JRC Scientific and Technical Reports

Proficiency test on the determination of 3-MCPD esters in edible oil

Final Report

Lubomir Karasek, Thomas Wenzl and Franz Ulberth

EUR 24356 EN - 2010

JRC EUROPEAN COMMISSION

irmm Institute for Reference Materials and Measurements

The mission of the JRC-IRMM is to promote a common and reliable European measurement system in support of EU policies.

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
Address: Retieseweg 111, 2440 Geel, Belgium
E-mail: thomas.wenzl@ec.europa.eu
Tel.: +3214571320
Fax: +3214571343

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

EUR 24356 EN
ISBN 978-92-79-15710-3
ISSN 1018-5593
DOI 10.2787/2587

Luxembourg: Publications Office of the European Union

Printed in Belgium

Summary

The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) was requested by the Directorate General Health and Consumers (DG SANCO) to organise a proficiency test on the determination of 3-chloropropane-1,2-diol esters (3-MCPD esters) in edible oils. The aim of this proficiency test was to scrutinise the capabilities of official food control laboratories, private food control laboratories as well as laboratories from food industry to determine the 3-MCPD esters content of edible oils. The study was announced in July 2009 by the JRC IRMM and DG SANCO.

The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" and ISO Guide 43. Three test materials were dispatched to the participants: contaminated palm oil, spiked extra virgin olive oil and a 3-MCPD standard solution in sodium chloride.

The palm oil test material was supplied by the European Federation of the Oil and Proteinmeal Industry (FEDIOL). The spiked olive oil was prepared by gravimetrical addition of 3-MCPD-1,2-dioleate to blank extra virgin olive oil, which was purchased from local retail markets in Belgium.

Altogether 41 laboratories from 11 EU Member States, Switzerland and Macedonia subscribed for participation in the study. The participants were asked to determine the 3-MCPD esters content of the test samples by application of their in-house analysis methods. The laboratories were requested to report the results via a web-interface into a secured databank. In total, 34 laboratories, representing official control laboratories, industry and other interested parties reported results to the organisers of the study.

Details regarding the applied analytical methods were requested from the participants too. Twenty six participants filled in and returned a questionnaire with details of their analysis method back to the organisers.

The assigned value for the 3-MCPD esters content of the palm oil test material was established by isotope dilution gas chromatography – tandem mass spectrometry with bracketing calibration (GC-MS/MS). The assigned value of the spiked sample of extra virgin olive oil was derived from the gravimetrical preparation data. The level of the target standard deviation for proficiency assessment was established based on the results of previous studies organised by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR). A value of 20 % for the relative standard deviation was considered fit for the purpose.

The performance of laboratories in the analysis of the 3-MCPD standard solution in sodium chloride was expressed as the relative bias from the gravimetrically established preparation value. A significant contribution of instrument calibration to the deviation of the results for the oil samples from the assigned values was detected for some of the participants by comparing the relative bias of the results for the oil samples with that of the 3-MCPD standard solution in sodium chloride.

The performance of laboratories in the determination of 3-MCPD esters in edible oils was expressed by z-scores. They are considered satisfactory if the values of $|z|$ are ≤ 2. The percentage of satisfactorily performing laboratories was 56 % for palm oil, and 85 % for spiked extra virgin olive oil test samples.

The study revealed that the application of a particular analysis procedure might lead to strong positive bias.

Contents

1. Introduction

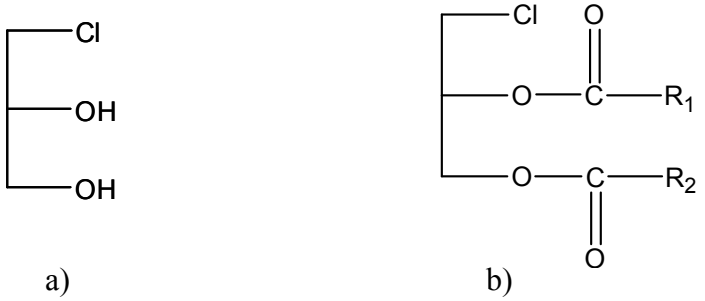
3-Chloropropane-1,2-diol (3-MCPD, Figure 1.1 a) is a well known contaminant in various foods such as acid hydrolysed vegetable protein (HVB), soy sauce, different food ingredients and bakery products.

For 3-MCPD in HVB and soy sauce, maximum levels of 20 µg/kg have been established by Commission Regulation (EC) No 1881/2006 [1]. Provisions for methods of sampling and analysis for the official control of 3-MCPD are laid down in Commission Regulation (EC) No 333/2007 [2].

3-MCPD esters were recently detected in a variety of different foodstuffs, especially in refined vegetable oils and products made of refined vegetable oils. High levels (above 4 mg/kg) were found in hydrogenated fats, palm oil and solid frying fats [3]. Esters of 3-MCPD with higher fatty acids (Figure 1.1 b) are formed at high temperatures during the refining process of edible oils and fats, mainly during the deodorisation step.

The Scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) was asked by the European Commission for a statement regarding the findings of high levels of 3-MCPD esters in refined edible oils. Taking into account the opinion of the German Institute for Risk Assessment (BfR) on 3-MCPD esters [3], the CONTAM panel preliminarily assumed 100 % release of the 3-MCPD moiety from its esters in humans through the action of gut lipases [4].

The International Life Science Institute (ILSI) in cooperation with the European Commission organised a workshop on 3-MCPD esters in food products on 5 - 6 February 2009 in Brussels. The topics addressed were: the assessment of risks posed by 3-MPCD esters in food; analysis and method validation; occurrence, exposure and toxicology; formation routes and mitigation options [5]. During the workshop it was concluded that the presence of 3-MCPD esters in food is a topic of potential concern, which requires close follow-up and urgent initiatives by the authorities and food business operators on, among others, the availability of a validated method of analysis, including sample preparation, for the determination of 3-MCPD esters in different foodstuffs to obtain reliable and comparable analytical results.



a) b)

Figure 1.1. Structure of 3-MCPD (a) and 3-MCPD esters (b)

The JRC - IRMM was requested by the Directorate General Health and Consumers (DG SANCO) to organise an interlaboratory comparison in order to assess the ability of laboratories in Europe to determine the 3-MCPD esters content of edible oils.

The interlaboratory comparison was free of charge for the participants. The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories", further-on denoted as "Harmonised Protocol" [6] and ISO Guide 43 [7]. It was announced by DG SANCO to the competent authorities of EU Member States, EEA countries and candidate countries. Information concerning the application procedure for the study was also made available on the homepage of the JRC-IRMM (http://irmm.jrc.ec.europa.eu). Registration of participants was facilitated via a special web-interface (Annex 1).

Altogether 41 laboratories from 11 EU Member States, Switzerland and from Macedonia subscribed for participation in the study. Receipt of the test samples was confirmed by the participants via the sample receipt form (see Annex 2).

The participants were asked to determine the 3-MCPD esters as total 3-MCPD content of the test samples by application of their usual in-house analysis methods. The laboratories were requested to report the results via the web-interface into a secured databank:

http://www.irmm.jrc.be/imepapp/jsp/loginResult.jsp

2. Test Material

2.1 Preparation

The contaminated palm oil sample was received from the European Federation of the Oil and Proteinmeal Industry (FEDIOL). The blank extra virgin olive oil sample (3-MCPD esters content below 30 µg/kg) was purchased from a local retail market in Belgium. The material was stored at room temperature.

The contaminated palm oil material was heated to 55 °C, stirred for 1 hour, filled in 10 mL amber glass ampoules with added small magnetic stirring bars, and sealed under inert atmosphere at the IRMM Reference Materials Unit.

The spiked extra virgin olive oil sample was prepared gravimetrically by addition of a 3-chloropropane-1,2-dioleate standard (GC purity 99.55 %), which was synthesised on request at the Institute of Chemical Technology in Prague, to blank extra virgin olive oil, stirred

overnight, filled in 10 mL amber glass ampoules and sealed under inert atmosphere at the IRMM Reference Materials Unit.

The 3-MCPD standard solution was prepared gravimetrically by addition of the 3-chloropropane-1,2-diol standard (Sigma-Aldrich, Bornem, Belgium) to an aqueous sodium chloride solution (200 g/L). The material was filled in 10 mL amber glass ampoules and sealed under inert atmosphere at IRMM.

All ampoules got unique identifiers and were stored at room temperature.

2.2 Homogeneity of the test samples

Sufficient homogeneity was assumed for the test solution of the 3-MCPD standard in sodium chloride as it consisted of a well mixed solution of the analyte in a solvent of relatively low viscosity.

Homogeneity of the contaminated palm oil and the spiked extra virgin olive oil test materials was evaluated according the Harmonised Protocol [6].

The contents of ten randomly selected test sample vials were analysed in duplicate by gas chromatography mass spectrometry (GC-MS) after hydrolysis, derivatisation with phenylboronic acid and liquid-liquid extraction. This method was previously validated in a collaborative trial organised by the German Institute for Risk Assessment (BfR), modified and standardised by the German Society for Fat Science (DGF) [8-9]. In brief, portions of 0.1 g of oil sample were placed into 10 ml amber glass screw cap vials, dissolved in *t*-butylmethylether/ ethylacetate (80/20, v/v) and after addition of a deuterated internal standard (3-MCPD-d_5) treated for 15 min with 0.5 mL of 1-propanol/sulphuric acid (100/0.5, v/v) in an ultrasonic bath at 45 °C. The hydrolysis of 3-MCPD esters was carried out by addition of sodium methoxide (0.5 mL) solution in methanol (0.5 mol/L). The samples were vigorously shaken and left at room temperature for 10 min. The reaction was stopped by addition of 3 mL of acetic acid (3.3 %, v/v) in 20 % sodium chloride. Then 3 mL of *n*-hexane were added and the organic phase was removed and discarded, another 3 mL of *n*-hexane were added, the samples were shaken and organic phase was discarded. The derivatisation of 3-MCPD was carried out by addition of 250 µL of phenylboronic acid to the vial with the sample. The vial with the sample was heated up to 90 °C for 20 minutes, then it was left to cool down at the room temperature. The derivative of 3-MCPD with PBA, 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane (Figure 2.1), was extracted from the reaction mixture by shaking with 3 mL of

n-hexane. The final determination of the total 3-MCPD was performed by gas chromatography-tandem mass spectrometry (GC-MS/MS). The final *n*-hexane extract containing the 3-MCPD derivative was injected (1 µL) into the GC in splitless mode. The separation of analytes was carried out on a capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm, 5 % phenyl, 95 % polymethylsiloxane). Identification and quantification of analytes was performed by internal standardisation using 3-MCPD-d_5 as an internal standard. GC-MS/MS was operated in selected reaction monitoring (SRM) mode. The transition 196>147 (derivative of native 3-MCPD) and 201>150 (derivative of deuterium labelled 3-MCPD) were used for quantitation. Transition 198>147 was used for confirmation of the analyte identity.

For confirmation of the results acquired by the described method [8], another, different sample preparation protocol was applied. The oil samples were subjected to acidic methanolysis by sulphuric acid (1.8 % in methanol, v/v) [10-11], derivatised by PBA and analysed by GC-MS/MS.

Both of the above mentioned protocols apply a treatment of the sample with sulphuric acid at the beginning of the sample preparation. This approach avoids the generation during the analysis of additional 3-MCPD from fatty acid esters of glycidol, which can be present in high amounts in the refined edible oil samples [12]. However, glycidyl esters are completely degraded by acid treatment.

