

An Overview of Indirect Methods for the Analysis of MCPD Esters and Glycidyl Esters in Fats and Oils

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INTRODUCTION

Direct and indirect analytical methods are the two general approaches in quantifying MCPD esters and glycidyl esters. Direct methods determine the individual MCPD and glycidyl esters while indirect methods determine free MCPD and glycidol that have been liberated from their derivative analogues. Both methods have advantages and disadvantages. The direct methods, obviously, have the advantage of being more direct, requiring a minor degree of sample preparation and thereby having little chance of being impaired by errors originating from cumbersome preparation procedures. Besides, direct methods provide detailed information on the chemical structure of the esters. However, they have a disadvantage when proper quantification requires that several reference and standard compounds to be available for use. To make things worse, for the unknown glycidyl derivatives that may be present in any sample or matrix, the compounds might not be detected because there is no prior knowledge of their analytical characteristics. In contrast, despite the major drawback of the need for long sample preparation steps, indirect analytical methods allow for the determination of all MCPD and glycidyl derivatives as long as they undergo transesterification to release their free forms. The complexity of the analytes composition requires selective separation and advanced de-

fewer standards and simpler instruments (e.g. gas chromatography/mass spectrometry equipment) are required.

The American Oil Chemists' Society (AOCS) has benchmarked three indirect methods as official methods, namely: i) Cd 29a-13: Determination of 2- and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by acid transesterification ii) Cd 29b-13: Determination of bound monochloropropanediol- (MCPD-) and bound 2,3-epoxy-1-propanol (glycidol-) by gas chromatography/mass spectrometry (GC/MS), and iii) Cd 29c-13: Fatty-acid-bound 3-chloropropane-1,2,diol (3-MCPD) and 2,3-epoxy-propane-1-ol (glycidol) determination in oils and fats by GC/MS (Differential measurement). These methods were selected because they are suitable for routine monitoring, perform well and give reliable results, close to the known content

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tection equipment which become a limitation to small-scale laboratories. Thus, the indirect analytical approach seems to be better suited for a wider group of researchers and for routine analyses because

of the carefully manufactured authentic reference standards.

INDIRECT METHOD – 3-MCPD ESTERS

Generally, there are two types of indirect methods that are widely used: alkaline or acidic catalysed transesterification. Both of these methods share a common analytical protocol which is summarised below:

The addition of an internal standard (free or the esterified form of 3-MCPD- d_5) into the oil sample is required for its accurate quantification. This is then followed by cleavage of the 3-MCPD esters to form 3-MCPD by means of transesterification. Salting out is a process where salting out agents such as sodium chloride and sulphate salts are added during or after the neutralisation step, aiming to facilitate the extraction of lipophilic compounds (fatty acid methyl esters or FAMES) from the transesterification mixture. Hexane/heptane which functions as a solvent is then added repeatedly to wash off FAMES and impurities. Derivatisation prior to the final GC/MS analysis is a very critical step as it offsets the undesirable characteristics (*viz.* low volatility and high polarity) of 3-MCPD. Phenylboronic acid is the most common derivatisation agent used because it is very selective and reacts specifically with diols to form non-polar cyclic derivatives that are extractable with non-polar solvents (Divinova *et al.*, 2004).

Modifications of each individual step by different laboratories lead to a number of in-house methods as summarised in *Table 1*.

The alkaline-catalysed transesterification approach is relatively convenient compared with the acid-catalysed transesterification process owing to the short duration of its reaction time. However, the reliability of the method is highly dependent on the transesterification time. 3-MCPD is unstable under alkaline conditions and gives rise to glycidol. Hrnčirik *et al.* (2011) and Kuhlmann (2011) both reported the degradation of 3-MCPD under alkaline conditions in their independent studies. 3-MCPD recovery after 1, 3, 9 and 10 min of transesterification was about 83%-95%, 75%, 50%, and 40% respectively. Besides, the specificity of the alkaline method has been questioned when sodium chloride is used as the salting out agent (Weiβhaar, 2008). The presence of glycidol in refined oils leads to the undesirable formation of additional 3-MCPD, thus resulting in an overestimation of 3-MCPD esters.

