
**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**

*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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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.

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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 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.

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