The homogeneity of the test samples was proven by subjecting the results of the duplicate measurements to one-way analysis of variance (ANOVA). The variation of the 3-MCPD esters content between the ten different sample vials was not significantly larger than the variation within the vials. All analyses complied with the provisions given by the Harmonised Protocol. Hence it was concluded that the palm and extra virgin olive oil test materials were sufficiently homogeneous.

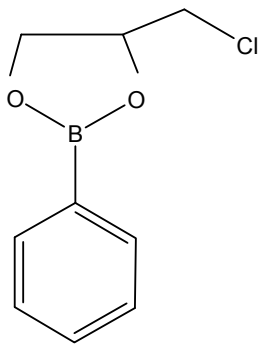


Figure 2.1. Product of the derivatisation of 3-MCPD with phenylboronic acid, 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane

2.3 Stability of the test samples

The 3-MCPD esters content of the palm oil and spiked extra virgin olive oil test materials was monitored, using both of the above mentioned protocols, at the beginning of the study, during the study as well as after receipt of the results of the participants as it is suggested in the Harmonized Protocol [6]. Statistically significant differences of the results of analysis obtained before dispatch of samples and after termination of the study were not found, thus indicating the stability of the test materials. Test samples were kept at room temperature for the period of the study.

2.4 Dispatch of samples

All samples were packed in polystyrene boxes and sent via express mail. The samples were received mostly within 24 hours after dispatch. The participants were asked to fill in the sample receipt form (Annex 2) and to send it back to the organisers by e-mail or fax. The samples were dispatched from IRMM on 16 November 2009. Each participant received (together with the shipment) the sample receipt form, an accompanying letter with instructions for sample handling, measurement, and reporting (Annex 3), and four 10 mL amber glass ampoules containing the palm oil, the spiked extra virgin olive oil, blank extra virgin olive oil and the 3-MCPD standard solution in 20 % sodium chloride. The blank extra virgin olive oil was added to the set of test samples to support laboratories in method development.

3. Statistical evaluation of the results

3.1 Assigned value

Contaminated palm oil material

An assigned value for the 3-MCPD esters content of the palm oil test material was established by isotope dilution GC-MS/MS using the "bracketing technique" for calibration. The bracketing calibration method is frequently used for the establishment of reference values for the analyte contents of reference materials [13, 14].

The isotope labelled 3-chloropropane-1,2-dipalmitate-d_5 (Toronto Research Chemicals Inc., North York, Canada) was added to the sample at a level close to that of the naturally present 3-MCPD esters level in the test material, which was roughly estimated in a preceding analysis. Two standard solutions containing native 3-chloropropane-1,2-dipalmitate (Toronto Research Chemicals Inc., North York, Canada) were prepared in parallel:

Standard A: 3-chloropropane-1,2-dipalmitate concentration level between 10 and 20 % lower than roughly estimated 3-MCPD esters content of sample

Standard B: 3-chloropropane-1,2-dipalmitate concentration level between 10 and 20 % higher than roughly estimated 3-MCPD esters content of the palm oil sample.

The standards and the sample contained labelled 3-chloropropane-1,2-dipalmitate-d_5 at the same concentration level, which was close to the level of the estimated assigned value. The sample and the standards were analysed in the following sequence: Standard A – Sample - Standard B - Standard B -Sample - Standard A - Standard A – Sample - Standard B - Standard B – Sample - Standard A. The measurement scheme was repeated on a second day with freshly (starting from the pure substances) prepared standards; on both days the DGF Standard C-III 18 (09) protocol [8] was applied. The measurement scheme was repeated again on a third day by using the acidic hydrolysis method [10-11] with the modification that sodium chloride was substituted by ammonium sulphate. The assigned value corresponds to the average value of all sample measurements of the three days. The results produced by alkaline and acidic transesterification agreed within ± 6 %.

The 3-MCPD esters content of the sample was calculated for each standard-sample-standard triplet according to equation 3.1:

$$C = \left[\frac{(I_S - I_A) * (W_B - W_A)}{(I_B - I_A)} + W_A \right] * \frac{M_{Lab}}{M_S} \qquad \text{Equation 3.1}$$

C: 3-MCPD esters content of the test sample (mg/kg)
I_S: ion intensity ratio of unlabelled/labelled 3-MCPD esters measured in the test sample
I_A: ion intensity ratio of unlabelled/labelled 3-MCPD esters measured in Standard A
I_B: ion intensity ratio of unlabelled/labelled 3-MCPD esters measured in Standard B
W_A: mass ratio of unlabelled/labelled 3-MCPD esters measured in Standard A
W_B: mass ratio of unlabelled/labelled 3-MCPD esters measured in Standard B
M_{Lab}: mass of the labelled 3-MCPD-1,2-dipalmitate added to the sample (µg)
M_S: weight of the sample (g)

The combined uncertainty of the assigned value was estimated from the standard uncertainties of the different sources (weighing, purity of standards, and repeatability of measurements). Results of the determination of the assigned value by isotope dilution GC-MS/MS are shown in Annex 4.

Spiked extra virgin olive oil and 3-MCPD standard solution test samples

The spiked extra virgin olive oil has been prepared by gravimetrical addition of a 3-MCPD-1,2-dioleate to the blank extra virgin olive oil. The standard solution in 20 % sodium chloride was prepared by dilution of 3-MCPD standard with 20 % sodium chloride, therefore the assigned value for these two materials were derived from the gravimetrical preparations.
The uncertainties of the assigned values for spiked extra virgin olive oil, and for the 3-MCPD solution in 20 % sodium chloride were estimated from the standard uncertainties of the different preparation steps. The respective values are given in the tables 4.1, 4.3, 4.5, and 4.7.

3.2 Performance indicator and standard deviation for proficiency assessment

The performance of an individual laboratory *i* was expressed by the z_i-score, which was calculated according to equation 3.2:

$$z_i = \frac{x_i - \hat{X}}{\sigma_P} \qquad \text{Equation 3.2}$$

z_i: z-score of laboratory *i* for the respective sample; x_i reported result of laboratory *i* for that sample, expressed as the mean of multiple determinations; $\hat{X}$: assigned value for the respective sample, σ_P: standard deviation for proficiency assessment

The standard deviation for proficiency assessment was set by the organisers of the study to be fit for purpose, because the application of the modified Horwitz equation for the concentration level of total 3-MCPD in the samples would lead to relative standard deviations in the range of 11 to 12 %. The organiser considered such low values as too strict for such a complicated analysis procedures which includes, among others, a derivatisation step. Guidance on the magnitude of the standard deviation for proficiency assessment was given by the results of the method validation study by collaborative trial, which was organised by the BfR [8]. There the reproducibility standard deviations of the tested methods were in the range of 7 to 28 % [8]. Accounting for the additional variability introduced by the application of different procedures, a relative standard deviation of 20 % was considered reasonable for performance evaluation. The standard deviation for proficiency assessment was calculated for the individual test samples according to equation 3.3. The appropriateness of this level of tolerated variability of results was confirmed by calculation of the relative standard deviations for the mean values of the participants' results for the contaminated palm, and the spiked extra virgin olive oil test materials after exclusion of outliers. The calculated relative standard deviations were within the range of 20 % to 23 %.

$$\sigma_P = \frac{20 \times \hat{X}}{100} \qquad \text{Equation 3.3}$$

$\hat{X}$: assigned value for the respective sample, σ_P: standard deviation for proficiency assessment

z-Scores were calculated for the oil test samples only. The acceptability of a laboratory's performance was evaluated according to the following generally accepted limits [6, 7]:

$\lvert z\rvert \leq 2.0$	satisfactory
$2.0 < \lvert z\rvert < 3.0$	questionable
$\lvert z\rvert \geq 3.0$	unsatisfactory

The performance of an individual laboratory *i* in the analysis of the 3-MCPD standard solution in sodium chloride was expressed by the relative bias from the gravimetrically established value, which was calculated according to equation 3.4:

$$Rel.\,bias_i = \frac{x_i - \hat{X}}{\hat{X}} \times 100 \qquad \text{Equation 3.4}$$

Relative bias of laboratory *i* for the respective sample; x_i reported result of laboratory *i* for that sample, expressed as the mean of multiple determinations; $\hat{X}$: assigned value for the respective sample.

4. Performance assessment

4.1 General

Thirty four of the 41 laboratories that enrolled in the study reported results. However the deadline for the reporting of results had to be extended on request of some of the participants to 22 January 2010.

In order to assure confidentiality, the identities of the laboratories were coded by a unique number between 100 and 300.

Data of laboratories that reported measurement results for the 3-MCPD ester contents of the edible oil samples were considered in the statistical evaluations. Analysis procedure dependent differences in the performance of the laboratories were found for the contaminated palm oil test material. The individual procedures are colour coded in Figure 4.1 in order to allow easy distinction. However, method dependent differences in performance were not found for the spiked olive oil sample.

The distributions of the results were checked by Kernel density estimations. This analysis is also capable of determining multimodality [6, 15]. In general the results of analysis were not normally distributed, the data sets contained outliers and the respective Kernel density plots showed several modes (figures 4.2, 4.4, 4.6 and 4.8).

Details regarding the applied analytical methods were requested from the participants too. Twenty six participants filled in and sent the questionnaire with method details back to the organisers. The details of the applied analysis methods are given in Annex 5.

4.2 z-Scores of the participants

4.2.1 Contaminated palm oil

A summary of the statistical evaluation is presented in Table 4.1.

Fifteen laboratories out of 34 (44 %) reported results leading to |z|>2 for the contaminated palm oil test material. Laboratory mean values of the determinations of 3-MCPD esters in the palm oil test sample are tabulated with the corresponding z-scores in Table 4.2. Figure 4.1 shows the plot of z-scores in ascending order, with indication of the methods applied. The distribution of the results was checked for multimodality by Kernel density estimation (figure 4.2).

Positively biased results reported for the 3-MCPD esters content of the contaminated palm oil were probably caused by transformation of glycidyl esters to 3-MCPD during the analysis. Those laboratories which treated the sample with acid at the beginning of the sample preparation, achieved better z-scores, which could be reasoned by the complete degradation of glycidol esters.

Table 4.1: Summary statistics for the contaminated palm oil test sample

Number of results		34
Range of results	mg/kg	0.6 to 18.8
Median	mg/kg	10.19
Huber H15	mg/kg	10.82
Mean of results of participants	mg/kg	10.76
Mean of results of participants after removal of outliers (according to [15])	mg/kg	10.28
Assigned value (bracketing, isotope dilution GC-MS/MS)	mg/kg	**8.77**
Expanded uncertainty (k=2) of the assigned value	mg/kg	0.35
Robust standard deviation ($\hat{\sigma}$)	mg/kg	3.40
Target standard deviation (fitness for purpose, RSD_R= 20%)	mg/kg	**1.75**
Number (percentage) of results of |z| > 2.0		15 (44 %)

Table 4.2: Results of analysis and z-scores for the contaminated palm oil test sample; bold printed z-scores mark results outside the satisfactory range

Lab Number	reported result [mg/kg]	z - score	Lab Number	reported result [mg/kg]	z - score
120	10.1	0.7	**192**	12.4	**2.1**
123	18.8	**5.7**	**195**	8.1	-0.4
129	13.4	**2.6**	**198**	15.3	**3.7**
132	12.3	2.0	**201**	12.6	**2.2**
138	10.5	1.0	**204**	13.0	**2.4**
150	8.9	0.1	**207**	10.3	0.9
156	15.5	**3.8**	**210**	12.6	**2.2**
159	8.1	-0.4	**213**	3.0	**-3.3**
162	16.5	**4.4**	**216**	14.1	**3.1**
165	8.7	-0.1	**219**	8.6	-0.1
168	6.7	-1.2	**222**	0.6	**-4.7**
171	14.5	**3.2**	**225**	9.9	0.7
174	9.2	0.2	**228**	9.1	0.2
177	8.3	-0.3	**231**	10.9	1.2
180	18.6	**5.6**	**234**	8.3	-0.2
186	7.0	-1.0	**237**	9.2	0.2
189	13.7	**2.8**	**240**	7.3	-0.9

Figure 4. 1: Plot of participants' z-scores for the contaminated palm oil test sample. The different analysis procedures applied are colour coded.