On the other hand, acid transesterification-based methods show good robustness and specificity as they give comparable results regardless of variations in analytical conditions, such as the type of salting out agent used (Ermacora and Hrnčirik, 2012), or the derivatisation procedure (Seefelder *et al.*, 2008). No degradation of 3-MCPD was observed when using the abovementioned method (Hrnčirik *et al.*, 2011). However, the only limitation is the long transesterification time of 16 hr, which can be very time-consuming. The Joint Research Centre of the European Commission (Karasek *et al.*, 2010) has conducted a proficiency test on 3-MCPD esters analysis in oils. The study found that all results obtained by acid transesterification

methods were satisfactory, while larger variation were observed for specific alkaline transesterification (acid pre-treatment or chloride-free). The laboratories which adopted the non-specific alkaline-catalysed transesterification method failed to produce satisfactory results.

INDIRECT METHOD- GLYCIDYL ESTERS

The first indirect quantification method for glycidyl esters was proposed by Weiβhaar and Perz (2010), and has been presented by the German Society for Fat Science (DGF) as its official method (DGF, 2009). The principle of this method is based on calculation of the difference between two independent quantifications: the first pretreatment (A) quantifies the sum of 3-MCPD and 3-MCPD forming substances which involve glycidyl esters (after their conversion to 3-MCPD) and MCPD esters, while the second pretreatment (B) is specific to 3-MCPD esters. Nevertheless, this approach is only applicable and meaningful if pretreatment A ensures a complete conversion of glycidol into 3-MCPD, and if pretreatment B is free from any conversion of glycidol. The value obtained from the subtraction of B from A has to be corrected by multiplication with a non-stoichiometric factor of 0.67. The non-stoichiometric factor reflects and assumes the complete transformation of glycidol into 3-MCPD during sample preparation (Kuhlmann, 2011).

The other more elaborate alternative method introduced by Kuhlmann (2011) enables the parallel determination of both glycidyl

TABLE 1. INDIRECT ANALYTICAL METHODS FOR MCPD AND GLYCIDYL ESTERS

Type of transesterification	Analyte	Comments	References
Acidic (H ₂ SO ₄ /MeOH)	3-MCPD esters	- Long transesterification time of 16 hr - Good robustness - High specificity	Divinova <i>et al.</i> (2004), Zelinkova <i>et al.</i> (2006) Seefelder <i>et al.</i> (2008)
Acidic (H ₂ SO ₄ /MeOH) with pre-treatment (NaBr/H ⁺) for the conversion of glycidyl esters into 3-mono-bromopropanediol (3-MBPD) esters	3- and 2-MCPD esters, glycidyl esters	- Short transesterification time (5-10 min) - Salting out with sodium chloride, thus leading to the conversion of glycidol to 3-MCPD - Drawback: Low specificity and not specific to bound 3-MCPD alone (resulting in overestimation of 3-MCPD)	Ermacora and Hrnčirik (2013)
Alkaline, non-specific (DGF C-III 18(09) A) (NaOCH ₃ /MeOH)	Sum of 3-MCPD and glycidyl esters		DGF (2009)
Alkaline, non-specific (DGF C-VI 17(10) B) (NaOCH ₃ /MeOH)	Sum of 3-MCPD and glycidyl esters	- Short transesterification time (5-10 min) - Modification of DGF C-III 18 (09) A	DGF (2011a)
Alkaline, with pretreatment (DGF C-III 18(09) B) (NaOCH ₃ /MeOH)	3-MCPD esters	- Short transesterification time (5-10 min) - Pretreatment step by adding sulphuric acid/propanol mixture to selectively eliminate glycidyl esters - Withdrawn in early 2011 as the pretreatment step is insufficient and not reliable	DGF (2009)
Alkaline, chloride-free (DGF C-VI 18(10)) (NaOCH ₃ /MeOH)	3-MCPD esters	- Short transesterification time (3-5 min) - Substitution of sodium chloride with other salts	DGF (2011b)
Alkaline, mild (SGS) (NaOH/ MeOH)	3- and 2-MCPD esters, glycidyl esters	- Long transesterification time of 18 hr - Low temperature (-22°C) applied to eliminate undesirable conversion of MCPD to glycidol - Complex analytical protocol - Subfreezing conditions are strictly required	Kuhlamann (2011)
Alkaline with pre-treatment (CH ₃ OH/ H ⁺) for the conversion of glycidyl esters into 3-methoxypropane-1, 2-diol (3-MPD) esters.	3-MCPD esters and glycidyl esters	- The optimised conditions are 1 min for transesterification and 15 min for derivatisation, both at ambient temperature - This is to minimise the undesirable conversion of glycidyl esters and 3-MCPD esters into glycidol	Küsters <i>et al.</i> (2011)

