INTERNATIONAL STANDARD

ISO 18363-1

First edition
2015-08-15

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

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 —

Partie 1: Méthode par transestérification alcaline rapide et mesure pour le chloro-3 propane-1,2-diol (3-MCPD) et par mesure différentielle pour le glycidol

Reference number
ISO 18363-1:2015(E)

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

Contents

Page

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

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).

Note 2 to entry: The presence of free glycidol in fats and oils is unlikely because of the instability of this compound.

4 Principle

For the determination of the sum of bound 3-MCPD and bound glycidol as free 3-MCPD (assay A), an aliquot of the sample is spiked with surrogate standard d_5-3-MCPD-1,2-bis-palmitoyl ester and dissolved in *tertiary*-butyl methyl ether (*t*BME). The addition of a diluted solution of sodium hydroxide or sodium methoxide in methanol will release free 3-MCPD and free glycidol. This reaction is stopped by the addition of an excess amount of sodium chloride in acidic solution. Under acidic conditions, free glycidol reacts with inorganic chloride to additional 3-MCPD and a small amount of 2-MCPD. Undesired non-polar compounds in the sample are removed by double extraction of the aqueous phase with isohexane. The analyte, together with the surrogate standard, is 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, concentrate the analytes and transfer them into an inert organic solvent the sample extract is then placed over a small amount of anhydrous sodium sulfate and evaporate until dry under a stream of nitrogen before being finally re-dissolved in isooctane for the measurement by GC-MS.

For the determination of bound 3-MCPD, a second aliquot of the sample (assay B) is spiked with a surrogate standard d_5-3-MCPD-1,2-bis-palmitoyl ester and dissolved in *t*BME. The addition of a diluted solution of sodium hydroxide or sodium methoxide in methanol will release free 3-MCPD and free glycidol. This reaction is stopped by the addition of an excess amount of an acidic chloride-free salt solution (e.g. sodium sulfate, ammonium sulfate, sodium bromide). Under chloride-free acidic conditions, free glycidol reacts to different products depending on the salt used, but does not generate additional 3-MCPD. The additional sample preparation and measurement is performed as described for assay A.

For the calculation of bound glycidol, the difference of both determinations (assay A and B) is multiplied by a non-stoichiometric factor reflecting the transformation of glycidol to 3-MCPD. This procedure is based on the assumption that the difference between the determinations is caused only by the exclusive occurrence of glycidyl esters.

Quantification of the analytes is carried out using d_5-3-MCPD-1,2-bis-palmitoyl ester as surrogate standard. Therefore, no control of the completeness of the ester cleavage is necessary. A matrix calibration over the complete method is carried out periodically and serves for the determination of method linearity, relative recovery and in the case of bound glycidol for the determination of the transformation factor. As long as the method is not changed, this calibration does not necessarily have to be done every working day.

Samples which do contain 3-MCPD only and no glycidol result in lower values (approximately 2 % to 10 %) when using procedure B instead of procedure A. The difference is due to an isotope effect in procedure B. This effect can be determined by dosing a non-contaminated oil sample with equal amounts of 3-MCPD-1,2-bis-dipalmitoylester and d_5-3-MCPD-1,2-bis-palmitoylester before being divided into two aliquots. The aliquots are then analysed three times according to procedure A and procedure B. From the determinations, a correction factor may be determined, which is the ratio of the results from procedure A and B. The isotope effect also appears in the presence of bound glycidol.

As 3-MCPD can occur in certain polymers used for wet strengthening resins and for other purpose, it is recommended that a blank sample be analysed every day in order to control undesired contamination. The blank shall be oil which does not contain any 3-MCPD or glycidol, e.g. a virgin vegetable oil. Contamination might also occur from 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 Standard and reference compounds

5.2.1 Working solution surrogate standard: d_5-3-MCPD-1,2-*bis*-palmitoyl ester (e.g. 26,87 µg/mL in toluene; equivalent 5,0 µg/mL free 3-MCPD); other representative d_5-3-MCPD-1,2-*bis*-esters might be used instead as well.

5.2.2 3-MCPD-1,2-*bis*-palmitoyl ester (26,6 µg/mL in toluene; equivalent 5,0 µg/mL free 3-MCPD); other representative 3-MCPD-1,2-bis-esters might be used instead as well.

5.2.3 3-MCPD, $w \geq 99{,}0$ %.

5.2.4 Glycidyl stearate, $w \geq 95{,}0$.

5.3 Solvents

5.3.1 Toluene.

5.3.2 *tertiary*-butyl methyl ether (*t*BME), (2-Methoxy-2-methylpropane).

5.3.3 Methanol.

5.3.4 Isohexane (2-methylpentane).

5.3.5 Ethyl acetate.

5.3.6 Diethyl ether.

5.3.7 Isooctane.

5.4 Other reagents

5.4.1 Sodium hydroxide solution, ρ = 20 g/L in methanol or sodium methoxide solution, ρ = 25 g/L in methanol.

5.4.2 Acidified sodium chloride solution (ρ = 200 g/L). Add 35 mL of sulfuric acid (25 %) to 1 L of aqueous sodium chloride solution (200 g/L). 600 µL of this solution shall neutralize 200 µL of sodium hydroxide or sodium methoxide solution (5.4.1) and adjust the pH-value to the acidic range. The use of other acids (like acetic acid for example) is possible, but their suitability shall be tested by spiking experiments or analysis of ring test material.

5.4.3 Acidified sodium bromide solution, (ρ = 600 g/L). Add 35 mL of sulfuric acid (25 %) to 1 L of aqueous sodium bromide solution (600 g/L). 600 µL of this solution shall neutralize 200 µL of sodium hydroxide or sodium methoxide solution (5.4.1) and adjust the pH-value to the acidic range. The