SLOVENSKI STANDARD
oSIST prEN ISO 18363-4:2021

01-februar-2021

Živalske in rastlinske maščobe in olja - Ugotavljanje na maščobno kislino vezanih kloropropanediolov (MCPD) in glicidola z GC/MS - 4. del: Metoda z uporabo hitrega alkalnega preestrenja in meritev 2-MCPD, 3-MCPD in glicidola z GC-MS/MS (ISO/DIS 18363-4:2020)

Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS (ISO/DIS 18363-4:2020)

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Tierische und pflanzliche Fette und Öle - Bestimmung von fettsäuregebundenem Chlorpropandiol (MCPD) und Glycidol mittels GC/MS - Teil 4: Verfahren mittels schneller alkalischer Umesterung und Messung für 2-MCPD, 3-MCPD und Glycidol (ISO/DIS 18363-4:2020)

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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 4: Méthode par transestérification alcaline rapide et mesure pour le 2-MCPD, le 3-MCPD et le glycidol par CPG-SM/SM (ISO/DIS 18363-4:2020)

Ta slovenski standard je istoveten z: **prEN ISO 18363-4**

ICS:

67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils

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

Part 4:
Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS

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oSIST prEN ISO 18363-4:2021

Contents

Page

iTeh STANDARD PREVIEW
(standards.iteh.ai)
oSIST prEN ISO 18363-4:2021
https://standards.iteh.ai/catalog/standards/sist/e6e1828a-1450-42d4-940d-347df5de6e27/osist-pren-iso-18363-4-2021

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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*.

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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
- Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol (MS/MS)

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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 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.

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ISO 18363-2 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

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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.

ISO 18363-4 describes a rapid procedure based on fast alkaline cleavage of the MCPD and glycidyl esters. The released glycidol is subsequently converted into 3-MBPD. The pH of the fast alkaline cleavage generally causes the released MCPD to partially convert to glycidol during the cleavage of the esters, leading to overestimation of the glycidyl ester content of the sample. By adding two distinct isoptopically labelled ester-bound 3-MCPD and glycidol internal standards it is possible to quantify the amount of labelled glycidol resulting from the degradation of the released internal standard. This information can be used to correct for overestimation of the glycidyl ester induced glycidol by 3-MCPD induced glycidol. The same two internal standards are used for quantification of the bound MCPD and glycidol, requiring a single sample preparation to quantify bound 2-MCPD-, 3-MCPD- and glycidol esters. In analogue with Parts 1 to 3, the released MCPD's and 3-MBPD are derivatized with phenylboronic acid before GC-MS/MS analysis. In contrast to the other parts of ISO 18363, part 4 requires GC-MS/MS instrumentation to unambiguously detect each of the (isotopically labelled) MBPD's required for correct quantification of the glycidyl ester induced glycidol. ISO-1363-4 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 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.

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

Part 4:
Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS

1 Scope

This part of ISO 18363 describes a rapid 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 alkaline catalysed ester cleavage and derivatization of cleaved (free) analytes with phenylboronic acid (PBA) prior to GC-MS/MS analysis.

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. 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.

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Milk and milk products (or fat coming from milk and milk products), infant formulas, emulsifiers, free fatty acids and other fats and oils derived matrices are excluded from the scope of this international standard.

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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*

ISO 5555, *Animal and vegetable fats and oils — Sampling*

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

3.1
Bound 2-MCPD
The amount of 2-MCPD cleaved from its esterified (bound) forms by alkaline-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).

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3.2
Bound 3-MCPD
The amount of 3-MCPD cleaved from its esterified (bound) forms by alkaline-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
The amount of glycidol cleaved from its esterified (bound) forms by alkaline-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 toluene and tert-butyl-methyl-ether, and the internal standards (3-MCPD-$^{13}C_3$ diester and pentadeuterated glycidyl ester) are added. The sample is then cooled down to 10 ºC before the alkaline transesterification is initiated by the addition of a sodium methoxide solution in methanol. After 12 min incubation at 10 ºC, the sample mixture is acidified with an acidic solution of sodium bromide to convert the released glycidol to 3-MBPD. The fatty acid methyl esters generated during the transesterification are removed by duplicate extraction of the organic layer. Finally, the purified sample – containing cleaved (free) analytes – is derivatized with phenylboronic acid prior to GC-MS/MS analysis[1].

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The quantification of ester bound 2- and 3-MCPD is based on the 2-MCPD/3-MCPD-$^{13}C_3$ and 3-MCPD/3-MCPD-$^{13}C_3$ signal ratio, respectively. The quantification of ester bound glycidol is based on the 3-MBPD/3-MBPD-d5 signal ratio. The amount of 3-MBPD-$^{13}C_3$ formed after the transesterification reaction signifies the amount of released 3-MCPD-$^{13}C_3$ that has degraded to glycidol due to the conditions of the alkaline transesterification. Because no difference in degradation speed between 3-MCPD and 3-MCPD-$^{13}C_3$ has been observed, it is then used to correct for overestimation of the glycidyl ester induced glycidol caused by this degradation of 3-MCPD. Under the conditions used the 2-MCPD is considered stable and thus will not significantly contribute to possible glycidol overestimation[2].

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

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 1,2-Dipalmitoyl-3-chloropropanediol (PP-3-MCPD), purity ≥ 95% (e.g., supplied by Toronto Research Chemicals Inc., North York, ON, Canada[1)] or synthesized from 3-MCPD and palmitoyl chloride as described by Kraft et al.[1]).

1) The Standard and reference compound supplier is an example of a suitable supplier of reference standards that is commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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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 should be preferred as they are the most abundant in the majority of oils/fats).

