International Standard

ISO 18363-2

Second edition
2025-01

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

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 lente et mesure pour le 2-MCPD, le 3-MCPD et le glycidol

Reference number
ISO 18363-2:2025(en)

© ISO 2025



COPYRIGHT PROTECTED DOCUMENT

© ISO 2025
All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

Published in Switzerland

Contents

Page

Foreword ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Principle ... **2**
5 Reagents ... **3**
5.1 General ... 3
5.2 Solvents and chemicals ... 3
5.3 Standard and reference compounds ... 3
5.4 Working solutions ... 4
5.5 Other solutions ... 4
6 Apparatus ... **4**
7 Sample ... **5**
7.1 Sampling ... 5
7.2 Preparation of the test sample ... 5
8 Procedure ... **5**
8.1 Spiking with surrogate standard and homogenization ... 5
8.2 Ester cleavage and glycidol transformation ... 5
8.3 Matrix removal ... 6
8.4 Derivatization ... 6
8.5 Gas chromatography/mass spectrometry references ... 6
9 Expression of results ... **7**
9.1 Determination of bound glycidol ... 7
9.2 Determination of bound 2-MCPD ... 8
9.3 Determination of bound 3-MCPD ... 9
9.4 Determination of the degree of diester cleavage ... 9
9.5 Quality control ... 9
10 Notes ... **10**
Annex A (informative) **Examples of relevant chromatograms and data evaluation using "low-MCPD" palm oil** ... **12**
Annex B (informative) **Results of interlaboratory tests** ... **19**
Bibliography ... **21**

© ISO 2025 – All rights reserved

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of 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 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*, 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).

This second edition cancels and replaces the first edition (ISO 18363-2:2018), of which it constitutes a minor revision to align the Introduction with ISO 18363-4:2021.

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.

© ISO 2025 – All rights reserved

Introduction

The ISO 18363 series[1] 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 each individual method.

ISO 18363-1[2] is a differential method equivalent to the DGF standard C-VI 18 (10)[3] and identical to AOCS Official Method Cd 29c-13[4]. 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 of this document, 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,[5] but again, a validation study must be undertaken before the analysis of this analyte.

This document (i.e. ISO 18363-2) represents AOCS Official Method Cd 29b-13.[6][7] For information on corresponding validation data, see Annex B. 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. This document 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 isotope-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. 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 the analysis of these matrices. Any free analytes within the sample are included in the results, but the document does 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 refined vegetable oils and fats.

ISO 18363-3[8] represents AOCS Official Method Cd 29a-13.[9][10] 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 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

© ISO 2025 – All rights reserved

(esterified) analytes, but if required, ISO 18363-3 can also be 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 of this document, research has not shown any evidence of a free analyte content as high as the esterified analyte content in refined vegetable oils and fats.

ISO 18363-4[11] 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, this document 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, ISO 18363-4 requires GC-MS/MS instrumentation to unambiguously detect each of the (isotopically labelled) MBPDs required for correct quantification of the glycidyl ester induced glycidol. ISO 18363-4 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 ISO 18363-4 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 refined vegetable oils and fats.

© ISO 2025 – All rights reserved

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 document specifies 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-catalysed ester cleavage, transformation of the released glycidol into monobromopropanediol (MBPD) and derivatisation of the 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, in the event that free analytes are present, they would contribute proportionately to the results. The results always being the sum of the free and the bound form of a single analyte.

This method is applicable to solid and liquid fats and oils. This document can also apply to animal fats and used frying oils and fats, but a validation study is 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 document.

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:

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

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

3.1
bound 2-MCPD
sum of all 2-MCPD-derivatives that are cleaved by alkaline-catalysed alcoholysis

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

© ISO 2025 – All rights reserved