
Živalske in rastlinske maščobe ter olja - Določevanje maščobno kislinsko vezanih kloropropanoliolov (MCPDs) in glicidola z GC/MS - 4. del: Metoda z uporabo hitre alkalne transesterifikacije in meritev 2- MCPD, 3-MCPD in glicidolov z GC-MS/MS (ISO 18363-4:2021)

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 18363-4:2021)

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Tierische und pflanzliche Fette und Öle - Bestimmung von fettsäuregebundenem Chloropropaniol (MCPD) und Glycidol mittels GC/MS - Teil 4: Verfahren mittels schneller alkalischer Umesterung und Messung für 2-MCPD, 3-MCPD und Glycidol (ISO 18363-4:2021)

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 18363-4:2021)

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67.200.10	Rastlinske in živalske maščobe in olja	Animal and vegetable fats and oils
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(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
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Tierische und pflanzliche Fette und Öle - Bestimmung
von fettsäuregebundenem Chlorpropanediol (MCPD)
und Glycidol mittels GC/MS - Teil 4: Verfahren mittels
schneller alkalischer Umesterung und Messung für 2-
MCPD, 3-MCPD und Glycidol (ISO 18363-4:2021)

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European foreword

This document (EN ISO 18363-4:2021) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 307 "Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2022, and conflicting national standards shall be withdrawn at the latest by February 2022.

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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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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

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 307, *Oilseeds, vegetable and animal fats and oils and their by-products – Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 different parts 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)^[9] and identical to AOCs Official Method Cd 29c-13^[6]. In brief, 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. Consequently, 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 must 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 must be undertaken before the analysis of this analyte.

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ISO 18363-2 represents the AOCs Official Method Cd 29b-13^[5]. In brief, 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 are 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 is 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 also applies to animal fats and used frying oils and fats, but a validation study must be undertaken before the analysis of these matrices. Any free analytes within the sample are included in the results, but the document does not allow a 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^[4]. In brief, 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. ISO 18363-3 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. ISO 18363-3 is applicable to the determination of ester-bound 2-MCPD, 3-MCPD and glycidol in refined

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and non-refined vegetable oils and fats. It also applies to animal fats and used frying oils and fats, but a validation study must be undertaken before the analysis of these matrices. The method is suited for the analysis of bound (esterified) analytes, but if required ISO 18363-3 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 are included in the results and the amount of free analytes can be calculated as the 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.

This document specifies 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 isotopically 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 ISO 18363-1, ISO 18363-2 and ISO 18363-3, the released MCPDs and 3-MBPD are derivatized with phenylboronic acid before GC-MS/MS analysis. In contrast to the other parts of the ISO 18363 series, this document requires GC-MS/MS instrumentation to unambiguously detect each of the (isotopically labelled) MBPDs required for correct quantification of the glycidyl ester induced glycidol. This document is applicable to the determination of ester-bound 3-MCPD, 2-MCPD and glycidol in refined and unrefined vegetable oils and fats. It also applies to animal fats and used frying oils and fats, but a validation study must be undertaken before analysis of these matrices. Any free analytes within the sample are 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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