BS ISO 18363-1:2015

BSI Standards Publication

Animal and vegetable fats and oils — Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS

Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol

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National foreword

This British Standard is the UK implementation of ISO 18363-1:2015.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oilseeds, animal and vegetable fats and oils and their by-products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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INTERNATIONAL STANDARD

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Animal and vegetable fats and oils — Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS —

Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol

Corps gras d'origines animale et végétale — Détermination des esters de chloropropanediols (MCPD) et d'acides gras et des esters de glycidol et d'acides gras —

Partie 1: Méthode par transestérification alcaline rapide et mesure pour le chloro-3 propane-1,2-diol (3-MCPD) et par mesure différentielle pour le glycidol

Reference number
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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

ISO 18363 consists of the following parts, under the general title *Animal and vegetable fats and oils — Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS*

— *Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol*

The following parts are under preparation:

— *Part 2: Method using alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol*

— *Part 3: Method using acid transesterification and measurement for 2-MCPD, 3-MCPD and glycidol*

Introduction

ISO 18363 is a set of International Standards which can be used for the determination of ester-bound MCPD and glycidol. There are currently three International Standards which have been proposed and this introduction is a description of these methods, which can be used by the analyst to decide which methods are suitable for their application. The detailed application of each method is contained within the scope of the individual method.

This part of ISO 18363 is a differential method equivalent to the DGF standard C-VI 18 (10) and identical to AOCS Official Method Cd 29c-13. Briefly, it is based on a fast alkaline catalysed release of 3-MCPD and glycidol from the ester derivatives. Glycidol is subsequently converted into induced 3-MCPD. It consists of two parts. The first part (A) allows the determination of the sum of ester bound 3-MCPD and ester bound glycidol, whereas the second part (B) determines ester-bound 3-MCPD only. Both assays are based on the release of the target analytes 3-MCPD and glycidol from the ester bound form by an alkaline catalysed alcoholysis carried out at room temperature. In part A, an acidified sodium chloride solution is used to stop the reaction and subsequently convert the glycidol into induced 3-MCPD. Thus, 3-MCPD and glycidol become indistinguishable in part A. In part B, the reaction stop is achieved by the addition of an acidified chloride-free salt solution which also prevents the conversion of glycidol into induced MCPD. Thereby, part B allows the determination of the genuine 3-MCPD content. Finally, the glycidol content of the sample is proportional to the difference of both assays (A – B) and can be calculated when the transformation ratio from glycidol to 3-MCPD has been determined. This part of ISO 18363 is applicable for the fast determination of ester bound 3-MCPD and glycidol in refined and non-refined vegetable oils and fats. This part of ISO 18363 can also apply to animal fats and used frying oils and fats, but a validation study has to be undertaken before the analysis of these matrices. Any free analytes within the sample would be included in the results, but the standard does not allow the distinction between free and bound analytes. However, as of publication, research has not shown any evidence of a free analyte content as high as the esterified analyte content in refined vegetable oils and fats. In principle, this part of ISO 18363 can also be modified in such a way that the determination of 2-MCPD is feasible, but again, a validation study has to be undertaken before the analysis of this analyte.