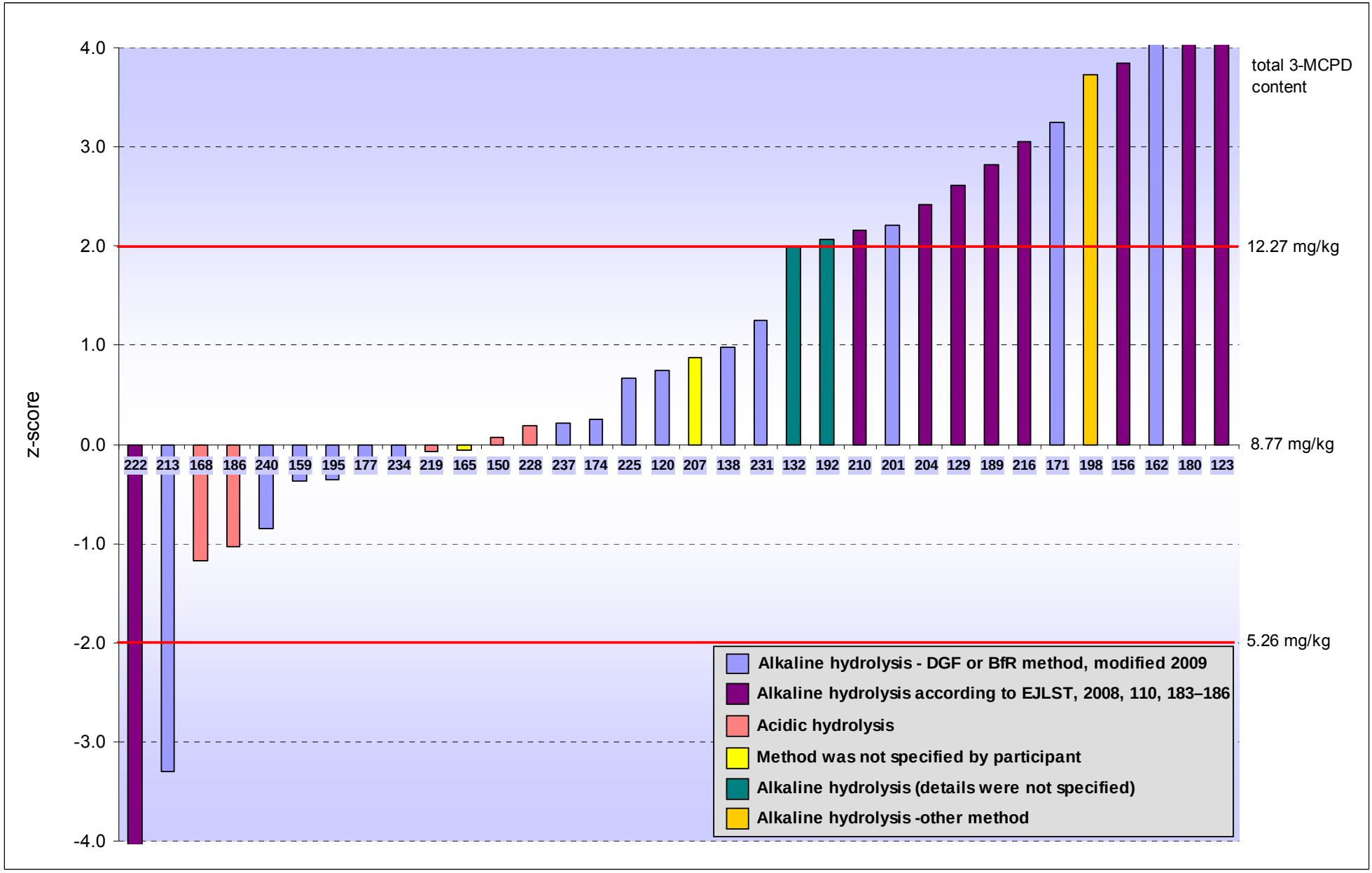
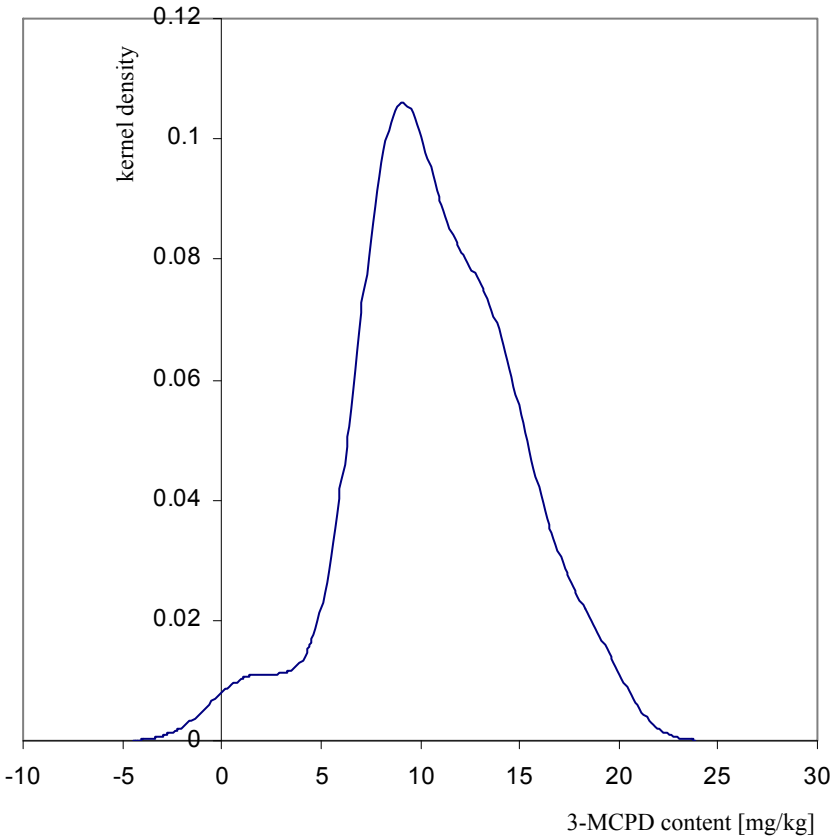


Figure 4.2: Kernel density plot of the participants' results for the contaminated palm oil test sample



4.2.2 Spiked extra virgin olive oil

A summary of the statistical evaluation is presented in Table 4.3. Five laboratories out of 34 (15.3 %) reported results with |z|>2. Laboratory mean values of the determinations of total 3-MCPD in the spiked extra virgin olive oil test sample are tabulated with the corresponding z-scores in Table 4.4. As this sample was not refined, it can be assumed that it was free of glycidol esters. This is supported by the performance of the laboratories, which did not show any method dependent differences. Hence method dependent colour coding of the results was abandoned. Figure 4.3 shows the plot of z-scores in ascending order.

The distribution of the results was checked for multimodality by Kernel density estimation (Figure 4.4).

Table 4.3: Summary statistics for the spiked extra virgin olive oil test sample

Number of results		34
Range of results	mg/kg	2.38 to 14.78
Median	mg/kg	4.40
Huber H15	mg/kg	4.53
Mean of results of participants	mg/kg	4.85
Mean of results of participants after removal of outliers (according to [15])	mg/kg	4.45
Assigned value (established gravimetrically)	mg/kg	**4.58**
Expanded uncertainty (k=2) of the assigned value	mg/kg	0.21
Robust standard deviation ($\hat{\sigma}$)	mg/kg	0.57
Target standard deviation (fitness for purpose, RSD_R 20%)	mg/kg	**0.92**
Number (percentage) of results of |z| > 2.0		5 (15.3 %)

Table 4.4: Results of analysis and z-scores for the spiked extra virgin olive oil test sample; bold printed z-scores mark results outside the satisfactory range

Lab Number	reported result [mg/kg]	z - score	Lab Number	reported result [mg/kg]	z - score
120	5.16	0.6	**192**	4.30	-0.3
123	5.45	1.0	**195**	4.03	-0.6
129	4.20	-0.4	**198**	7.95	**3.7**
132	3.80	-0.8	**201**	4.89	0.3
138	6.00	1.6	**204**	2.40	**-2.4**
150	4.51	-0.1	**207**	4.79	0.2
156	7.35	**3.0**	**210**	4.08	-0.5
159	4.46	-0.1	**213**	14.78	**11.2**
162	4.70	0.1	**216**	4.26	-0.4
165	4.76	0.2	**219**	4.99	0.5
168	3.49	-1.2	**222**	2.38	**-2.4**
171	4.09	-0.5	**225**	4.74	0.2
174	4.35	-0.2	**228**	5.42	0.9
177	4.35	-0.2	**231**	4.94	0.4
180	4.75	0.2	**234**	4.24	-0.4
186	3.12	-1.6	**237**	4.25	-0.4
189	4.35	-0.2	**240**	3.76	-0.9

Figure 4.3: Plot of participants' z-scores for the spiked extra virgin olive oil test sample

