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

Part 3: Method using acid transesterification and measurement for 2-MCPD, 3-MCPD and 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 par CPG/SM —

Partie 3: Méthode par transestérification acide et mesure pour le 2-MCPD, le 3-MCPD et le glycidol

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Contents

Page

Foreword

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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 voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

A list of all parts in the ISO 18363 series can be found on the ISO website.

Introduction

The ISO 18363 series is a family of International Standards which can be used for the determination of ester-bound MCPD and glycidol. This introduction describes the methods specified in the three documents currently published or proposed so that the analyst can decide which methods are suitable for application. The detailed application of each method is contained within the scope of the individual method.

ISO 18363-1 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. ISO 18363-1 is applicable to the fast determination of ester-bound 3-MCPD and glycidol in refined and non-refined vegetable oils and fats. ISO 18363-1 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 document 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, ISO 18363-1 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.

ISO 18363-2 (proposed) will represent AOCS Official Method Cd 29b-13. Briefly, it will be based on a slow alkaline release of MCPD and glycidol from the ester derivatives. Glycidol is subsequently converted into 3-MBPD. ISO 18363-2 will consist of two sample preparations that differ in the use of internal standards. Both preparations will be used for the determination of ester-bound 2-MCPD and 3-MCPD. In part A, a preliminary result for ester-bound glycidol will be 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 will serve 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 will be able to be monitored. Both assays A and B will be 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 will be 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 will prevent the undesired formation of 3-MCPD from glycidol in the case of samples which contain naturally occurring amounts of chloride. ISO 18363-2 will be applicable to the determination of ester-bound 3-MCPD, 2-MCPD, and glycidol in refined and unrefined vegetable oils and fats. It will also apply to animal fats and used frying oils and fats, but a validation study will have to be undertaken before the analysis of these matrices. Any free analytes within the sample would be included in the results, but the document will not allow the distinction between free and bound analytes. However, as of publication of this document, research has not shown any evidence of a free analyte content as high as the esterified analyte content in vegetable oils and fats.

This document represents AOCS Official Method Cd 29a-13. Briefly, it is based on the conversion of glycidyl esters into 3-MBPD esters and a slow acid catalysed release of MCPD and MBPD from the ester derivatives. This document 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. This document can be applied for the determination of ester-bound 2-MCPD, 3-MCPD, and glycidol in refined and non-refined vegetable oils and fats. It 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, this document 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 of this document, research has not shown any evidence of a free analyte content as high as the esterified analyte content in vegetable oils and fats.

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

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

1 Scope

This document specifies a procedure for the simultaneous determination of 2-MCPD esters (bound 2-MCPD), 3-MCPD esters (bound 3-MCPD) and glycidyl esters (bound glycidol) in a single assay, based on acid catalysed ester cleavage and derivatization of cleaved (free) analytes with phenylboronic acid (PBA) prior to GC/MS analysis.

This document is applicable to solid and liquid fats and oils. For all three analytes the limit of quantification (LOQ) is 0,1 mg/kg and the limit of detection (LOD) is 0,03 mg/kg.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. 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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
bound 2-MCPD
amount of 2-MCPD cleaved from its esterified (bound) forms by acid catalysed transesterification according to the reference method

Note 1 to entry: The content of 2-MCPD is calculated and reported as a mass fraction, in milligrams per kilogram (mg/kg).

3.2
bound 3-MCPD
amount of 3-MCPD cleaved from its esterified (bound) forms by acid catalysed transesterification 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).

3.3
bound glycidol
amount of glycidol cleaved from its esterified (bound) forms by acid catalysed transesterification according to the reference method

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

4 Principle

The oil/fat sample is dissolved in tetrahydrofuran, and the internal standards (pentadeuterated 3-MCPD diester and pentadeuterated glycidyl ester) are added. During the first step of sample preparation, glycidyl esters are converted into 3-MBPD monoesters by the addition of an acidified solution of sodium bromide. Upon completion of the reaction, the organic phase, containing 2- and 3-MCPD esters and 3-MBPD esters, is separated and evaporated to dryness. In the second step the residue is dissolved in tetrahydrofuran and the acid transesterification is initiated by the addition of an acid alcoholic solution. After 16 h incubation at 40 °C, the sample mixture is neutralized and the fatty acid methyl esters generated during the transesterification are removed. Finally, the purified sample [containing cleaved (free) analytes] is derivatized with phenylboronic acid prior to GC/MS analysis.