The second part of the proposed International Standards for the determination of ester-bound MCPD and glycidol represents the AOCS Official Method Cd 29b-13. Briefly, it is based on a slow alkaline release of MCPD and glycidol from the ester derivatives. Glycidol is subsequently converted into 3-MBPD. The second part of the proposed International Standards consists of two sample preparations that differ in the use of internal standards. Both parts can be used for the determination of ester bound 2-MCPD and 3-MCPD. In part A, a preliminary result for ester bound glycidol is determined. Because the 3-MCPD present in the sample will be converted to some minor extent into induced glycidol by the sample preparation, part B serves to quantify this amount of induced glycidol that is subsequently subtracted from the preliminary glycidol result of part A. By the use of isotopically labelled free MCPD isomers in assay A and isotopically labelled ester bound 2-MCPD and 3-MCPD in part B, the efficiency of ester cleavage can be monitored. Both assays A and B are based on the release of the target analytes 2-MCPD, 3-MCPD, and glycidol from the ester bound form by a slow alkaline catalysed alcoholysis in the cold. In both sample preparations, the reaction is stopped by the addition of an acidified concentrated sodium bromide solution so as to convert the unstable and volatile glycidol into 3-MBPD which shows comparable properties to 3-MCPD with regard to its stability and chromatographic performance. Moreover, the major excess of bromide ions prevents the undesired formation of 3-MCPD from glycidol in the case of samples which contain naturally occurring amounts of chloride. The second part of the proposed standards is applicable for the determination of ester bound 3-MCPD, 2-MCPD, and glycidol in refined and unrefined vegetable oils and fats. The second part of the proposed International Standards can also apply to animal fats and used frying oils and fats, but a validation study has to be undertaken before the analysis of these matrices. Any free analytes within the sample would be included in the results, but the standard does not allow the distinction between free and bound analytes. However, as of publication, research has not shown any evidence of a free analyte content as high as the esterified analyte content in vegetable oils and fats.

The third part of the proposed International Standards for the determination of ester-bound MCPD and glycidol represents the AOCS Official Method Cd 29a-13. Briefly, it is based on the conversion of glycidyl esters into 3-MBPD esters and a slow acidic catalysed release of MCPD and MBPD from the

ester derivatives. The third part of the proposed International Standards is based on a single sample preparation in which glycidyl esters are converted into MBPD monoesters, and subsequently, the free analytes 2-MCPD, 3-MCPD, and 3-MBPD are released by a slow acid-catalysed alcoholysis. The 3-MBPD represents the genuine content of bound glycidol. The third part of the proposed International Standards can be applied for the determination of ester bound 2-MCPD, 3-MCPD, and glycidol in refined and non-refined vegetable oils and fats. The third part of the proposed International Standards can also apply to animal fats and used frying oils and fats, but a validation study has to be undertaken before the analysis of these matrices. The method is suited for the analysis of bound (esterified) analytes, but if required, the third part of the proposed International Standards can be also performed without the initial conversion of glycidyl esters. In such a setup, both free and bound 2-MCPD and 3-MCPD forms would be included in the results and the amount of free analytes can be calculated as a difference between two determinations performed in both setups. However, as of publication, research has not shown any evidence of a free analyte content as high as the esterified analyte content in vegetable oils and fats.

INTERNATIONAL STANDARD ISO 18363-1:2015(E)

Animal and vegetable fats and oils — Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS —

Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol

1 Scope

This part of ISO 18363 describes a procedure for the indirect determination of 3-MCPD esters (bound 3-MCPD) and possible free 3-MCPD after alkaline catalysed ester cleavage and derivatization with phenylboronic acid (PBA). Furthermore, this part of ISO 18363 enables the indirect determination of glycidyl esters (bound glycidol) under the assumption that no other substances are present that react at room temperature with inorganic chloride to generate 3-MCPD.

This part of ISO 18363 is applicable to solid and liquid fats and oils. Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this part of ISO 18363.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
bound 3-MCPD
sum of all 3-MCPD derivatives being cleaved by alkaline catalysed alcoholysis (especially fatty acid esters) according to the reference method

Note 1 to entry: The content of 3-MCPD is calculated and reported as a mass fraction, in milligrams per kilogram (mg/kg).

Note 2 to entry: In fats and oils, the amount of free 3-MCPD which can possibly occur is generally negligibly low but has a bearing on the result.

3.2
bound glycidol
sum of all glycidyl derivatives being cleaved by alkaline catalysed alcoholysis (especially fatty acid esters) according to the specified standard

Note 1 to entry: The content of glycidol is calculated and reported as a mass fraction, in milligrams per kilogram (mg/kg).

Note 2 to entry: The presence of free glycidol in fats and oils is unlikely because of the instability of this compound.

