{A\ (m/z\ 502)}{A\ (m/z\ 507)}$$

R	Response ratio of standard/internal standard
A	Response area

To set up the calibration function, plot the response ratio of the 3-MCPD standard and the d_5-3-MCPD internal standard against the concentration of the 3-MCPD standard (µg). Calculate the calibration function by means of linear regression.

$$R = a * m_{3-MCPD} + b$$

R	Response ratio
a	Slope of the regression line
b	Intercept of the regression line
$m_{3\text{-MCPD}}$	Absolute amount of 3-MCPD (µg) in the derivatization preparation of the standard

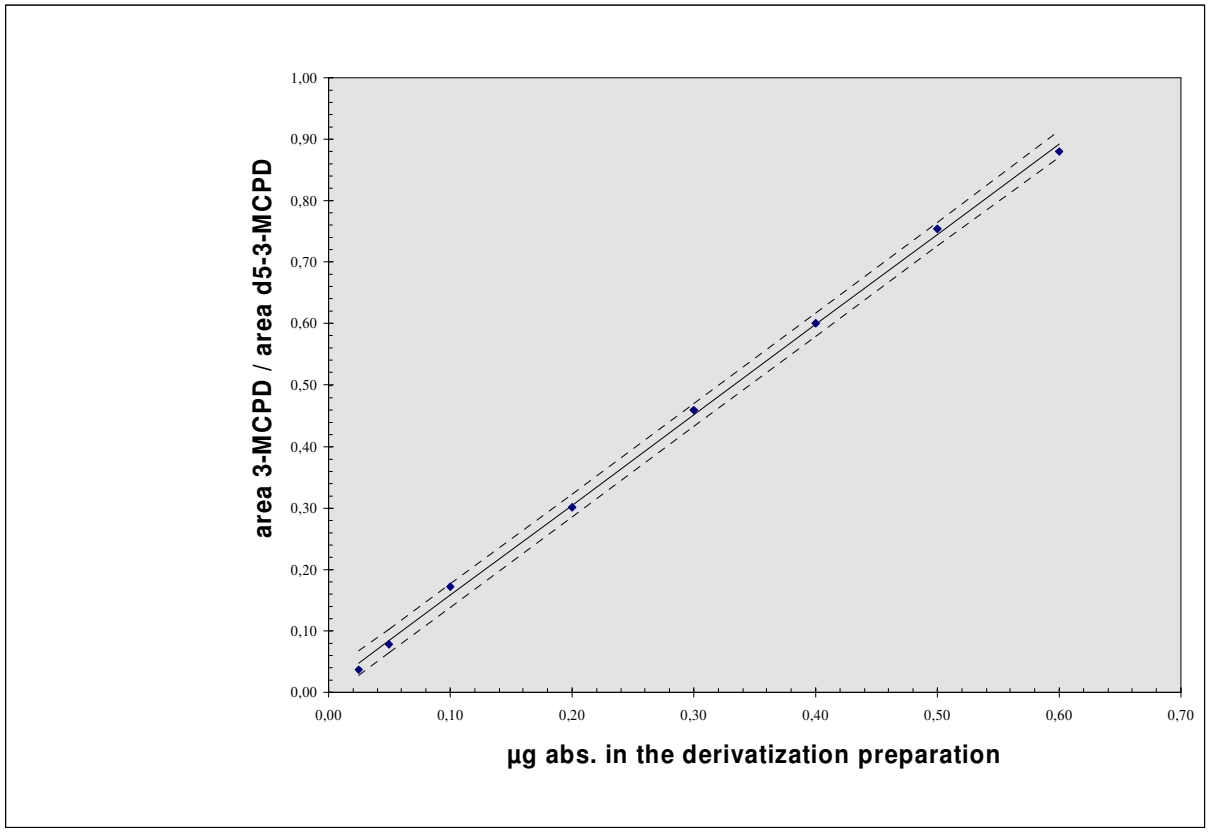

Figure 1Model for a calibration line

9.1.2 Calculation of the 3-MCPD concentrations in the derivatization preparation of the samples

The 3-MCPD concentration of the sample is stated in µg as absolute amount of 3-MCPD in the derivatization preparation.

$$m_{3MCPD} = \frac{(R_{\text{Sample}} - b)}{a}$$

$m_{\text{3-MCPD}}$	Absolute amount (µg) 3-MCPD in the sample extract
R_{Sample}	Response ratio of analyte/internal standard determined in the sample extract

9.1.3 Calculation of the 3-MCPD concentrations in the sample (mg/kg)

$$\omega = \frac{m_{3-MCPD}}{m}$$

ω	3-MCPD concentration stated in mg/kg
m	Sample amount stated in g

The concentration should be given with an accuracy of one significant digit.