esters, 3- and 2- MCPD in oil matrices. It is based on an improved alkaline-catalysed release of MCPD and glycidol, followed by a transformation of glycidol into monobromopropanediol (MBPD) by using a concentrated aqueous solution of sodium bromide, derivatisation with phenylboronic acid (PBA), and analysis by GC/MS. 3-MBPD is a highly stable compound that shows excellent GC/MS behaviour. It possesses a structure homologous to 3-MCPD which thus allows the same sample preparation, derivatisation and analytical conditions for both analytes. In order to avoid the generation of additional glycidol out of the liberated MCPD during the alkaline-catalysed transesterification, the author reported that performing a mild alkaline-catalysed ester cleavage at -25°C with a more diluted solution of sodium hydroxide in methanol can be an effective and promising solution. This is based on the assumption that the transformation of 3-MCPD to glycidol requires a higher activation energy than the alkaline-catalysed cleavage that releases MCPD and glycidol from their derivatives. However, the transesterification time has to be prolonged to 16 hr to ensure the completion of ester cleavage.

Kuster *et al.* (2011) also developed a method for the simultaneous determination and differentiation of glycidyl esters and 3-MCPD esters in different foodstuffs, such as fat-rich products like soup and gravy powders, cookies, bread and mayonnaise, other than just in edible oils and fats. This method adopts a simple and rapid extraction step to separate the esters, followed by conversion (ring-opening) of the glycidyl moiety into esters of 3-methoxypropane-1,

2-diol (3-MPD) by acidic methanolysis prior to rapid alkaline-catalysed transesterification at ambient temperature, and subsequent ester cleavage by sodium methoxide. All the optimised conditions and parameters of transesterification (1 min at ambient temperature) and derivatisation (15 min at ambient temperature) are aimed at minimising the undesirable conversion of glycidyl esters and 3-MCPD esters into glycidol, or else, glycidol will be subsequently converted into as well as being determined as 3-MCPD phenyl boronate during derivatisation. In other words, this reaction renders glycidyl esters and 3-MCPD esters indistinguishable. To avoid this interaction, acidic alcoholysis by methanol and sulphuric acid is performed to transform glycidyl ester to 3-MPD ester prior to transesterification, and finally into 3-MPD phenyl boronate after derivatisation. Thus, this is a critical step to enable the differentiation between 3-MCPD esters and glycidyl esters. Besides, transesterification time of 1 min is found to be the minimum time required for completion of ester cleavage and the least glycidol formation. The derivatisation procedure by phenylboronic acid (PBA) at ambient temperature instead of the usual derivatisation temperature of 80°C (or higher) is applied in this approach to prevent the derivatisation of undesirable glycidol which forms inevitably during the previous transesterification. The optimised derivatisation at ambient temperature shows no differences in sensitivity and precision compared to the one carried out at 80°C . Moreover, it is shown to be highly reproducible with a relative standard deviation of less than 5%.

In 2013, Ermacora and Hrnčirik introduced a new indirect

method for the parallel quantitation of 2-MCPD, 3-MCPD and glycidol in oils and fats by GC/MS. This method is based on the acid-catalysed conversion of glycidyl esters into 3-monobromopropanediol (3-MBPD) monoesters by using an acidified aqueous solution of sodium bromide at 50°C for 15 min. The epoxide ring of glycidyl esters is expected to be opened by the nucleophilic attack (in this case, bromide), and this process is induced and accelerated under acidic conditions together with increased temperatures. The solution then undergoes acid catalysed transesterification, extraction and discarding of the fatty acid methyl esters (FAMES) followed by derivatisation of free diols (MCPD and MBPD) with PBA prior to GC/MS analysis.