4 Principle

For the determination of the sum of bound 3-MCPD and bound glycidol as free 3-MCPD (assay A), an aliquot of the sample is spiked with surrogate standard d_5-3-MCPD-1,2-bis-palmitoyl ester and dissolved in *tertiary*-butyl methyl ether (*t*BME). The addition of a diluted solution of sodium hydroxide or sodium methoxide in methanol will release free 3-MCPD and free glycidol. This reaction is stopped by the addition of an excess amount of sodium chloride in acidic solution. Under acidic conditions, free glycidol reacts with inorganic chloride to additional 3-MCPD and a small amount of 2-MCPD. Undesired non-polar compounds in the sample are removed by double extraction of the aqueous phase with isohexane. The analyte, together with the surrogate standard, is transferred into an organic phase by multiple extraction of the aqueous phase with diethyl ether, ethyl acetate, or a mixture of both solvents. Derivatization takes place in the organic phase by reaction with PBA. In order to remove excess amounts of PBA, concentrate the analytes and transfer them into an inert organic solvent the sample extract is then placed over a small amount of anhydrous sodium sulfate and evaporate until dry under a stream of nitrogen before being finally re-dissolved in isooctane for the measurement by GC-MS.

For the determination of bound 3-MCPD, a second aliquot of the sample (assay B) is spiked with a surrogate standard d_5-3-MCPD-1,2-bis-palmitoyl ester and dissolved in *t*BME. The addition of a diluted solution of sodium hydroxide or sodium methoxide in methanol will release free 3-MCPD and free glycidol. This reaction is stopped by the addition of an excess amount of an acidic chloride-free salt solution (e.g. sodium sulfate, ammonium sulfate, sodium bromide). Under chloride-free acidic conditions, free glycidol reacts to different products depending on the salt used, but does not generate additional 3-MCPD. The additional sample preparation and measurement is performed as described for assay A.

For the calculation of bound glycidol, the difference of both determinations (assay A and B) is multiplied by a non-stoichiometric factor reflecting the transformation of glycidol to 3-MCPD. This procedure is based on the assumption that the difference between the determinations is caused only by the exclusive occurrence of glycidyl esters.

Quantification of the analytes is carried out using d_5-3-MCPD-1,2-bis-palmitoyl ester as surrogate standard. Therefore, no control of the completeness of the ester cleavage is necessary. A matrix calibration over the complete method is carried out periodically and serves for the determination of method linearity, relative recovery and in the case of bound glycidol for the determination of the transformation factor. As long as the method is not changed, this calibration does not necessarily have to be done every working day.

Samples which do contain 3-MCPD only and no glycidol result in lower values (approximately 2 % to 10 %) when using procedure B instead of procedure A. The difference is due to an isotope effect in procedure B. This effect can be determined by dosing a non-contaminated oil sample with equal amounts of 3-MCPD-1,2-bis-dipalmitoylester and d_5-3-MCPD-1,2-bis-palmitoylester before being divided into two aliquots. The aliquots are then analysed three times according to procedure A and procedure B. From the determinations, a correction factor may be determined, which is the ratio of the results from procedure A and B. The isotope effect also appears in the presence of bound glycidol.

As 3-MCPD can occur in certain polymers used for wet strengthening resins and for other purpose, it is recommended that a blank sample be analysed every day in order to control undesired contamination. The blank shall be oil which does not contain any 3-MCPD or glycidol, e.g. a virgin vegetable oil. Contamination might also occur from consumables, e.g. screw lid vials or filter. Baking the glassware at 400 °C to 500 °C can reduce this problem. A better solution is the use of non-contaminated materials.

5 Reagents

5.1 General

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational, and personal safety measures shall be followed.

Unless otherwise stated analytically, pure reagents shall be used; water shall comply with grade 3 of ISO 3696.

5.2 Standard and reference compounds

5.2.1 Working solution surrogate standard: d_5-3-MCPD-1,2-*bis*-palmitoyl ester (e.g. 26,87 µg/mL in toluene; equivalent 5,0 µg/mL free 3-MCPD); other representative d_5-3-MCPD-1,2-*bis*-esters might be used instead as well.