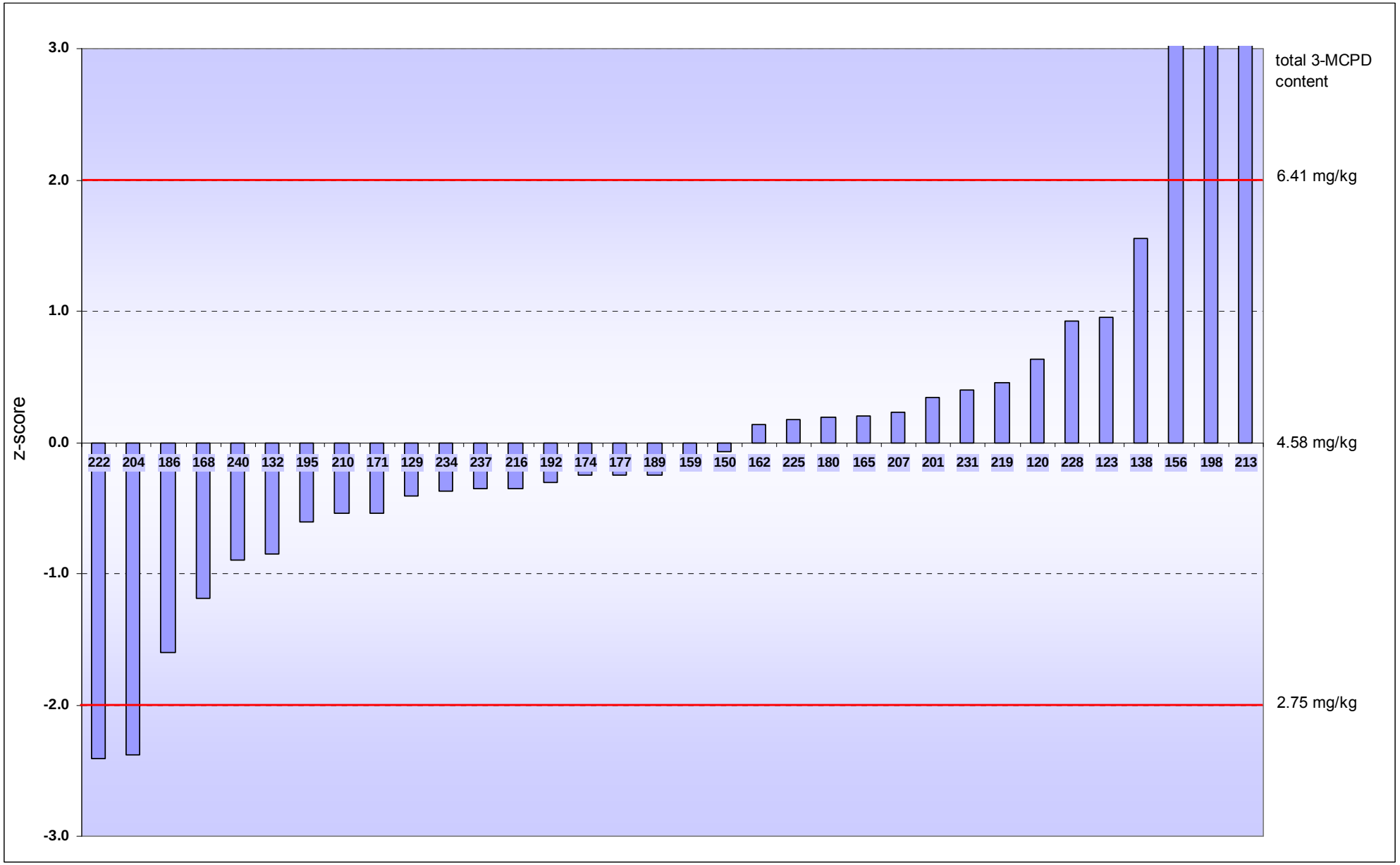
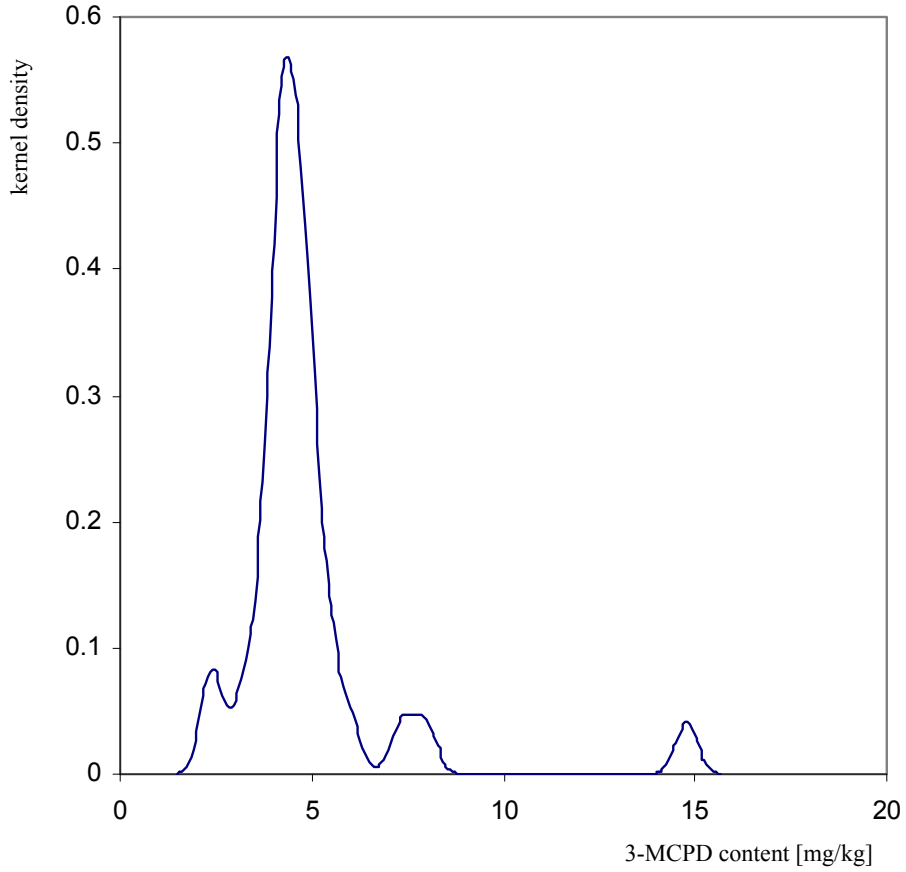


Figure 4.4: Kernel density plot of the participants' results for the extra virgin olive oil test sample



4.2.4 Solution of 3-MCPD in sodium chloride

Thirty laboratories reported results for the solution of 3-chloropropane-1,2-diol in 20 % sodium chloride. A summary of the statistical evaluation is presented in Table 4.7. Laboratory mean values of the determinations of 3-MCPD standard solution are tabulated with the corresponding relative bias in Table 4.8. Figure 4.7 shows the plot of relative bias from the assigned value in ascending order. The respective Kernel density plot is depicted in Figure 4.8.

Some participants submitted the results in units other than requested. These results were transferred into the requested units by application of the density of 20 % sodium chloride solution of 1.14779 g/mL and the density equation.

Table 4.7: Summary statistics for 3-MCPD solution in 20 % sodium chloride

Number of results		30
Range of results	ng/mL	0.936 to 850
Median	ng/mL	422
Huber H15	ng/mL	441
Mean of results of participants	ng/mL	443
Mean of results of participants after removal of outliers (according to [15])	ng/mL	435
Assigned value (established gravimetrically)	ng/mL	**417**
Expanded combined uncertainty (k=2) of the assigned value	ng/mL	11
Number (percentage) of results of rel. bias > 20 %		11 (37)

Table 4.8: Results of analysis and relative bias for 3-MCPD standard solution

Lab Number	reported result [ng/mL]	relative bias [%]	Lab Number	reported result [ng/mL]	relative bias [%]
123	506	**21.3**	189	674	**61.7**
129	420	0.8	192	385	-7.6
132	850	**103.9**	195	395	-5.2
138	471	13.0	201	425	1.9
150	354	-15.2	204	380	-8.8
156	300	**-28.0**	207	496	19.0
159	390	-6.4	210	405	-2.8
162	705	**69.1**	213	0.936	**-99.8**
165	428	2.7	216	257	**-38.4**
168	594	**42.5**	219	389	-6.6
171	474	13.7	222	361	-13.5
174	447	7.1	225	470	12.8
177	633	**51.9**	228	445	6.8
180	415	-0.4	231	377	-9.6
186	640	**53.5**	234	202	**-51.5**

Figure 4.7: Plot of participants' relative bias from the gravimetrically established value of 3-MCPD content of the 20 % sodium chloride solution

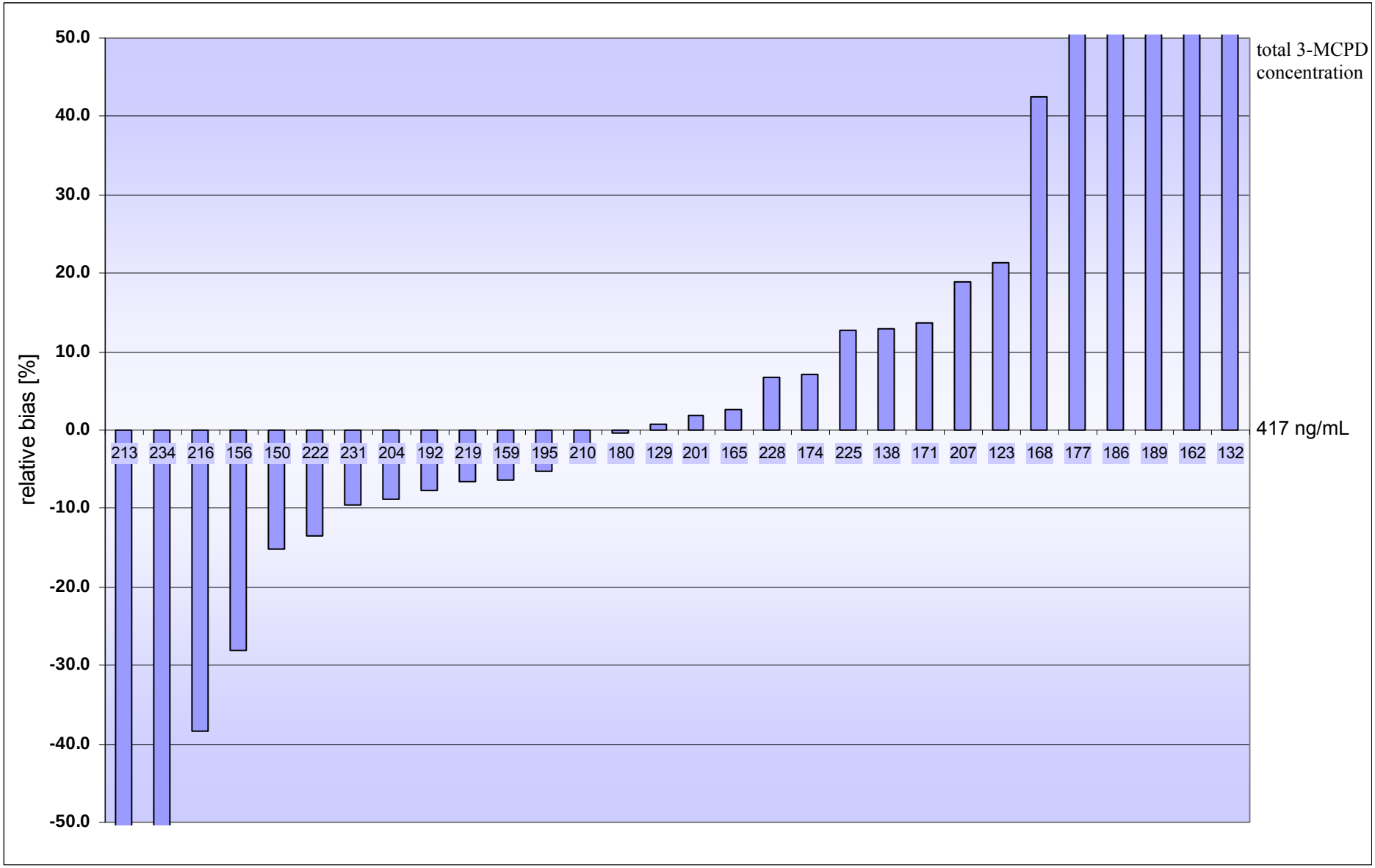
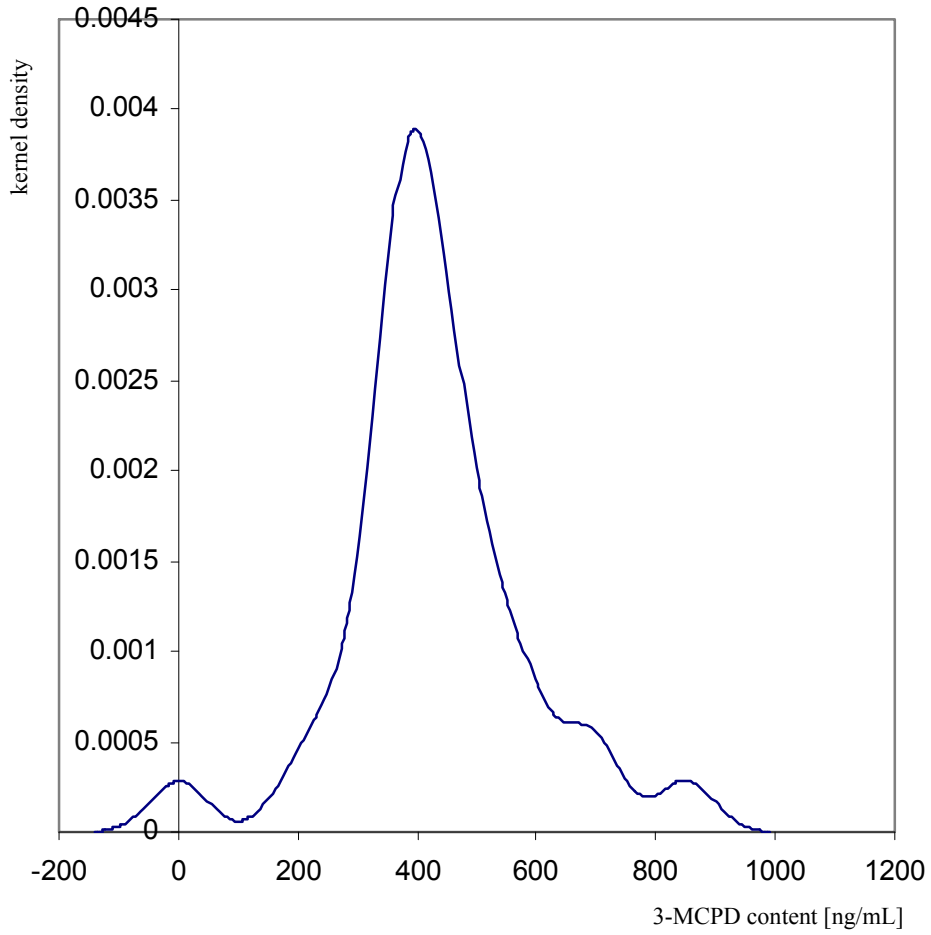