10 Selected Chromatograms

The following figures illustrate GC-MS measurement with chemical ionisation.

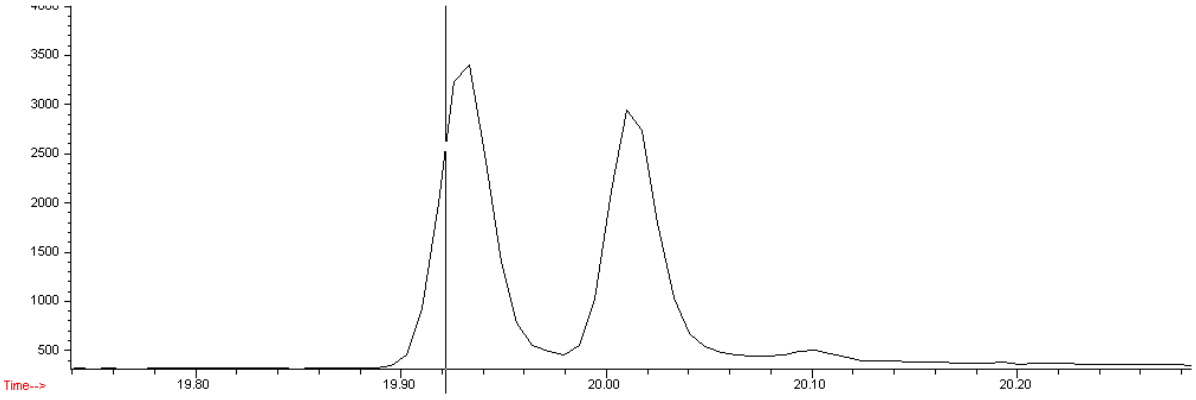

Figure 2 **TIC of a standard solution with 0.4 µg 3-MCPD und 0.4 µg of d_5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d_5-3-MCPD**