5.2.2 3-MCPD-1,2-*bis*-palmitoyl ester (26,6 µg/mL in toluene; equivalent 5,0 µg/mL free 3-MCPD); other representative 3-MCPD-1,2-bis-esters might be used instead as well.

5.2.3 3-MCPD, $w \geq 99{,}0$ %.

5.2.4 Glycidyl stearate, $w \geq 95{,}0$.

5.3 Solvents

5.3.1 Toluene.

5.3.2 *tertiary*-butyl methyl ether (*t*BME), (2-Methoxy-2-methylpropane).

5.3.3 Methanol.

5.3.4 Isohexane (2-methylpentane).

5.3.5 Ethyl acetate.

5.3.6 Diethyl ether.

5.3.7 Isooctane.

5.4 Other reagents

5.4.1 Sodium hydroxide solution, ρ = 20 g/L in methanol or sodium methoxide solution, ρ = 25 g/L in methanol.

5.4.2 Acidified sodium chloride solution (ρ = 200 g/L). Add 35 mL of sulfuric acid (25 %) to 1 L of aqueous sodium chloride solution (200 g/L). 600 µL of this solution shall neutralize 200 µL of sodium hydroxide or sodium methoxide solution (5.4.1) and adjust the pH-value to the acidic range. The use of other acids (like acetic acid for example) is possible, but their suitability shall be tested by spiking experiments or analysis of ring test material.

5.4.3 Acidified sodium bromide solution, (ρ = 600 g/L). Add 35 mL of sulfuric acid (25 %) to 1 L of aqueous sodium bromide solution (600 g/L). 600 µL of this solution shall neutralize 200 µL of sodium hydroxide or sodium methoxide solution (5.4.1) and adjust the pH-value to the acidic range. The

use of other acids (for example, acetic acid) is possible, but their suitability shall be tested by spiking experiments or analysis of ring test material.

5.4.4 Sodium sulfate anhydrous, grained.

5.4.5 Phenylboronic acid, saturated in diethyl ether with precipitation.

5.4.6 Nitrogen (e.g. quality 5,0, volume fraction *purity* = 99,999 %).

6 Apparatus

6.1 Eppendorf pipettes (e.g. 10 µL to 100 µL, 10 µL to 200 µL, 100 µL, to 1 000 µL).

6.2 Piston stroke and volumetric pipettes, various sizes.

6.3 Volumetric flasks, various sizes.

6.4 Analytical balance, readability 0,000 1 g, weighing precision 0,001 g.

6.5 Screw cap vials (approximately 2 mL in capacity) and screw caps with Polytetrafluoroethylene (PTFE)-coated septa.

6.6 Pasteur pipettes and pipette bulbs.

6.7 Micro inserts (approximately 200 µL in capacity) for screw cap vials (approximately 2 mL in capacity).

6.8 Nitrogen blow-off equipment.

6.9 GC-MS-system with temperature programmable injector.

6.10 Fused-silica-GC-column, stationary phase 50 % diphenyl- 50 % dimethylpolysiloxane, length 30 m, ID 0,25 mm, film thickness 0,25 µm, low bleed for MS purpose, with pre-column.

The precolumn, which periodically should be exchanged, retards non-volatile components and thereby serves to prolong the lifetime of the main column.

7 Sample

7.1 Sampling

Sampling is not part of this method. A recommended sampling method is given in ISO 5555.

7.2 Preparation of the test sample

Liquid samples shall be used without additional treatment. Solid or turbid fats shall be carefully melted at approximately 80 °C in a drying oven or water bath. For high-melting fats, the temperature shall be carefully increased in 10 °C steps until the melting process starts.

Solid samples which contain higher amounts of water, resulting in a phase separation after melting, shall not be melted but used as such or the fat contingent is isolated by liquid/liquid-extraction. The latter procedure is recommended if the samples might contain relevant amounts of chloride as it might be the case, for instance, in some margarine or in butter.