Figure 4.8: Kernel density plot of the participants' results for the 3-MCPD solution in 20 % sodium chloride



5. Conclusions

- 34 participants reported results for the palm oil test material, 56 % of them were within the satisfactory performance range (z-score ≤ |2.0|).
- 34 participants reported results for the spiked extra virgin olive oil test material, 85 % of them were within the satisfactory performance range.
- 30 participants reported results for the 3-MCPD standard solution in sodium chloride, a relative bias of less than 20 % was achieved by 63 % of them, and a relative bias of less than 30 % was achieved by 70 % of participants.
- The critical steps in the analysis of 3-MCPD esters in oil samples are linked to the method of esters hydrolysis and instrument calibration.
- A number of biased results reported for the 3-MCPD ester content of palm oil is probably caused by transformation of glycidyl esters to 3-MCPD. Method dependant differences in the performance were not found for the spiked virgin olive oil sample and among laboratories, which treated the sample with acid at the beginning of sample preparation.
- A number of laboratories stated that they just stepped into this field of analysis; therefore they were at the time of the interlaboratory comparison test still busy with the in-house validation of analytical methods, and had a lack of experience with this type of analysis.
- Application of a well defined harmonised analysis procedure might serve preventing bias caused by the measurement of glycidol esters and might also minimise inconsistencies related to instrument calibration and data analysis.
- The study showed the importance of continuous participation in interlaboratory comparison schemes in order to achieve comparability of results. It is recommended to repeat the study after a period of time.

Acknowledgements

The organisers of the study would like to thank Mrs. Claire-Lise Bechert, FEDIOL for the supply of test material and Mrs. Anne-Mette Jensen for her support in the provision of test materials and the Reference Materials Unit at IRMM, in particular Mr. Håkan Emteborg, for ampouling of the test samples.

6. References

[1] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, OJ L 364, 20.12.2006, p. 5, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20090701:EN:PDF

[2] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, OJ L 88, 29.3.2007, p. 29–38, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF

[3] Säuglingsanfangs- und Folgenahrung kann gesundheitlich bedenkliche 3-MCPD-Fettsäureester enthalten, Stellungnahme Nr. 047/2007, Bundesinstitut für Risikobewertung (BfR) 2007, http://www.bfr.bund.de/cm/208/saeuglingsanfangs_und_folgenahrung_kann_gesundheitlich_bedenkliche_3_mcpd_fettsaeureester_enthalten.pdf

[4] Statement of the Scientific Panel on Contaminants in the Food chain (CONTAM) on a request from the European Commission related to 3-MCPD esters, Question number: EFSA-Q-2008-258, Adopted: 28 March 2008, http://www.efsa.europa.eu/en/scdocs/scdoc/1048.htm

[5] Workshop on 3-MCPD esters in food products, International Life Sciences Institute (ILSI), 5-6 February 2009, Brussels (Belgium). http://www.ilsi.org/Europe/Publications/Final%20version%203%20MCPD%20esters.pdf

[6] M. Thompson, S.L.R. Ellison, R. Wood: The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, 78, (2006), 145-196.

[7] International Organization for Standardization. ISO Guide 43: Proficiency testing by interlaboratory comparisons, Part 1: Development and operation of proficiency testing schemes, 1994, Geneva, Switzerland.

[8] Deutsche Gesellschaft für Fettwissenschaft: DGF Standard Method C III 18 (2009): Determination of ester-bound 3-chloropropane-1,2-diol (3-MCPD esters) and 3-MCPD forming substances in fats and oils by means of GC-MS. *Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen*, Wissenschaftliche Verlagsgesellschaft, Stuttgart (Germany) 2009.

[9] R. Weißhaar: Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. *Eur. J. Lipid Sci. Technol.* 2008, 110, 183–186.

[10] Z. Zelinkova, B. Svejkovska, J. Velisek, M. Dolezal: Fatty acid esters of 3-chloropropane-1,2-diol in edible oils. *Food Additives and Contaminants* 2006, 23, 1290–1298.

[11] B. Svejkovska, O. Novotny, V. Divinova, Z. Reblova, M. Dolezal, J. Velisek: Esters of 3-Chloropropane-1,2-diol in Foodstuffs. *Czech J. Food Sci.* 2004, 22, 190–196.

[12] R. Weißhaar, R. Perz: Fatty acid esters of glycidol in refined fats and oils. *Eur. J. Lipid Sci. Technol.* 2010, 112, 158–165.

[13] M. Sargent, R. Harte, C. Harrington, Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry (IDMS). RSC Analytical Methods Committee, LGC Limited, 2002

[14] CCQM-K6: Key Comparison on the Determination of Cholesterol In Serum, Final Report, NIST, 2001,

[15] Analytical Methods Committee, Robust statistics: a method of coping with outliers, Technical brief No 6, Apr 2001. http://www.rsc.org/pdf/amc/brief6.pdf

Annex

Annex 1: Announcement of Study

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
IRMM

Geel, 10/07/2009
ARES(2009)164531

Dear Madame/Sir,

We would like to inform you that we are ready to launch the inter-laboratory comparison study (ILC) on the **determination of 3-chloropropane-1,2-diol (3-MCPD) esters in edible oil**.

Participation is open to all analytical laboratories and is free of charge.

Detailed information about the design of the comparison and the results submission will be provided together with the dispatch of samples.

Registration for laboratories is available via web page:

https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=319

Deadline for the registration is 15 September 2009.

Mr. Karasek and me are at your disposal for any clarification you may wish!
For more information you can contact: JRC-IRMM-Contaminants@ec.europa.eu

Please note that registration can only be done via the link above.

With best regards

Thomas Wenzl

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 343; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

Annex 2: Sample receipt form

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
IRMM

Geel, 07.11. 2008

Inter-laboratory comparison study
on the determination of mineral oil in sunflower oil

SAMPLE RECEIPT FORM

Name of Participant	
Organisation	
Address	

Please check if the samples (consisting of four 50 mL serum bottles and one 10 mL glass ampoule) have been received undamaged.

Date of sample receipt	
The sample has been received undamaged	Yes ☐ / No ☐

Please store the sample at room temperature!

Please return the completed form by email to: **Lubomir.Karasek@ec.europa.eu**

or by fax to: **+32-14-571-783**

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 343; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

Annex 3: Study description

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
IRMM

Geel, 12.11. 2009
ARES (2009) 320257

Dear Sir/Madame,

The inter-laboratory comparison study on the determination of 3-chloropropane-1,2-diol esters (3-MCPD esters) in edible oil starts on **16 November 2009** with the dispatch of samples.
Please, **store the test samples at room temperature** in order to maintain sample integrity!

Outline of the study

Test samples

You will receive:

- an ampoule with 8 mL of naturally contaminated palm oil,
- an ampoule with 8 mL of extra virgin olive oil spiked with 3-MCPD ester,
- an ampoule with 8 mL of blank extra virgin olive oil, which was used for the preparation of spiked sample
- an ampoule with 8 mL of a solution of 3-MCPD in 20% NaCl.

Please take notice that none of the test samples contains any internal standard.

The **total 3-MCPD** content of the **contaminated palm oil** and **spiked extra virgin olive oil** shall be expected within the range of **1 mg/kg to 20 mg/kg**.
The concentration of 3-MCPD in 20 % **sodium chloride solution** shall be expected within the range of **100 ng/mL to 1000 ng/mL**.

Please note, that the **palm oil** is solid at room temperature. It is recommended to warm it up to a temperature between 50 °C and 55 °C and stir it until the oil becomes a completely clear liquid. Each ampoule with the palm oil sample contains a small magnetic stirring bar in order to support mixing.

You are requested to perform duplicate analysis per test material applying a method of your choice.

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 343; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

The content of **total 3-MCPD** has to be reported for each sample. The result shall be reported in **mg/kg** for contaminated palm oil and spiked olive oil samples, and in **ng/mL** for the 3-MCPD solution in 20 % NaCl solution.

The mean values of the replicate analyses will be applied for calculation of performance indicators.

A set of questions regarding the applied analysis method shall be answered as well.

For more information you can contact: JRC-IRMM-Contaminants@ec.europa.eu

Results have to be reported via the web-interface:
https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do

The login key and the sample keys will be sent to you by separate email.

Deadline for reporting of results is 31 December 2009

Mr. Karasek (Lubomir.Karasek@ec.europa.eu; Tel.: +32 14 571301) and myself are at your disposal for any clarification you may wish!

With best regards

Thomas Wenzl

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 343; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

Annex 4: Determination of the reference value – palm oil sample

Table 4.1: Results of isotope dilution GC-MS/MS with bracketing calibration

Material	Day 1	Day2	Day 3
3-MCPD-1,2-dipalmitate - Standard A [µg/mL]	44.818	41.967	41.967
3-MCPD-1,2-dipalmitate - Standard B [µg/mL]	60.976	60.472	60.472
3-MCPD-1,2-dipalmitate-d_5 - IS [µg/mL]	52.493	52.505	52.505
3-MCPD theoretical conc.* - Standard A [µg/mL]	8.435	7.898	7.898
3-MCPD theoretical conc.* - Standard B [µg/mL]	11.475	11.381	11.381
3-MCPD-d_5 theoretical conc.* - IS [µg/mL]	10.317	10.229	10.229
IS amount [µg]	1.0232	1.0234	1.0234
Sample 1 [mg/kg]	9.756	8.606	8.096
Sample 2 [mg/kg]	9.796	8.646	8.406
Sample 3 [mg/kg]	8.731	8.662	8.863
Sample 4 [mg/kg]	8.699	8.225	8.692
average per day [mg/kg]	9.246	8.535	8.514
average [mg/kg]			**8.765**
uncertainty (k=2) [mg/kg]			0.35

* Concentration of free 3-MCPD assuming complete hydrolysis of the ester

Day 1-2: DGF Method [8]
Day 3: Modified acidic hydrolysis [10-11]

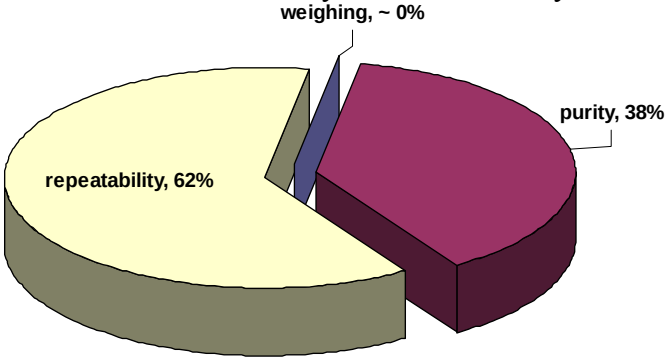
Table 4.2: Molar masses of substances applied for bracketing calibration

analyte	Mol. mass [g/mol]	theoretical ratio 3-MCPD ester/3-MCPD (100 % hydrolysis)
3-MCPD	110.539	
3-MCPD-d_5	115.469	
3-MCPD-dipalmitate	587.36	5.3136
3-MCPD-dipalmitate-d_5	592.39	5.1303

Table 4.3: Estimation of uncertainty of the assigned value

sources of uncertainty	relative standard uncertainty [%]	combined relative uncertainty [%]	expanded relative uncertainty [%] (k=2)	expanded uncertainty [mg/kg] (k=2)
weighing steps	0.0006			
purity of substances	1.041	1.996	3.990	**0.350**
repeatability of measurements	1.703			

Figure 4.1: Contribution of sources of uncertainty to the uncertainty of the assigned value



Annex 5: Analytical methods applied by the participants

The details of the applied analysis methods are tabulated as they were reported by the participants. The presented data were not at all edited. Not tabulated information was not submitted. It should be noted that the authors do neither claim completeness nor correctness of the given information.