The researchers has validated the AOCS method Cd 29a-13 for the detection and quantification of 3-MCPD esters and glycidyl esters in palm oil based on several parameters: limit of detection (LOD), limit of quantitation (LOQ), recovery and precision. Quantitative analysis was carried out by monitoring quantifier ions at m/z 147 and qualifier ions at m/z 196 and 198 for the phenylboronic derivative of 3-MCPD, while for the phenylboronic derivative of 3-MCPD- d_5 , the quantifier and qualifier ions were monitored at m/z 150 and 201, respectively. In the case of glycidyl esters, quantifier ions at m/z 147 and qualifier ions at m/z 245 were monitored for the phenylboronic derivative of 3-MBPD; while for the phenylboronic derivative of 3-MBPD- d_5 , the quantifier and qualifier ions were monitored at m/z 150 and 245, respectively. The calibration curves for 3-MCPD esters and glycidyl esters showed excellent linearity with a correlation

coefficient (R^2) above 0.999 (Figures 1 and 2). The LOD for bound MCPD and glycidol was 0.05 and 0.10 mg kg⁻¹, respectively, while LOQ for both compounds were \leq 0.20 mg kg⁻¹. Mean recovery was 98.7%-101.8% for 3-MCPD esters and 90.0%-98.9% for glycidol esters with relative standard deviation (RSD) values of less than 10%.

CONCLUSION

Indirect methods are more suitable for routine analyses of bound MCPD and glycidol in oil samples as they require significantly fewer chemical standards as well as simple analytical instruments. The indirect acid transesterification method is

more reliable and robust compared with the alkaline transesterification method for the determination of 3-MCPD esters because these esters tend to degrade under alkaline conditions. The presence of glycidyl esters can result in an overestimation of the 3-MCPD ester content when sodium chloride is used as the salting agent. The AOCS Official Method Cd 29a-13 is an indirect acid-catalysed transesterification method that enables the simultaneous determination of fatty acid esters of 2-MCPD, 3-MCPD and glycidol in edible oils and fats. The method has been validated and it is found to be applicable and suitable for monitoring of these process contaminants in palm oil.

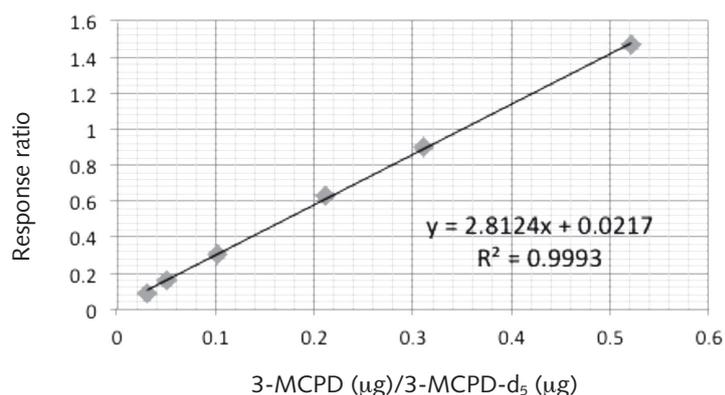


Figure 1. Matrix-based calibration curve of PP-MCPD in a blank sample.

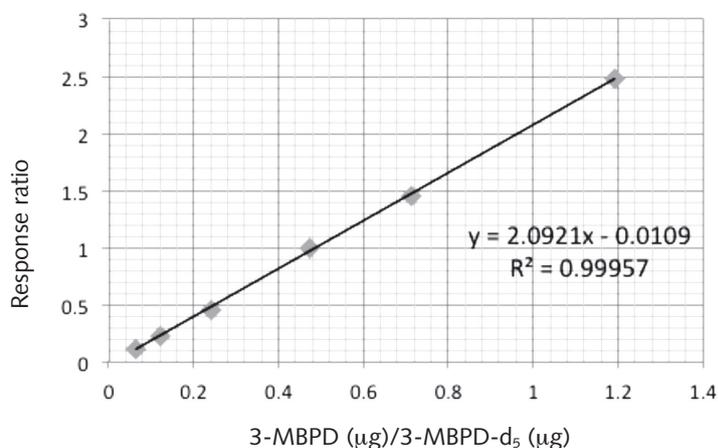


Figure 2. Matrix-based calibration curve of glycidyl palmitate in a blank sample.

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