8 Procedure

8.1 Spiking with surrogate standard and homogenization

Weigh, to the nearest 0,5 mg, two 100 mg portions of the sample into two 2 mL screw cap vials. Add to each vial 100 µL of surrogate standard solution (5.2.1) and 100 µL of *t*BME (assays A and B). Shake both assays until the sample is completely dissolved. In the case of high melting sample material, the reaction vessel should be warmed.

8.2 Ester cleavage and glycidol transformation

8.2.1 Add to each assay 200 µL of sodium hydroxide or sodium methoxide solution (5.4.1), seal the vials, and shake for a short time.

8.2.2 The reaction time for ester cleavage is 3,5 min to 5,5 min. Turbid solutions or solutions with precipitates shall occasionally be shaken resulting in a clear solution in most cases. The ester cleavage is stopped by the addition of the following:

— 600 µL of acidified sodium chloride solution (5.4.2) to one vial (assay A);

— 600 µL of acidified chloride free salt solution (5.4.3) to the second vial (assay B).

CAUTION — It is very important to follow the above given reaction time as in alkaline solution and at higher temperature possibly liberated free 3-MCPD and d_5-3-MCPD reacts to glycidol and d_5-glycidol.

8.2.3 Add to both assays 600 µL of isohexane, seal the vials, and shake vigorously. Both vials are allowed to remain at room temperature for approximately 5 min. In this step, in assay A glycidol reacts under acidic catalysis to 3-MCPD and partly to 2-MCPD. The completeness of this reaction depends on the acidity of the reaction solution and so does the ratio of the formed MCPD isomers. If the acidity is not strong enough due to the use of a weak acid or the use of too basic reaction components, the reaction time and temperature shall be increased. Otherwise, the reaction will not be complete and reproducible.

8.3 Matrix clean up

Shake both assays vigorously and then separate and discard the organic phases using Pasteur pipettes. Repeat this step with another 600 µL of isohexane for both assays.

8.4 Derivatization

8.4.1 Extract the aqueous phases (8.3) three times with each 600 µL of a mixture of diethyl ether/ethyl acetate (e.g. 600 µL/mL diethyl ether; 400 µL/mL ethyl acetate) by means of new Pasteur pipettes. The composition and quantity of extraction solvent and the number of extractions can vary. Combine the organic extracts of every assay without the aqueous phases in a new screw cap vial containing a small amount of anhydrous sodium sulfate (5.4.4), add 10 µL to 100 µL of the derivatization reagent (5.4.5). The amount of the derivatization reagent shall be adapted to the capability of the chromatographic system. If the drying agent gets sticky, the solution shall be transferred into a new screw cap vial with another portion of anhydrous sodium sulfate (5.4.4). Both assays shall be processed immediately according to 8.4.2.

8.4.2 To complete the derivatization reaction and to remove excess reagent, evaporate both assays to dryness using a gentle stream of nitrogen. Redissolve soluble fractions subsequently in approximately 500 µL of isooctane. For GC-MS measurement, one part of each redissolved assay is transferred in a 200 µL micro insert.

8.5 Gas chromatography/mass spectrometry references

8.5.1 Injection volume: 1 µL to 2 µL.

8.5.2 Carrier gas: helium 4,6 (purity = 99,996 %) or better, const. flow 1 mL/min to 1,4 mL/min.

8.5.3 PTV temperature programme: e.g. 85 °C, with 300 °C/min to 165 °C, 10 min isothermal, with 300 °C/min to 320 °C, 8 min isothermal.

8.5.4 Injector: e.g. splitless, purge flow 50 mL/min 0,5 min to 1 min, septum purge 3 mL/min.

8.5.5 GC oven temperature programme: the following conditions have been found to be suitable but should be adapted depending on the apparatus used and column: 85 °C, isothermal 0,5 min, with 6 °C/min to 150 °C, with 12 °C/min to180 °C, with 25 °C/min to 280 °C, isothermal 7 min.