Table 5.1: Number of samples analysed by laboratories per year for the 3-MCPD esters content

Lab Code	Number of samples per year			
	< 20	20 - 50	51 - 200	> 200
120	X			
123				
129	X			
132		X		
150				X
156				X
159				X
168				X
177		X		
180		X		
186				X
189	X			
195				X
198				
201		X		
204			X	
210				X
213	X			
216				
219	X			
222		X		
225			X	
228			X	
231			X	
234			X	
237			X	

Table 5.2: Sample preparation details

Lab Code	Sample weight in [g]	Sample preparation, dissolution, solvents applied				
		dissolution in organic solvent	destruction of glycidol with acid	salting out with solution of NaCl	salting out with solution of other salt	details:
120	0.1 - 0.2	X				*tert*-butyl methyl ether
123	0.1	X				Dissolve in 0.5 ml of solvent mixture (tBME and Ethyl Acetate 8:2)
129	0.1	X				sample is dissolved in t-butyl methyl ether and ethyl acetate (80+20)
132	0.1	X				0.5 mL *tert*. Butylmethylether/Ethylacetat (8 + 2)
150	0.1	X				
156	1	X				Sample solved in TBME/EtAc. Internal standard added.
159A	0.1	X			X	
159B	0.1			X		
168	0.1	X				100 mg of sample is dissolved in tetrahydrofuran (1mL) containing internal standard
177	0.1	X				The sample is dissolved in 0,5 ml tert. Butylmethylether and 400 ng internal standard (d5-3-MCPD) is added.
180	0.25	X				dissolve in 5 mL tert.- Butyl methyl ether
186	0.1	X	X		X	salt: (NH4)2SO4
189	0.1	X		X		To the sample (100 mg) we add: 0.5 mL solvent mix A (8 mL t-BME + 2 mL ethyl acetate) _ 50 µL IS solution (20 µg/mL) _ sodium methoxide for transesterification _ 3 mL hexane and 3 mL solvent mix B (1 mL acetic acid in 30 mL NaCl solution) _ PBA solution (derivatization in the aqueous phase, discarding the organic phase) _ and 3 mL hexane for extracting of 3-MCPD derivative .
195	0.1	X				
198	1	X				olive oil: 1 mL of a solution made with 1 g of oil sample in 50 mL of t-butylmethylether / ethylacetate 8/2. 0.2 µg of d5-3-MCPD was added as internal standard. Palm oil: 1 mL of a solution made with 1 g of oil sample in 100 mL of t-butmethylether / ethylacetate 8/2. 0.2 µg of d5-3-MCPD was added as internal standard.
201	0.1		X			
204	0,1	X		X		
210	0.1			X		
213	0.1 - 0.3	X				

Table 5.2: continued

216	0.1	X				0.1 g fat dissolve in 0.5 mL of solvent mixture (tBME/EtAc, 8:2) and add of 0.2 mL of internal standard solution (3MCPD-d5, c= 10ug/mL in EtAc)
219	0.5	X				0.5 g of sample dissolve in 10 ml tetrahydrofurane
222	0.1	X				Isolation of fat with TBME at room temperature, dissolve 0.1 g of fat in 1.0 ml TBME/Ethylacetate
225	0.1					The sample is solved in 0.5 mL t-BME and 20µL Internal Standard (3-MCPD-d5) are added.
228	0.1					The sample is solved in 0.5 mL tert-butylmethylether and 20µL internal standard (3-MCPD-d5) are added.
231	0.1	X				dissolve in MtBE
234	0.1	X				Dilution in MTBE
237	0.1			X		DGF C-III 18 (09), Option B

Table 5.3: Hydrolysis of esters – method details

Lab Code	Hydrolysis, cleavage of esters			
	alkaline transesterification with sodium methoxide	acidic transesterification with sulphuric acid	other	details:
120	X			
123	X			Addition 1 ml of Sodium Methylate, c=0.5 mol/l in Methanol.Add 3 ml hexane and 3 ml solvent mixture (acetic acid and NaCL-solution). Remove the organic phase, add 3 ml hexane and remove the organic phase.
129	X			sodium methylate, 0.5 mol/l
132			X	methanolic Natrium methylate NaOCH3 0,5 mol/l
150		X		
156			X	For transesterification methanolic sodium methylate is used. Extraction with acetic acid, Na2SO4-solution and hexane. Only aqueous phase is used for derivatization.
159A	X			0.2 ml (0.5 mol/l sodium methoxide in methanol); Incubation: 9-10 min room temperature constant shaking; 0.6 ml stop reagent: (10 g (NH4)2SO4 in 25 ml water + 25% H2SO4 (50+3v/v)); 20s vortex; defattening n-hexane; extraction ethylacetate
159B	X			1 ml (0.5 mol/l sodium methoxide in methanol); Incubation: 9 min room temperature constant shaking; 3 ml n-hexane + 3 ml stop reagent: (30 ml Sodium chloride in water (200 g/l)+ 1 ml acetic acid); 5s vortex; Defattening with n-hexane, SPE
168		X		Chemicals: methanol (LiChrosolv), sulphuric acid (purity>95%). Procedure: 1,8mL of hydrolysing reagent (1,8% (v/v) sulphuric acid in methanol) is added to the sample. Conditions for the hydrolysis: 16hrs at 40C.
177	X			The sample solution is transesterified at room temperature for 9-10 minutes by adding 0,2 ml Sodiummethylate-solution (c=0,5 mol/l) in methanole. The reaction is stopped by adding 0,6 ml of a solution of ammoniumsulfate and sulfuric acid (10 g (NH4)2SO4 i
180	X			Add 0,2 ml NaOCH3 20% and allow to react for 5 min. Stop reaction by adding 0,2 ml glacial acetic acid and extract analytes with 5ml sodium chloride solution 20%
186		X		H2SO4+CH3OH 16h, 40°C
189	X			As it is described above in the "Details", after the addition of Internal Standard, we add 1ml sodium methoxide solution, allowing the mixture to stand for 10 minutes at RT.
195	X			Natriummethylatlösung, c=0,5 mol/L: 0,27 g NaOCH3 dissolved in 10 ml MeOH

Table 5.3: continued

198	X			One ml of sodium methoxide 0,5M in methanol was added. The mixture was left for ten minutes. Then we added 0,5ml of AcOH/MeOH 1/4. Shaken. Add 3ml of NaHCO3 saturated solution and 0,2g NaCl. Extract with heptane (twice 4ml), Discard heptane. Concentrate to remove the methanol. Extract 3-MCPD with ethylacetate,Dry the ethylacetate solution with Na2SO4 before silicagel SPME purification (elution of 3-MCPD with ethanol/ethylacetate 6/100), Concentrate up to about 0,1mL.
201	X			
204	X			Hydrolysis with 1 ml 0,5 mol sodium methoxide, clean-up with 2x3 ml n-hexane, add. acetic acid/NaCl solution
210	X			
213	X			DGF Standard Method Section C fats C-III 18 (09)
216	X			After addition of 1 mL sodium methoxide (0.5 mol/L in methanol) stand for 10 min at room temperature. After this, add in 3 mL n-hexane and 3 mL 3.3% acetic acid in 20% NaCl, extract, remove upper organic phase, add further 3 mL n-hexane, discard upper phase.
219		X		To 1 ml oil solution in THF add 2 ml solution sulfuric acid in methanol (18 ml 96% sulfuric acid in 982 ml of methanol). Mix and place in termoblock for 15 hours at 45oC. After heating, cool to room temperature and neutralised with 800 ul of saturated sol
222	X			Add 1.0 ml of 0.5 m Na-OCH3-solution in Methanol and 0.10 ml ISTD-Solution (MCPD-D3, 25 µg/ml), allow to stand for 10 min, add 0.10 ml Acetacid, 3.0 ml NaCl-solution (200g/L) and 3.0 ml iso-Hexan, shake for 1 min, discharge organic layer, repeat extraktion one time
225	X			The reagent for the hydrolysis consists of 2.7 g sodium methoxide in 100 mL methanol. 0.2 mL solution are added to the samples. The samples are mixed for 10 seconds on a vortex mixer. Afterwards the samples are incubated for 10 minutes. After this time the reaction is stopped by adding 0.6 mL solution of ammonium sulfate in aqueous sulphuric acid (10 g ammoniumsulfate in 25 mL deionised water and 1,5 mL 25% sulphuric acid) and by mixing for 20 seconds on a vortex mixer. With the addition of 1 mL isohexane and shaking on a vortex mixer for 10 seconds the samples are degreased. To improve the phase separation the samples are centrifugated for 2 minutes at 207xg at room-temperature. The upper phase is rejected. The degreasing is repeat for one time. Afterwards the 3-MCPD is extracted for two times with 0.6 mL acetic ether.