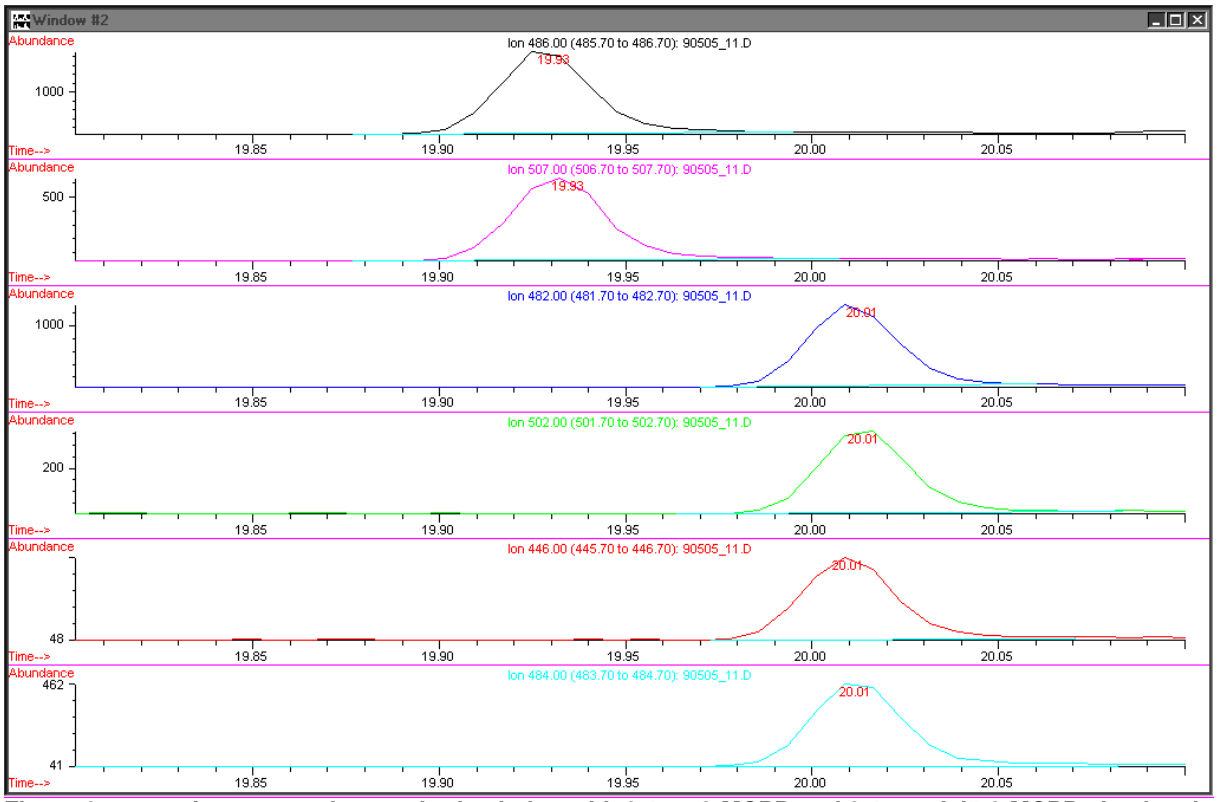

Figure 3 **Ion traces of a standard solution with 0.4 µg 3-MCPD and 0.4 µg of d5-3-MCPD absolute in the derivatization preparation (4 mg/kg 3-MCPD and 4 mg/kg d5-3-MCPD)**