8.5.6 Mass spectrometric detector: electron-impact (EI), selected ion monitoring (SIM), detected ion traces, 149/150/201/203 for surrogate standard, 146/147/196/198 for analyte. Generally, the corresponding ion traces 150 and 147 or 201 and 196 are targeted for quantification, whereas the other ion traces serve as qualifiers.

NOTE The use of chromatographic back-flush-technique is not necessary.

8.5.7 For quantification, suitable software with automatic analysis is recommended.

9 Expression of results

9.1 Quantification of the sum of 3-MCPD that has been released from bound 3-MCPD, as well as having been generated from glycidyl esters is carried out by multiplying the ratio of signal areas of the analyte and the isotopic labelled surrogate standard based on corresponding ion traces with the spiking level of the isotopic labelled surrogate standard in assay A, as given by Formula (1):

$$w_{3-\mathrm{MCPD}(\mathrm{A})} = \frac{SF_{\mathrm{A}} \times w_{\mathrm{d}_5-3-\mathrm{MCPD}(\mathrm{A})}}{SF_{i\mathrm{A}}} \quad (1)$$

where

$w_{3\text{-MCPD(A)}}$ is the mass fraction, in mg/kg, of 3-MCPD in assay A;

$w_{\mathrm{d}_5-3-\mathrm{MCPD(A)}}$ is the mass fraction, in mg/kg, of d_5-3-MCPD assay A;

SF_{A} is the area of 3-MCPD in assay A;

$SF_{i\mathrm{A}}$ is the area of d_5-3-MCPD in assay A.

9.2 Quantification of the contingent bound 3-MCPD is carried out by multiplying the ratio of signal areas of the analyte and the isotopic labelled surrogate standard based on corresponding ion traces with the spiking level of the isotopic labelled surrogate standard in assay B, as given by Formula (2):

$$w_{3-\mathrm{MCPD(B)}} = \frac{SF_{\mathrm{B}} \times w_{\mathrm{d_5-3-MCPD(B)}}}{SF_{i\mathrm{B}}} \quad (2)$$

where

$w_{3\text{-MCPD(B)}}$ is the mass fraction, in mg/kg, of 3-MCPD in assay;

$w_{\mathrm{d_5-3-MCPD(B)}}$ is the mass fraction, in mg/kg, of d_5-3-MCPD in assay B;

SF_{B} is the area of 3-MCPD in assay B;

$SF_{i\mathrm{B}}$ is the area of d_5-3-MCPD in assay B.

Report the results to one decimal place.

9.3 The transformation factor that quantifies the 3-MCPD that has been generated from glycidol under the described reaction conditions may be determined by a matrix calibration in the presence of chloride (assay A). Therefore, a non-contaminated oil or fat sample is processed according to Clause 7 (assay A) after spiking with different amounts of glycidol (introduced e.g. as glycidyl stearate) in a way that equidistant concentration levels will result. The reciprocal value of the resulting calibration curve $y = mx + n$ equates to the transformation factor t, as given by Formula (3):

$$t = \frac{1}{m} \quad (3)$$

where

y is the mass fraction of $w_{3\text{-MCPD}}$;

x is the mass fraction of w_{glycidol};

m is the slope of the line;

n is the y-intercept.

9.4 The content of glycidol, w_{glycidol}, is the difference of the result obtained in Formula (1) and Formula (2) multiplied with the transformation factor t [by Formula (3)], gives Formula (4):

$$w_{\mathrm{glycidol}} = t \times \left[w_{3\text{-MCPD(A)}} - w_{3\text{-MCPD(B)}} \right] \quad (4)$$

where

w_{glycidol} is the mass fraction, in mg/kg, of glycidol in the sample.

10 Precision

10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test might not be applicable to concentration ranges and matrices other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the values of *r* given in Table A.1.

10.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will, in not more than 5 % of cases, exceed the values of *R* given in Table A.1.