Table 5.3: continued

228		X		The reagent for the hydrolysis consists of 1.8 mL sulphuric acid in 100 mL methanol. 1.8 mL solution are added to the samples. The samples are mixed for 10 seconds on a vortex mixer. Afterwards the samples are shaked with an overhead-mixer for two hours. Then the samples are heated on 40°C in a drying cabinet for at least 16 hours (max 20 hours). After this time the reaction is stopped by adding 0.5 mL solution of saturated monosodium carbonate in deionised water and by mixing for 20 seconds on a vortex mixer. With the addition of 1 mL isohexane and shaking on a vortex mixer for 10 seconds the samples are degreased. To improve the phase separation the samples are centrifuged for 2 minutes at 207xg at room-temperature. The upper phase is rejected. The degreasing is repeat for one time.
231	X			c = 0,5 mol/l sodium methylate in methanol, clean up with ethyl acetate
234			X	Hydrolysis with Sodium methanolate
237	X			DGF C-III 18 (09), Option B

Table 5.4: Derivatisation – method details

Lab Code	Derivatisation							
	PBA	Heptafluorobutyryl imidazol	Acetone	Other	Remarks	reaction temperature [°C]	reaction time [min]	volume [mL]
120				X	Heptafluorobutyric anhydride (HFBA)	70	20	0.05
123	X					80	20	0.250
129	X					80	20	0.25
132	X							
150	X					25	5	0.2
156	X					80	20	0.250
159A	X				PBA in diethylether (saturated), evaporated to dryness	ambient	2	0.1
159B	X				Reaction in ultrasonic bath	ambient	2	0.2
168	X				0,25mL of phenylboronic acid solution (prepared by dissolving 5g PBA in acetone:water 19:1 v/v) is added to the sample	80	20	0.25
177	X				Derivasation takes place in approximately 1,2 ml ethylacetate solution with 100 µl reagent (diethylether saturated with PBA) in an ultrasonic bath. The solution is then evaporated to dryness and dissolved again in 500 µl isooctane (= solution for GC-MS an	20	3	0,1
180	X					90	30	0.2
186	X					85	20	1
189	X				*** 50µl PBA solution (20 µg/ml)	80	20	***
195	X					20	2-3	0.1
198			X		reagent: toluene 4-sulphonic acid 1mg/ml in acetone; After derivatisation the mixture was filtered through a basic aluminium oxide cartridge. The filtrate was injected	40	90	1
201	X					80	20	0.5
204	X					80	20	0.25
210	X					80	20	0.25
213	X							

Table 5.4: continued

216	X		X		Add 0.5 mL PBA (2.5 g PBA in 19 mL acetone and 1 mL water), close tightly and heat at 80°C for 20 min, then cool to room temperature. Extract by shaking it with 3 mL isooctane, dry with sodium sulphate and transfer to GC vial.	80	20	0.5
219	X				PBA: 1,5 PBA dissolve in 6 ml of acetone/water (19+1 v/v)	90	20	0.4
222	X					90	20	0.25
225	X				ca. 0.4 g phenylboronic acid saturated in diethylether. The derivatisation occurs with an ultrasonic treatment.	room temperature	3	0.1
228	X				5g PBA are solved in 19 mL acetone and 1 mL deionised water. The derivatisation occurs with an ultrasonic treatment.	room-temperature	3	0.25
231	X			X	reaction at room temperature, using of ultra sonic, clean up with heptane	room	3	1
234	X					21	3	0.1
237	X				DGF C-III 18 (09), Option B	80	20	0.5

Table 5.5: Extraction of derivatised 3-MCPD and sample pre-concentration

Lab Code	extraction and pre-concentration			
	Final solvent	Sample pre-concentration YES	Sample pre-concentration NO	Final volume of sample [mL]
120	*iso*octane		X	0.250
123	hexane		X	3
129	hexane		X	3
132	hexane		X	3
150	hexane		X	1
156	*n*-hexane	X		0.25
159A	acetone		X	0.2
159B	*n*-hexane		X	0.6
168	hexane	X		0.4
177	isooctane	X		0.5
180	hexane		X	2
186	*n* -hexane		X	2
189	hexane		X	3
195		X		ca .0.5
198	acetone		X	about 1
201	hexane		X	3
204	*n*-hexane		X	3
210	*n*-hexane		X	3
213			X	
216	isooctane		X	3
219	hexane		X	2
222	*i*-octane		X	1.0
225	isooctane	X		0.25
228	isooctane	X		0.25
231	heptane	X		0.5
234	acetone	X		0.5
237	hexane		X	3

Table 5.6: Method of the final determination

Lab Code	Method of determination				
	GC-MS	GC-MS/MS	Other	Details	Instrument manufacturer
120	X				Varian
123	X				HP GC-6890 MS-5973
129	X				Hewlett Packard
132	X				Thermo Quest Trace MS
150	X				Agilent
156		X			Varian
159A	X				Agilent
159B	X				Agilent
168	X				Agilent Technologies, Palo Alto, CA, USA
177	X				Shimadzu QP2010 Plus
180	X				Agilent
186	X				Agilent
189	X				SCHIMADZU GCMS-QP 2010
195	X				Agilent
198	X				HP
201	X				Agilent Technologies
204	X				Agilent Technologies
210	X				Agilent
213	X				Agilent GC-7890A MS-5975C
216	X				Agilent Technologies 5975C
219	X				Varian
222	X				Agilent Technologies
225	X				Perkin Elmer
228	X				Perkin Elmer
231			X	GC-HRMS	Finnigan
234	X				Agilent
237	X				Thermo Scientific

Table 5.7: Injection technique - details

Lab Code	Injection technique					
	PTV	splitless	split	on-column	Remark	Injection volume [µL]
120		X				1
123		X				2
129		X				2
132	X	X				2
150		X				1
156	X					1
159A		X				2
159B		X				2
168		X			Injection mode: pulsed splitless	1
177		X			Liner-Temperature 180 °C	1
180		X				2
186		X				0.2
189		X				1.5
195	X					2
198		X				2
201	X				PTV injector operated in pulsed splitless mode	2
204	X	X				2
210	X	X				2
213		X				2
216		X			inlet temperature 250°C, pressure 252.76 kPa	1
219		X				1
222		X			250 °C temperature	1
225		X				2
228		X				2
231		X				0.5
234		X				2
237	X				Back flush technique	2

Table 5.8: GC conditions

Lab Code	GC column					Carrier gas			
	Stationary phase	Supplier	Length [m]	ID [mm]	Film thickness [µm]	Carrier gas type	flow rate [mL/min]	constant flow	constant pressure
120	DB-XLB	J&W	60	0.25	0.25	Helium	1	X	
123	(5%-phenyl)-methylpolysiloxane	Agilent	30	0.250	0.25	Helio	1.2	X	
129	DB-5MS	Agilent Technologies	30	0.25	0.25	helium	2	X	
132	DB-5-MS		30	0.25	0.25	He	1.2	X	
150	Equity 1	Supelco	30	0.25	1	He	0.8	X	
156	HP 5MS (5%-Phenyl)-methylpolysiloxane	Agilent Technologies	30	0.25	0.25	Helium	1.2	X	
159A	DB-5MS	J&W (Agilent)	30	0.25	0.25	He	1.2	X	
159B	DB-5MS	J&W (Agilent)	30	0.25	0.25	He	1.2	X	
168	bonded, poly(dimethylsiloxane)	Sigma-Aldrich Supelco, Bellefonte, PA, USA	30	0.25	1	Helium	0.8	X	
177	Rxi-5ms	Restek	30	0,25	0,25	helium	0,8		X
180	HP-5MS (5 % phenyl 95 % dimethylpolysiloxane)	Agilent	30	0.25	0.25	helium	0.7	X	
186	HP-5MS	Agilent	30	0.25	0.25	helium	1.0	X	
189	DB-5 ms	SUPELCO	30	0.25	0.25	Helium	0.9	X	
195	5%diphenyl/95%dimethylpolysiloxane		30	0.25	0.25	He	1.2	X	
198	HP innowax	Agilent	60	0.25	0.25	He	1	X	
201	DB-17MS	J & W Scientific	30	0.25	0.25	He	1.2	X	
204	Multiresidue 1 (MR-1)	Phenomenex	30	0.25	0.25	helium	1	X	
210	DB5-MS	agilent	30	0.25	0.25	He	2.8	X	
213	SPB5		30	0.25	0.25	H2	0.8	X	
216	(5%-Phenyl)-methylpolysiloxane	J&W Scientific	25	0.25	0.25	Helium	4		X

Table 5.8: continued

219	polydimethylsiloxane	Varian	30	0,25	0,2	Helium	1	X	
222	DB-5 MS	J&W	30	0.25	0.25	He	1		X
225	Crossbond 5% diphenyl / 95% dimethyl polysiloxane	Restek	30	0.25	0.25	Helium	1.2	X	
228	Crossbond 5% diphenyl / 95% dimethyl polysiloxane	Restek	30	0.25	0.25	Helium	1.2	X	
231	DB5-MS	Agilent	60	0.250	0.25	He	0.5	X	
234	ID-HT5	SGE	25	0.22	0.1	He	1.2		X
237	Rtx-5MS	Restek	30	0.25	0.25	He	1.2	X	

Table 5.9: GC oven conditions and retention time

Lab Code	GC oven temperature programme	Retention time [min]	Remark
120	50 °C hold 1 min -> 2 °C/min 90 °C -> 20 °C/min 270 °C hold 10 min	22.5	
123	60ºC 1 min; 6ºC/min to 190ºC; 20ºC/min to 300ºC	16.7	
129	80 °C x 1 min x 20 °C/minx190 °C x 0 x 15 °C/min x 280 °C x 7 min	7.6	
132	60°C (1 min), 6°C/min, 190 °C, 20 °C/min, 280°C (15 min)	17.44	
150	80 (1 min), 80-300 (10°C/min), 300 (5 min)	12	
156	60°C/1min; 25°C/min; 190°C/0min; 35°C/min; 300°C/5min	5.6	
159A	start 60°C (1min); 6°C/min 190°C; 30°C/min 280°C (10min)	17.2	
159B	start 60°C (1min); 6°C/min 190°C; 30°C/min 280°C (10min)	17.2	
168	80°C (1min), 80°C- 170°C (0min) at 10°C/min, 170°C- 200°C (0min) at 3°C/min, 200°C- 280°C (15min) at 15°C/min	16	
177	initial temp.: 100 °C, 1 min; ramp 1: 10 °C/min to 180 °C; ramp 2: 20 °C/min to 300 °C; 300 °C for 5 min	9.0	
180	50°C (hold time 1min) to 210 (10°C/min) to 300°C (hold time 5min) (30°C/min)	14	
186	60°C(6°C/min)-190°C(5°C/min)-280°C(10°C/min, hold on 10min)	15.142	3-MCPD-d5-15.072min,3-MCPD-15.142min
189	80 °C (1min) _ to 300 °C (by rate 10 °C/min) _ 300 °C (27min)	10.95	
195	60°C for 1min then 6°C/min to 190°C for 0 min then 30°C/min to 280°C for 0 min	16.3	
198	50 to 150°C at 7°C/min; then 20°C /min up to 240°C	13	
201	60 °C for 1.2min., 6 °C/min.to 175 °C, 60 °C/min.to 280 °C, hold 7.88min.	19.16	
204	60 °C, 0,5 min.; 60 to 160 °C (5 °C/min); 160 to 320 °C (40 °C/min)	20.3	
210	75°C 1min, 10°C/min to 174°C, 100°C/min to 320°C for 10min	10	
213	60°C 2 min stop 50 °C/min to 100°C 2 min stop 7°C/min to 290°C 10 min stop	13-14	
216	60°C, 1 min, 6°C/min to 162°C, 30°C/min to 282°C, 10 min. Total runtime 30 min.	15.9	constant pressure 252.76 kPa for 18 min, then backflush
219	80°C (hold 1 min) -> 200°C (rate 10°C/min) -> 270°C (rate 20°C/min, hold 13.5 min)	11.5	

Table 5.9: continued

222	60/2-5-150/1-25-300/15	20	
225	60°C (1 min), 6 °C/min till 190°C, 30°C/min till 280°C (10 min)	16.6	
228	60°C (1 min), 6°C/min till 190°C, 30°C/min till 280°C (10 min)	16.6	
231	80 degree C to 320 degree C	12	
234	60°C 1min; 6°C/min 190°C; 20°C/min 320°C 11,33min	13.8	
237	60°C (1 min) – 6°C/min to 190°C – 20°C/min to 280°C	16.5	