The quantification of 2- and 3-MCPD esters (expressed as bound 2- and 3-MCPD) is based on the 2-MCPD/3-MCPD-d5 and 3-MCPD/3-MCPD-d5 signal ratio, respectively. The quantification of glycidyl esters (expressed as bound glycidol) is based on the 3-MBPD/3-MBPD-d5 signal ratio.

This method allows the simultaneous quantification of all three analytes in a single assay.

5 Reagents

WARNING — This document requires 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.

5.1 Standard and reference compounds

5.1.1 1,2-Dipalmitoyl-3-chloropropanediol (PP-3-MCPD), purity ≥ 95 % (e.g. from a supplier or synthesized from 3-MCPD and palmitoyl chloride as described by Reference [1]).

NOTE 1,2-Dipalmitoyl-3-chloropropanediol can be substituted by 1,2-dioleyl-3-chloropropanediol or other fatty acid diesters of 3-MCPD with similar chain length (C16-C18 are preferred as they are the most abundant in the majority of oils/fats).

5.1.2 1,3-Dipalmitoyl-2-chloropropanediol (PP-2-MCPD), purity ≥ 95 % (e.g. synthesized from 2-MCPD and palmitoyl chloride as described by Reference [1]).

NOTE In analogy with the recommendations given for PP-3-MCPD, 1,3-dipalmitoyl-2-chloropropanediol can be substituted by other fatty acid diesters of 2-MCPD with similar chain length (C16-C18 are preferred as they are the most abundant in the majority of oils/fats).

5.1.3 Pentadeuterated 1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD-d5), purity ≥ 95 %.

NOTE The same consideration applied to 1,2-dipalmitoyl-3-chloropropanediol is valid also for its pentadeuterated analogue, see the Note in 5.1.1.

5.1.4 Glycidyl palmitate (Gly-P), purity ≥ 98 %.

NOTE Glycidyl palmitate can be substituted by glycidyl oleate or other fatty acid esters of glycidol with similar chain length (C16-C18 are preferred as they are the most abundant in the majority of oils/fats).

5.1.5 Pentadeuterated glycidyl palmitate (Gly-P-d5), purity ≥ 98 %.

NOTE The same consideration applied to glycidyl palmitate is valid also for its pentadeuterated analogue, see the Note in 5.1.4.

5.2 Standard solutions

5.2.1 General

All standard solutions can be prepared with either toluene (5.3.5) or tetrahydrofuran (5.3.1). Toluene is preferred for standard solutions containing glycidyl esters.

5.2.2 Stock solutions (1 mg/ml)

a) Weigh 10 mg of PP-3-MCPD (5.1.1) in a 10 ml volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

b) Weigh 10 mg of PP-2-MCPD (5.1.2) in a 10 ml volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

c) Weigh 10 mg of PP-3-MCPD-d5 (5.1.3) in a 10 ml volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

d) Weigh 10 mg of Gly-P (5.1.4) in a 10 ml volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

e) Weigh 10 mg of Gly-P-d5 (5.1.5) in a 10 ml volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

NOTE Stock solutions are stable for at least three months when stored at −18 °C.

5.2.3 Working solutions

a) Calibration I (PP-3-MCPD, 55 µg/ml). Pipette 550 µl of the stock solution [5.2.2 a)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

b) Calibration II (PP-3-MCPD, 5,5 µg/ml). Pipette 1 ml of the Calibration I solution [5.2.3 a)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

c) Calibration III (PP-2-MCPD, 55 µg/ml). Pipette 550 µl of the stock solution [5.2.2 b)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

d) Calibration IV (PP-2-MCPD, 5,5 µg/ml). Pipette 1 ml of the Calibration III solution [5.2.3 c)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

e) Calibration V (Gly-P, 100 µg/ml). Pipette 1 ml of the stock solution [5.2.2 d)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

f) Calibration VI (Gly-P, 10 µg/ml). Pipette 1 ml of the Calibration V solution [5.2.3 e)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

g) Internal standard I (PP-3-MCPD-d5, 40 µg/ml). Pipette 400 µL of the stock solution [5.2.2 c)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

h) Internal standard II (Gly-P-d5, 50 µg/ml). Pipette 500 µL of the stock solution [5.2.2 e)] into a 10 ml volumetric flask and fill up to the mark with the solvent.

NOTE 1 As an alternative to the preparation of separate standard solutions for each analyte, the three [5.2.3 a), c), and e)] can be combined in one single standard solution at high concentration of all three analytes ("mixed Calibration I"). To prepare the mixed solution, pipette 550 µl of PP-3-MCPD stock solution [5.2.2 a)], 550 µl of PP-2-MCPD stock solution [5.2.2 b)], and 1 ml of Gly-P stock solution [5.2.2 d)] into a 10 ml volumetric flask and fill up to the mark with the solvent. Also, the solutions 5.2.3 b), d), and f) can be combined in one single standard solution at low concentration of all three analytes ("mixed Calibration II"). To prepare the mixed solution, pipette 1 ml of mixed Calibration I into a 10 ml volumetric flask and fill up to the mark with the solvent.