11 Test report

The test report shall include at least the following information:

a) all information necessary for the complete identification of the sample;

b) sampling method used, if known;

c) test method used, with reference to this part of ISO 18363, i.e. ISO 18363–1. It is mandatory to state whether assay A or B was used. Without this declaration, the method is deemed to be not fulfilled;

d) result(s) obtained;

e) if the repeatability has been checked, the final quoted result obtained;

f) any operating details not specified in this part of ISO 18363, or regarded as optional, together with details of any incidents which might have influenced the test result(s).

IMPORTANT — It is herewith explicitly stated that for the determination of the true amount of 3-MCPD, only method assay B shall be used, whereas method assay A shall be used to determine the combined amount of 3-MCPD and glycidol. The method is not fulfilled if 3-MCPD results are reported without indicating whether they are based on assay A or B.

Annex A
(informative)

Results of an collaborative trial

The precision of the method is the result of an interlaboratory study organized by the Joint Committee of DIN and DGF for the Analysis of fats, oils, fat products, related products and raw materials on an international basis. The study was carried out in 2010 on the following six samples.

Study on the following samples:

— A rapeseed oil extra virgin;

— B maize germ oil, refined;

— C deep-frying fat (hydrogenated fat);

— D walnut oil/extra virgin grape seed oil;

— E soybean oil/sunflower seed oil;

— F sample A with addition of 3 mg/kg free 3-MCPD.

The evaluation of the results was done according to ISO 5725-2. The results for samples A to F are given in Table A.1 and Table A.2.

Table A.1 — Summary of statistical results for assay A

Dimensions in mg/kg

Sum of 3-MCPD and glycidol	**A**	**B**	**C**	**D**	**E**	**F**
Number of participating laboratories	13	13	13	13	13	13
Number of laboratories retained after eliminating outliers	12	12	13	13	13	9
Number of individual test results of all laboratories on each sample	24	24	26	26	26	18
Mean value, m	**0,08**	**1,21**	**5,35**	**10,33**	**1,07**	**2,66**
Repeatability standard deviation, s_r	0,02	0,06	0,23	0,23	0,09	0,10
Repeatability coefficient of variation, $C_{V,r}$ (%)	25,6	4,6	4,4	2,3	8,1	3,9
Repeatability limit, r (2,8 s_r)	**0,06**	**0,15**	**0,66**	**0,65**	**0,24**	**0,29**
Reproducibility standard deviation (s_R)	0,10	0,27	0,97	1,51	0,21	0,45
Reproducibility coefficient of variation, $C_{V,R}$ (%)	131,7	22,6	18,2	14,6	20,0	16,9
Reproducibility limit, R (2,8 s_R)	**0,29**	**0,77**	**2,72**	**4,23**	**0,60**	**1,26**

Table A.2 — Summary of statistical results for assay

Dimensions in mg/kg

3-MCPD	A	B	C	D	E	F
Number of participating laboratories	13	13	13	13	13	13
Number of laboratories retained after eliminating outliers	12	13	13	13	12	12
Number of individual test results of all laboratories on each sample	24	26	26	26	24	24
Mean value, m	**0,09**	**0,46**	**2,31**	**8,12**	**0,62**	**2,37**
Repeatability standard deviation, s_r	0,04	0,07	0,11	0,22	0,04	0,17
Repeatability coefficient of variation, $C_{V,r}$ (%)	44,5	14,7	4,7	2,7	5,7	7,3
Repeatability limit, r **(2,8** s_r**)**	**0,11**	**0,19**	**0,30**	**0,62**	**0,10**	**0,48**
Reproducibility standard deviation, s_R,	0,12	0,17	0,54	1,41	0,18	0,57
Reproducibility coefficient of variation, $C_{V,R}$, (%)	130,9	37,7	23,6	17,4	28,5	24,1
Reproducibility limit, R **(2,8** s_R**)**	**0,34**	**0,48**	**1,52**	**3,95**	**0,50**	**1,60**

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