Table 5.10: MS conditions

Lab Code	MS settings		
	ionisation method	mass to charge ratios recorded	Remark
120	EI	40-470	data calculation over 275+289+453 (3-MCPD) and 257 (d5-3-MCPD)
123	EI+	201, 147, 196 and 91	
129	EI	147 (for quantification), 91, 196, 150 (int.st), 201 (int.st)	
132	EI, SIM	196, 147, 201	
150	EI	147; 150; 196; 201	
156	electron impact ionisation (EI)	m/z 201>150; 196>147; 198>147.6	
159A	EI	196 and 147 (3-MCPD), 201 (d5-3-MCPD)	Quantifier: 196
159B	EI	196 and 147 (3-MCPD), 201 (d5-3-MCPD)	Quantifier: 196
168	EI	147, 196, 198, 150, 201	147, 150: quantifier ions. 196, 198, 201: qualifier ions
177	EI	3-MCPD: quantifier: 196, qualifier: 198, 147, 146; d5-3-MCPD (Istd.): 201	
180	EI positive	m/z 147 (quantifier) + 196 (qualifier) for 3-MCPD and m/z 150 (quantifier) + 201 (qualifier) for 3-MCPD-d5	
186	SIM	3-MCPD-D5 : m/z = 201 3-MCPD: m/z = 196 (quantifier); 147 (qualifier)	
189	SIM	91, 147, 196 (3-mcpd ester derivative) and 93, 150, 201 (d5-3-mcpd ester derivative)	
195	EI	147,196(3-MCPD) 150,201(3-MCPD-d5)	
198	positive electron impact; SIM	135; 137; 140; 142	
201	EI+	147 (91, 196), 150 (93, 201)	
204	EI	3-MCPD (147, 196), d3-3-MCPD (150, 201)	
210	EI	196	
213	EI – SIM Mode	3mcpd m/z 196	
216	electron impact	3MCPD 196, 147, 91; 3MCPD-d5 201, 150, 93	
219	EI	70-210	
222	EI	198 – 196 – 145 – 146 (3-MCPD), 201 – 150 (ISTD)	

Table 5.10: continued

225	EI	196 m/z 3-MCPD, 201 m/z d5-3-MCPD for quantification; 147 m/z 3-MCPD, 201 m/z d5-3-MCPD for qualification	
228	EI	196 m/z 3-MCPD, 201 m/z d5-3-MCPD for quantification; 147 m/z 3-MCPD, 201 m/z d5-3-MCPD for qualification	
231	electron ionisation	196.0464; 198.0437; 201.0778; 203.0751	quantification and ratio masses for internal isotope labelled standard and native components
234	EI	196, 201, 147, 150	
237	EI	196, 147, 201 (ISTD)	

Table 5.11: Details on calibration

Lab Code	Calibration							Working range	
	External calibration	Details on external calibration	Internal standardisation	Details on IS	Amount of IS [µg]	IS added after weighting	IS added after sample prep	lower limit [mg/kg]	upper limit [mg/kg]
120			X	d5-3-MCPD	0.4	X			
123			X	d5-3MCPD	0.5	X		0.7	20
129			X	3-MCPD-d5, C/D/N Isotopes, Canada	0.5	X		1	10
132			X	3-MCPD-D5	0.525	X		0.5	20
150			X	3-MCPD-d5 dipalmitate	1	X		0.1	24
156			X	d5-MCPD-Ester	1	X		<0.15	5
159A			X	d5-3-MCPD	0.4	X		0.25	6.0
159B			X	d5-3-MCPD	0.4	X		0.25	6.0
168			X	3-MCPD-d5 dipalmitate	2	X		0.2	20
177			X	d5-3-MCPD, Calibration standards are prepared without transesterification and without matrix.	0.4	X		0.08	6
180	X	3-chloropropane-1,2-diol and 1,2-Dipalmitoyl -3-chlorpropane-d5 in *tert*-Butyl methyl ether			1	X		0.6	12.4
186			X	3-MCPD-D5	1		X	5	0.04
189			X	3-Chloro-1,2-propandiol-dipalmitate-d5	1 µg	X		1.2	31.3
195			X	3-MCPD-d5	0.4	X		0.25	6
198			X		see above	X			
201			X	3-MCPD, 3-MCPD-D5	1	X		0.25	30
204	X	range: 10ng/ml – 1000ng/ml	X	range: 10ng/ml - 1000ng/ml	2.5	X		0.3	25

Table 5.11: continued

210			X	3-MCPD d5	1	X		0.1	10
213			X	3-mcpd deuterated	1-2	X		0.5	20
216			X	3-Chloro-1,2-propanediol (propane-d5, 98%), Cambridge Isotope Laboratories, Inc.; 3-chloro-1,2-propanediol (98%), Fluka.	2	X			
219		method of standard (3-MCPD) addition	X	d5-3-MCPD	1.25		X	1	50
222			X	3-MCPD-d3	2.5	X		0.3	3
225	X	stock solution 3-MCPD : 100 mg 3-MCPD solved in 100 mL ethanol; this solution has to be diluted with tert-butylmethylether so that the solutions of calibration contains 0,025 µg till 0,6 µg 3-MCPD. This solutions are added to 0.4 µg internal standard, 1.2 mL acetic ether and 100 µl derivatisation reagent and they are mixed with a vortex mixer for 10 seconds.	X	fivefold deuterated 3-MCPD solved in tert-Butylmethylether (20µg/mL)	0.4	X		0.2	6.0
228	X	stock solution 3-MCPD : 100 mg 3-MCPD solved in 100 mL ethanol; this solution has to be diluted with tert-butylmethylether so that the solutions of calibration contains 0,025 µg till 0,6 µg 3-MCPD. These solutions are added to 0.4 µg internal standard, 1.8 mL ammonium sulfate solution (20g ammonium sulfate in 50 mL deionised water) and 250 µl derivatisation reagent and they are mixed with a vortex mixer for 10 seconds.	X	fivefold deuterated 3-MCPD solved in tert-Butylmethylether (20µg/mL)	0.4	X		0.2	6.0
231			X	3-MCPD-D5, deuterated standard solution	0,5	X		0.15	10
234	X				0.5	X		0.18	10.6
237			X	D5-3-MCPD (Promochem)	2	X		0.3	15

Table 5.12: Details on method quality control

Lab Code	Quality control		
	QC materials yes	QC materials no	Internal QC samples, spiking samples - details
120	X		Fat material obtained from the Bundesinstitut für Risikobewertung, Germany
123			
129	X		We use spiking with 3-MPCD.
132	X		vegetable oil spiked with 3-MCPD
150			
156	X		Sample with known concentration.
159A	X		Internal quality control materials: solid fat analysed continuously over one year in our lab
159B	X		Internal quality control materials: solid fat analysed continuously over one year in our lab
168	X		Control sample 1: Sample spiked with 3-MCPD dipalmitate, Control sample 2: Naturally contaminated sample
177	X		reference sample of BfR round robin test and spiking of one sample with 1,2 Bis-palmitol-3-chloropropanediol
180		X	
186		X	
189			**see Additional remarks, below.
195	X		
198		X	The method for 3-MCPD esters determination is still being studied, Not yet validated,
201	X		Blank extra virgin olive oil used for spiking of 3-MCPD
204		X	
210	X		internal reference material, olive oil (amount appr. 4.5 mg/kg fat)
213		X	
216		X	
219		X	
222	X		Spiked blank Sample
225	X		We used quality control material from BfR (federal institute for risk assessment) with a 3-MCPD-concentration of 3.0 mg/kg fat, that results from their proficiency test in 2009.
228	X		We used quality control material from BfR (federal institute for risk assessment in Germany) with a 3-MCPD-concentration of 3.0 mg/kg fat , that results from their proficiency test in 2009.
231	X		use of isotope labelled standards; spiking materials during the daily sample preparation
234	X		independent 3-MCPD spike-solution
237	X		Refined olive oil

Table 5.13: Method performance characteristics

Lab Code	Method performance					
	RSD_r [%]	Recovery [%]	Recovery correction Yes	Recovery correction No	LOD [mg/kg]	LOQ [mg/kg]
120				X		
123	8	0				0.7
129	0	90		X	0.5	1
132	9.2	98		X	0.1	0.5
150	5	97		X	0.1	0.3
156	13	107		X	0.05	0.15
159A	3-7			X	0.1	0.2
159B	5-10			X	0.2	0.3
168	2.3	94-99		X	0.05	0.2
177	2.5	85-115		X	0.08	0.21
180	5	101	X		0.1	0.4
186	7	93	X		0.05	0.15
189	0.85 - 1.69	**		X	0.4	1.2
195	3.16	ca. 102	X		0.08	0.21
198						
201	8	98		X	0.1	0.25
204	5	45	X		0.025	0.1
210	3.7	98		X	0.03	0.1
213	0	0			0	0
216	0	72	X		0	0.5
219	7.3			X	0.5	1
222	4	91		X	0.3	
225	12	116		X	0.08	0.21
228	12	92		X	0.09	0.23
231	15	80-120	X		0.05	0.15
234	15			X	0.09	0.18
237	not determined	45-65	X		0.1	0.3

Table 5.14: Additional remarks

Lab Code	Additional remarks to the PT
120	We could not fill out all requested data because our validation is still in process.
123	Method validation process. We haven't standard of 3-MCPD ester.
129	
132	
150	
156	
159A	This method was developed by BfR and was validated in a method validation study (the results will be published)
159B	This method is leaned on a method developed by R. Weißhaar (European Journal of Lipid Science and Technology 110, 183-186, 2008)
168	After the ester hydrolysis, the reaction is stopped with 0.5mL of NaHCO3 saturated solution, the most volatile solvents evaporated under nitrogen stream and 2mL of sodium sulphate solution (20%) is added for salting out.
177	We worked according to a given method of German BfR. This method was validated successfully by round-robin test in 2009. Statistical data is partly copied from BfR, because LOD and LOQ was not determined in the lab until now. The other data was established in our lab.
180	
186	
189	** The validation of this method has not yet been completed. In this comparison study, for the purposes of internal quality control we applied only standard solutions, blank matrix and blank reagent. We have estimated the repeatability at three concentration levels. We have not estimated the method recovery, due to the fact that 3-mcpd is produced by more than one kind of ester in a real sample. So we think that the results of this interlaboratory test will provide an estimation of our method recovery.
195	
198	
201	
204	
210	
213	
216	The results were calculated as sum of 3-MCPD esters and glycidyl esters. We analyse only 3-MCPD in soya sauce in our laboratory. Determination of 3-MCPD esters and glycidyl esters in fats and oils is new method for us and it has not been validated yet.
219	
222	
225	
228	
231	
234	
237	

European Commission

EUR 24356 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Proficiency test on the determination of 3-MCPD esters in edible oils
Author(s): Karasek L., Wenzl T., Ulberth F.
Luxembourg: Publications Office of the European Union
2010 – 62 pp. – 21 x 29.7 cm
EUR – Scientific and Technical Research series – ISSN 1018-5593
ISBN 978-92-79-15710-3
DOI 10.2787/2587

Abstract
The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) was requested by the Directorate General Health and Consumers (DG SANCO) to organise a proficiency test on the determination of 3-MCPD esters in edible oils. The aim of this test was to evaluate the comparability of analysis results gained by European laboratories.
The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" and ISO standard 43.
Altogether 41 laboratories from 11 EU Member States, Switzerland and Macedonia subscribed for participation in the study. The participants were asked to determine the 3-MCPD esters content of the test samples by application of their in-house analysis methods. In total, 34 sets of results were reported to the organisers of the study.
The performance of laboratories for the oil samples was expressed by z-scores and by relative bias for the 3-MCPD standard solution in sodium chloride.
The percentage of successful laboratories in the determination of the 3-MCPD esters in contaminated palm oil sample was 56 % and in spiked sample of extra virgin oil 85 %. The study revealed that the application of a particular analysis procedure might lead to strong positive bias.

How to obtain EU publications

Our priced publications are available from EU Bookshop (http://bookshop.europa.eu), where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents. You can obtain their contact details by sending a fax to (352) 29 29-42758.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

JRC
EUROPEAN COMMISSION

LA-NA-24356-EN-C

ISBN 978-92-79-15710-3

Publications Office