5.2.2 1,3-Distearoyl-2-chloropropanediol (SS-2-MCPD), purity ≥ 95% (e.g., supplied by Toronto Research Chemicals Inc., North York, ON, Canada[1)]).

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

5.2.3 Carbon-13 labelled- 1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD-$^{13}C_3$), purity ≥ 95% (e.g., supplied by Toronto Research Chemicals Inc., North York, ON, Canada[1)]).

NOTE The same consideration applied to 1,2-dipalmitoyl-3-chloropropanediol is valid also for its carbon-13 labelled analogue, see Note in 5.2.1.

5.2.4 Glycidyl stearate (Gly-S), purity ≥ 98% (e.g., supplied by Toronto Research Chemicals Inc., North York, ON, Canada[1)]).

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

5.2.5 Pentadeuterated glycidyl stearate (Gly-S-d5), purity ≥ 98% (e.g., supplied by Toronto Research Chemicals Inc., North York, ON, Canada[1)]).

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NOTE The same consideration applied to glycidyl palmitate is valid also for its pentadeuterated analogue, see Note in 5.2.4.

5.3 Standard solutions

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All standard solutions are prepared with toluene (5.4.4). All standards are prepared using ester-bound reference compounds (5.2). Concentrations are given in the free component equivalent concentration. For an exemplary calculation of ester-bound to free equivalent concentration conversion, please see Note 3.

5.3.1 Stock solutions

a) **Calibration Stock** (3-MCPD: 52.7µg/mL, glycidol: 52.2 µg/mL, 2-MCPD: 48.1 µg/mL): Weigh 14.0 mg of PP-3-MCPD (5.2.1), 12.0 mg of Gly-S (5.2.4) and 14.0 mg of SS-2-MCPD (5.2.2) in a 50 mL volumetric flask. Fill up to the mark, making sure that the standards are completely dissolved in the solvent.

b) **Spike Stock** (3-MCPD: 52.7µg/mL, glycidol: 52.2µg/mL, 2-MCPD: 34.4 µg/mL): Weigh 14.0 mg of PP-3-MCPD (5.2.1), 12.0 mg of Gly-S (5.2.4) and 10.0 mg of SS-2-MCPD (5.2.2) in a 50 mL volumetric flask. Fill up to the mark, making sure that the standards are completely dissolved in the solvent.

c) **PP-3-MCPD-$^{13}C_3$ Stock** (3-MCPD-$^{13}C_3$: 38.5 µg/mL): Weigh 20 mg of PP-3-MCPD-$^{13}C_3$ (5.2.3) in a 100 mL volumetric flask. Fill up to the mark, making sure that the standard is completely dissolved in the solvent.

d) **Gly-S-d5 stock** (Glycidol-d5: 45.8 µg/mL): Weigh 10 mg of Gly-S-d5 (5.2.5) in a 50 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 twelve months when stored at -18°C. Using an ultrasonic bath may help to ensure all standards are completely dissolved.

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5.3.2 Working solutions

a) **Calibration Working Solution I** (3-MCPD: 7.9 µg/mL, glycidol: 7.8 µg/mL, 2-MCPD: 7.2 µg/mL): Pipette 300 µL of the stock solution (5.3.1a) into a 2.5 mL GC vial containing 1700 µL of Toluene (5.4.4) and homogenize using a vortex mixer.

b) **Calibration Working Solution II** (3-MCPD: 3.2 µg/mL, glycidol: 3.1 µg/mL, 2-MCPD: 2.9 µg/mL): Pipette 120 µL of the stock solution (5.3.1a) into a 2.5 mL GC vial containing 1880 µL of Toluene (5.4.4) and homogenize using a vortex mixer.

c) **Calibration Working Solution III** (3-MCPD: 0.16 µg/mL, glycidol: 0.16 µg/mL, 2-MCPD: 0.14 µg/mL): Pipette 40 µL of the stock solution (5.3.2a) into a 2.5 mL GC vial containing 1960 µL of Toluene (5.4.4) and homogenize using a vortex mixer.

NOTE It is advisable to freshly prepare the calibration working solutions on the day they are to be used.

d) **Spike Solution** (3-MCPD: 1.05 µg/mL, glycidol: 1.04 µg/mL, 2-MCPD: 0.69 µg/mL): Pipette 5.0 mL of the spike stock solution (5.3.2b) into a 250 mL volumetric flask and fill up to the mark with the solvent.

e) **Internal Standard Solution:** (3-MCPD-$^{13}C_3$: 1.54 µg/mL, glycidol-d5: 0.92 µg/mL): Pipette 5.0 mL of the Gly-S-d5 (5.3.21d) and 10.0 mL of PP-3-MCPD-$^{13}C_3$ (5.3.1c) into a 250 mL volumetric flask and fill up to the mark with the solvent.

NOTE 1 The spike solution (5.3.2d) and internal standard solution (5.3.2e) can be stored in the refrigerator for at least 3 months.

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NOTE 2 The concentrations of all stock and standard solutions need to be corrected for the purity of the used standards.

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5.4 Other reagents

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5.4.1 Methanol, analytical grade

5.4.2 Iso-Octane, analytical grade

5.4.3 Acetone, analytical grade

5.4.4 Toluene, analytical grade

5.4.5 Tert-Butyl-Methyl-ether, analytical grade

5.4.6 Water, ultra-pure (e.g., obtained by using a Millipore Milli-Q purification system)

5.4.7 Sulfuric acid (purity ≥ 95%)

5.4.8 Phenylboronic acid (purity ≥ 97%)

5.4.9 Sodium bromide (purity ≥ 99.5%)

5.4.10 Sodium methoxide solution in methanol (25 wt.%)

5.4.11 Non-thermally treated, cold pressed vegetable oil (blank oil, see 9.3.1)

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