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 (ISO 18363-1:2015)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 18363-1:2021 sisaldab Euroopa standardi EN ISO 18363-1:2021 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 18363-1:2021 consists of the English text of the European standard EN ISO 18363-1:2021.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 15.12.2021.	Date of Availability of the European standard is 15.12.2021.
Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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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 (ISO 18363-1:2015)

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 1: Méthode par transestérification alcaline rapide et mesure pour le 3-MCPD et par mesure différentielle pour le glycidol (ISO 18363-1:2015)

Tierische und pflanzliche Fette und Öle - Bestimmung von fettsäuregebundenen Chlorpropandiol (MCPD) und Glycidol mittels GC/MS - Teil 1: Verfahren mittels schneller alkalischer Umesterung und Messung für 3 MCPD und Differenzmessung für Glycidol (ISO 18363 1:2015)

This European Standard was approved by CEN on 5 December 2021.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

The text of ISO 18363-1:2015 has been prepared by Technical Committee ISO/TC 34 "Food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 18363-1:2021 by 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 June 2022, and conflicting national standards shall be withdrawn at the latest by June 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 18363-1:2015 has been approved by CEN as EN ISO 18363-1:2021 without any modification.

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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.

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).