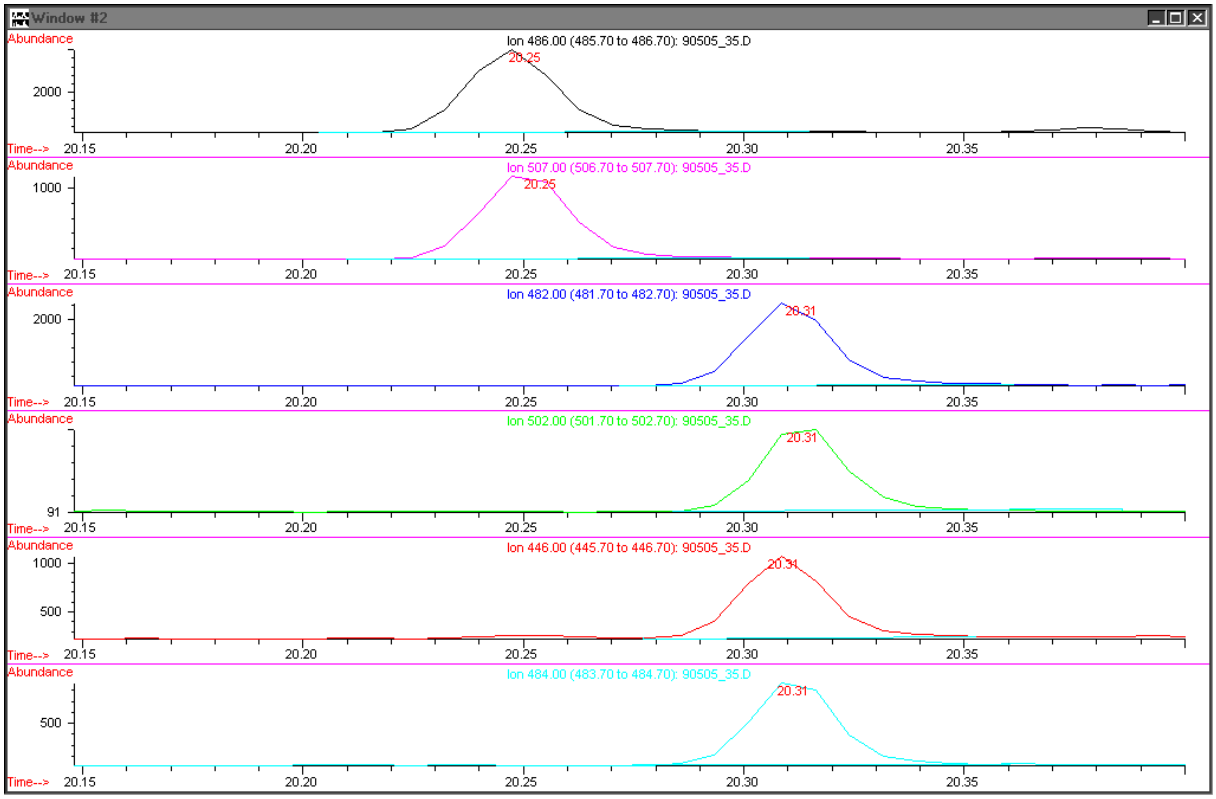

Figure 4 **Ion traces of a sample of solid fat (ca. 3 mg/kg 3-MCPD; spiked with 4 mg/kg d_5-3-MCPD**

11 Validation

11.1 Detection limit and quantification limit

The detection limit and the quantification limit were determined according to DIN 32 645 by means of a calibration function.

Characteristic data were obtained for both by spiking a blank sample with 3-MCPD: Oil samples devoid of analyte were spiked with 3-MCPD calibration solutions (3.1.4.2) which had a low 3-MCPD concentration (ranging between 0.1 and 0.6 mg/kg; equidistant intervals).

The determination of the detection limit was based on a significance level of $\alpha = 0.05$ and the determination of the quantification limit was based on a relative probability of error of 33 % (k=3). In accordance with the method of GC-MS measurement including chemical ionization, the ion traces m/z 502 (3-MCPD) and m/z 507 (d_5-3-MCPD) were used for this evaluation.

Table 2 presents the determined detection and quantification limit as well as the characteristic values of the linear regression.

Table 2 Detection limit and quantification limit according to DIN 32 645

Characteristic values/data	
Slope	**1.2456**
y-intercept	**0.008**
Coefficient of determination (r)	**0.9945**
Detection limit (mg/kg); (α=0.05)	**0.08**
Quantification limit (mg/kg); (probability of error 20 %)	**0.21**

11.2 Determination of the recovery rate

In order to determine the recovery rate, an oil sample devoid of analyte (a blank matrix was repeatedly confirmed as containing no 3-MCPD) was spiked with free 3-MCPD (3.1.1) as well as with 3-MCPD ester (1,2-bis-palmitol-3-chloropropane-1,2-diol; TRC) in three different concentrations, respectively.
Evaluation was performed by means of the ion traces m/z 482 (3-MCPD) and m/z 486 (d_5-3-MCPD). Table shows the mean value of the recovery rate relating to the three concentrations. The recovery rate was determined (in %) as a ratio based on the relation between the concentration actually found and the concentration that should have been expected.
After complete cleavage, 5.4 mg 3-MCPD ester correspond to 1 mg free 3-MCPD.

Table 3 Recovery rate

Added Analyte Concentration	**3-MCPD**	**Mean Value of Recovery**	Number of Samples
0.20 mg/kg 3-MCPD	0.2 mg/kg	**93.5 %**	4
1.00 mg/kg 3-MCPD	1.0 mg/kg	**82.1 %**	5
3.00 mg/kg 3-MCPD	3.0 mg/kg	**85.7 %**	5
1.06 mg/kg 3-MCPD ester	0.2 mg/kg	**103.8 %**	5
5.40 mg/kg 3-MCPD ester	1.0 mg/kg	**107.2 %**	5
16.20 mg/kg 3-MCPD ester	3.0 mg/kg	**98.5 %**	5

11.3 Laboratory precision

In order to determine the laboratory precision with regard to repeatability conditions, three fat samples with different 3-MCPD concentrations, respectively were subject to manifold analysis (performed by identical laboratory assistant with identical sample and identical laboratory apparatus (see Table 4).

Table 4 Laboratory precision related to repeatability conditions

Sample	**Number of determinations**	**Mean value 3-MCPD (mg/kg)**	**Standard deviation (mg/kg)**	**Coefficient of variation (%)**
Sample 1 (rape oil)	8	0.11	0.034	30.9
Sample 2 (safflower oil)	8	1.82	0.129	7.11
Sample 3 (solid fat)	7	3.09	0.197	6.36

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