NOTE 2 The internal standard solutions [5.2.3 g and h)] can also be combined in a single solution ("mixed internal standard"). To prepare the mixed solution, pipette 400 µl of PP-3-MCPD-d5 [5.2.2 c)] and 500 µl of Gly-P-d5 [5.2.2 e)] into a 10 ml volumetric flask and fill up to the mark with solvent.

5.3 Other reagents

5.3.1 Tetrahydrofuran, anhydrous.

5.3.2 Methanol, analytical grade.

5.3.3 n-Heptane, analytical grade.

5.3.4 Acetone, analytical grade.

5.3.5 Toluene, analytical grade.

5.3.6 Water, ultra-pure (e.g. obtained by using a purification system).

5.3.7 Sulfuric acid, purity ≥ 95 %.

5.3.8 Sodium hydrogen carbonate, purity ≥ 99 %.

5.3.9 Sodium sulfate, purity ≥ 99 %.

5.3.10 Phenylboronic acid, purity ≥ 97 %.

5.3.11 Sodium bromide, purity ≥ 99,5 %.

5.4 Reagent solutions

5.4.1 Acid aqueous solution of sodium bromide [sodium bromide 3 mg/ml, sulfuric acid 5 % (volume fraction)]. Prepare a concentrated aqueous solution of sodium bromide by dissolving 1 g of sodium bromide (5.3.11) in 10 ml of ultrapure water (5.3.6). Transfer 180 µl of the concentrated solution into an empty conical flask. Add 0.3 ml of sulfuric acid (5.3.7) and 5,5 ml of ultra-pure water (5.3.6). Shake vigorously.

It is advisable to freshly prepare the solution on daily basis.

5.4.2 Sodium hydrogen carbonate solution (0,6 %, mass concentration). Weigh 0,6 g of sodium hydrogen carbonate (5.3.8) in a 100 ml volumetric flask and fill up to the mark with ultra-pure water (5.3.6). Use an ultrasonic bath (6.3) to ensure the complete dissolution of the reagent.

NOTE As an alternative, the solution can be prepared by dilution of the sodium hydrogen carbonate saturated solution (5.4.4).

5.4.3 Sulfuric acid/methanol solution (1,8 %,volume fraction). Pipette 1,8 ml of sulfuric acid (5.3.7) in a 100 ml volumetric flask and fill up to the mark with methanol (5.3.2).

It is advisable to freshly prepare the solution on daily basis.

5.4.4 Sodium hydrogen carbonate solution (saturated). Weigh 9,6 g of sodium hydrogen carbonate (5.3.8) in a 100 ml volumetric flask and fill up to the mark with ultra-pure water (5.3.6). Use an ultrasonic bath (6.3) to ensure the dissolution of the reagent.

NOTE The saturated solution of sodium hydrogen carbonate can be substituted with a solution of the exact concentration (9 %, mass concentration) to improve the robustness of the method.

5.4.5 Sodium sulfate solution (20 %, mass concentration. Weigh 20 g of sodium sulfate (5.3.9) in a 100 ml volumetric flask and fill up to the mark with ultra-pure water (5.3.6). Use an ultrasonic bath (6.3) to ensure the complete dissolution of the reagent.

5.4.6 Phenylboronic acid solution (saturated). Weigh 3 g of phenylboronic acid (5.3.10) and add 12 ml of an acetone (5.3.4)/ultra-pure water (5.3.6) mixture (19/1 volume fraction). Shake vigorously.

NOTE The phenylboronic acid does not dissolve completely in the solvent mixture. Only the supernatant is used for the derivatization step (8.1.6).

6 Apparatus

6.1 Vortex mixer.

6.2 Oven, capable of 45 °C ± 5 °C.

6.3 Ultrasonic bath.

6.4 Evaporation unit (nitrogen).

6.5 Centrifuge.

6.6 GC/MS equipment consisting of

a) capillary gas chromatograph coupled with a quadrupole mass selective detector and data processing system, and

b) bonded, poly(dimethylsiloxane) capillary column (e.g. 30 m length x 0,25 mm i.d. x 1,0 µm film thickness).

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 carefully be melted at approximately 80 °C in a drying oven or water bath. For high-melting fats, the temperature shall carefully be increased in 10 °C steps until the melting process starts.