SLOVENSKI STANDARD
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Živalske in rastlinske maščobe in olja - Ugotavljanje na maščobno kislino vezanih kloropropanediolov (MCPD) in glicidola z GC/MS - 2. del: Metoda z uporabo počasnega alkalnega preestrenja in meritev 2-MCPD, 3-MCPD in glicidola (ISO/DIS 18363-2:2017)

Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 2: Method using slow alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol (ISO/DIS 18363-2:2017)

Tierische und pflanzliche Fette und Öle - Bestimmung von fettsäuregebundenen Chlorpropandiol (MCPD) und Glycidol mittels GC/MS - Teil 2: Verfahren mittels langsamer alkalischer Umesterung und Messung für 2-MCPD, 3-MCPD und Glycidol (ISO/DIS 18363-2:2017)

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 2: Méthode par transestérification alcaline et mesure pour le 2-MCPD, le 3-MCPD et le glycidol (ISO/DIS 18363-2:2017)

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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Foreword

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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

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

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

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, this 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.

This document 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. ISO 18363-2 consists 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 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. ISO 18363-2 is 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.

ISO 18363-3 represents 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. 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, 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 2:
Method using slow alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol

1 Scope

This part of ISO 18363 describes a procedure for the parallel determination of glycidol together with 2- MCPD and 3-MCPD present in bound or free form in oils and fats. The method is based on alkaline-catalyzed ester cleavage, transformation of the released glycidol into monobromopropanediol (MBPD) and derived free diols (MCPD and MBPD) with phenylboronic acid (PBA). Though free MCPD and glycidol are supposed to be present in fats and oils in low to negligible quantities only, significant content would increase proportionately the determination of bound analytes.

This method is applicable to solid and liquid fats and oils. This part of ISO 18363 can also apply to animal fats and used frying oils and fats, but a validation study must be undertaken before the analysis of these matrices.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this international standard.

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*[8]

3 Terms and definitions

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

3.1
bound 2-MCPD
the sum of all 2-MCPD-derivatives that are cleaved by alkaline-catalyzed alcoholysis.

Note 1 to entry: The content of bound 2-MCPD is reported in milligrams per kilogram (mg/kg).

3.2
bound 3-MCPD
the sum of all 3-MCPD-derivatives that are cleaved by alkaline-catalyzed alcoholysis.

Note 1 to entry: The content of bound 3-MCPD is reported in milligrams per kilogram (mg/kg).

3.3
bound glycidol
the sum of all glycidyl derivatives that are cleaved by alkaline-catalyzed alcoholysis.

Note 1 to entry: The content of bound glycidol is reported in milligrams per kilogram (mg/kg*).*

4 Principle

For the determination of bound 2-MCPD, bound 3-MCPD and bound glycidol as free 2-MCPD, free 3-MCPD and free 3-MBPD (3-Monobromopropanediol), two aliquots (A and B) of the sample are spiked with surrogate standards (d_5-2-MCPD, d_5-3-MCPD, d_5-glycidylester in assay A and d_5-2-MCPD-1,3-diester, d_5-3-MCPD-1,2-diester in assay B) and dissolved in diethyl ether. Both assays are processed in parallel and as follows: The addition of a diluted solution of sodium hydroxide or sodium methoxide in methanol in the cold will release free 2-MCPD, free 3-MCPD and free glycidol over night. This reaction is stopped by the addition of an excess amount of sodium bromide in acidic solution. Under acidic conditions, free glycidol reacts with inorganic bromide to form 3-MBPD and a small amount of 2-MBPD. Undesired non-polar compounds in the sample are removed by double extraction of the aqueous phase with isohexane. The analytes, together with the surrogate standards, are 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, the analytes are concentrated and transferred them into an inert organic solvent. The sample extract is then placed over a small amount of anhydrous sodium sulfate and evaporated to dryness under a stream of nitrogen before being finally redissolved in isooctane for the measurement by GC-MS.

The alkaline catalyzed transesterification in the cold minimizes the undesired transformation of 3-MCPD into glycidol that proceeds to a significant extend when the reaction is carried out at room temperature. Nevertheless, in case of large amounts of 3-MCPD being present, even a minor transformation into glycidol might increase the glycidol results from assay A artificially. In order to achieve the correct glycidol results, even in presence of high 3-MCPD content, assay B serves for the determination of the undesired 3-MCPD – glycidol transformation by determining the amount of d_5-glycidol that has been generated from d_5-3-MCPD-diester by the sample preparation. The corresponding transformation ratio is used for correcting the glycidol value derived from assay A. Another point to be taken into account is that 3-MCPD is converted approximately 1.2-fold faster via glycidol into 3-MBPD than 3-MCPD-d5 via glycidol-d5 into 3-MBPD-d5. Consequently, the isotopic factor I = 1.2 has to be considered for the quantitative determination of the amount of glycidol that has been generated accidentally from the non-labeled 3-MCPD by alkaline treatment in assay A.

Quantification of the analytes is carried out by internal one-point-calibration using the corresponding d_5-esters as surrogate standards. Therefore, no external calibration is necessary. Likewise no analyte recoveries have to be considered. However, the cleaving rates of MCPD mono- and diesters might be different and as only d_5-MCPD-diesters serve as internal standards, the degree of ester cleavage should have proceeded on a large scale. Therefore the degree of variations in ester cleavage is monitored by calculating the differences in 3-MCPD results between assay A and B.

As 3-MCPD can occur in certain polymers used for wet strengthening resins and for other purposes it might also occur from the use of 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 Solvents and chemicals

5.2.1 Toluene.

5.2.2 *tertiary*-Butyl methyl ether (*t*BME), (2-Methoxy-2-methylpropane).

5.2.3 Methanol.

5.2.4 *iso*-Hexane (2-methyl pentane)**.**

5.2.5 Ethyl acetate.

5.2.6 Diethyl ether.

5.2.7 *iso*-Octane.

5.2.8 Sodium sulfate anhydrous, granular.

5.3 Standard and reference compounds

5.3.1 2-MCPD.

5.3.2 2-MCPD-d_5.

5.3.3 2-MCPD-1,3-*bis*-stearoylester *.

5.3.4 2-MCPD-d_5-1,3-*bis*-stearoylester *.

5.3.5 3-MCPD.

5.3.6 3-MCPD-d_5.

5.3.7 3-MCPD-1,2-*bis*-palmitoylester *.

5.3.8 3-MCPD-d_5-1,2-*bis*-palmitoylester *.

5.3.9 Glycidyl oleate*.

5.3.10 Glycidyl-d_5 oleate*.

*Other commercially available fatty acid esters of the analytes may be substituted.

5.4 Working solutions**

5.4.1 2-MCPD: 10.0 µg/mL in methanol.

5.4.2 2-MCPD-d_5: 10.0 µg/mL in methanol.

5.4.3 3-MCPD: 10.0 µg/mL in methanol.

5.4.4 3-MCPD-d_5: 10.0 µg/mL in methanol.

5.4.5 2-MCPD-1,2-*bis*-stearoyl ester: 29.1 µg/mL; equivalent to 5.0 µg/mL free 3-MCPD in toluene.

5.4.6 2-MCPD-d_5-1,2- *bis*-stearoyl ester: 9.3 µg/mL in toluene; equivalent to 5.0 µg/mL free 3-MCPD.

5.4.7 3-MCPD-1,3- *bis*-palmitoyl ester: 26.6 µg/mL in toluene; equivalent to 5.0 µg/mL free 2-MCPD.

5.4.8 3-MCPD-d_5-1,3- *bis*-palmitoyl ester: 26.8 µg/mL in toluene; equivalent to 5.0 µg/mL free 2-MCPD.

5.4.9 Glycidyl oleate: 22.8 µg/mL in toluene; equivalent to 5.0 µg/mL free glycidol.

5.4.10 Glycidyl-d_5 oleate: 23.2 µg/mL in toluene; equivalent to 5.0 µg/mL free glycidol.

**Other concentrations of working solutions may be substituted.

5.5 Other solutions

5.5.1 Sodium hydroxide solution: Weigh 0.25 g freshly ground sodium hydroxide in a 100 mL plastic bottle. Add 100 mL methanol and tightly seal the bottle. Shake vigorously (vortex) until the sodium hydroxide has been dissolved completely. Store in a freezer at -22°C to -25°C. (see Notes, 10.1.1.).

5.5.2 Acidified sodium bromide solution: Weigh 600 g sodium bromide in a 1 L screw cap glass volumetric flask, add deionised water up to the 1 L mark. Acidify the mixture with 3 mL of *ortho*-phosphoric acid (85%), seal tightly and shake (vortex) until the solution is clear. 600 µL of this solution must neutralize 350 µL of sodium hydroxide solution (Solutions, 1) and adjust the pH-value to the acidic range (pH 3-1). Store the solution in a freezer at -22°C to -25°C. (see Notes, 10.1.1.).

5.5.3 Saturated solution of phenylboronic acid (PBA) in diethyl ether: Add approximately 200 mg PBA to 10 mL diethyl ether in a screw cap vial. Shake well, allow non-dissolved PBA to settle and remain as precipitate. For derivatization purposes, use only the clear supernatant.

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 0,0001 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 (approximately2 mL in capacity).