GERSTEL Application Note No. **191**, *2017*

Fully Automated Determination of 3-MCPD and Glycidol in Edible Oils by GC/MS Based on the Commonly Used Methods ISO 18363-1, AOCS Cd 29c-13, and DGF C-VI 18 (10)

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Keywords

3-MCPD, Glycidol, edible oil, lab automation, ISO 18363-1, AOCS Cd 29c-13, DGF C-VI 18 (10)

Abstract

3-MCPD and Glycidol and especially their fatty acid esters are process contaminants that are formed, for example, when edible oils and fats are refined. At least some of the above-mentioned substances are classified as potential human carcinogens, a fact which has prompted the introduction of rules and regulations that specify tolerable daily intake values and maximum levels in edible oils. Different analytical methods are available for the determination of these compounds. These methods follow two different strategies: Direct determination or, more commonly, indirect determination of the contaminants.

This AppNote describes a solution for fully automated determination of 3-MCPD and Glycidol in edible oils based on the reliable indirect method DGF C-VI 18 (10), similar to the ISO 18363-1 and AOCS Cd 29c-13 methods that are essentially identical. The edible oil sample is divided into two parts (assays A and B). Both are saponified using a Sodiumhydroxymethanol solution, but different quenching methods are used. In assay A, free Glycidol is converted to 3-MCPD using acidic quenching conditions in the presence of chloride. In contrast, for assay B, the quenching reagent is an acidic chloride free salt solution, in which free Glycidol is not converted into 3-MCPD. Following derivatization, the 3-MCPD amounts in both samples are determined by GC/MS as Phenylboronic acid (PBA) esters. Assay B is used to determine the amount of 3-MCPD in the sample while assay A provides the combined amounts of 3-MCPD and Glycidol. The amount of Glycidol is determined as the difference between the assay A and assay B results.

The work presented here involves an automated evaporation step as prescribed in the abovementioned official methods. This ensures that for most matrices, the required limits of detection can be reached using a single quadropole mass spectrometer (MSD). A further important aspect of the evaporation step is that it removes excess derivatization reagent, which could otherwise build up in the GC/MS system and influence system stability.

It is demonstrated that method ISO 18363-1, which is equal to both method AOCS Cd 29c-13 and method DGF C-VI 18 (10), can be automated using the GERSTEL MPS. The obtained results show good correlation with reference data. The excellent standard deviations achieved for the complete sample preparation and analysis workflow speak in favor of automation.

Introduction

3-Monochloropropanediol (3-MCPD), 2-Monochloropropanediol (2-MCPD) and Glycidol are contaminants that are present in a variety of food samples. These compounds are formed in fatty/salty foodstuffs whenever high temperatures are applied during processing. As an example, significant amounts of MCPD- and Glycidol fatty acid esters can be produced in the edible oil refining process, which can be divided into distinct steps as outlined in figure 1.

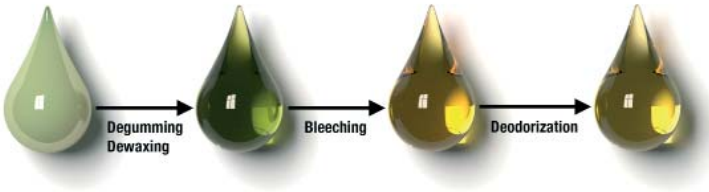

Figure 1. Refining process for production of edible oils.

In refining processes used for edible oil production, the final deodorization step is particularly critical and must be carefully controlled in order to avoid the formation of significant amounts of MCPD and Glycidol. The deodorization step is performed to remove unwanted odors and bittering agents from the oil. Varying the applied temperature during the deodorization process often merely changes the ratio of MCPD ester to Glycidol ester formed, but does not eliminate the formation of these compounds.

While toxicological studies on rats have shown that 3-MCPD causes tumors, the effect of 2-MCPD is less well known. 3-MCPD is labeled as a possible human carcinogen. In contrast Glycidol has already been classified as a probable human carcinogen.

For the determination of 3-MCPD, Glycidol and their esters, several different methods have been published. The two main approaches to determining the esters are the direct method using LC/MS and the indirect methods using GC/MS. The direct method has the disadvantage of having to deal with complex chemical compositions of the esters formed: The fatty acid distribution and the formation of both monoesters and diesters result in a wide variety of MCPD- and Glycidylesters being formed. This means that a lot of individual substances have to be quantified in order to determine the total amount of the contaminants. The situation is further complicated by the fact that quantification standards are unavailable. When looking at the toxicologically relevant part, it should be considered that during the intestinal resorption process, 3-MCPD-esters are split completely into free 3-MCPD. The Glycidol esters are also quantitatively converted to free Glycidol in the human body. For all these reasons, the indirect methods are currently being favored. The indirect methods basically all work according to the same principle. All esters are split into free MCPD and Glycidol, which are derivatized and determined by GC/MS.

In this AppNote a Sample Prep Solution based on the GERSTEL MultiPurpose Sampler is presented that provides completely automated determination of 3-MCPD and Glycidol in edible oils based on the DGF C-VI 18 (10) method, which again is very similar to the ISO 18363-1 and AOCS Cd 29c-13 methods. All of the mentioned methods are based on differential determination of Glycidol and 3-MCPD. The analysis is divided into two assays (A and B). The quenching reaction after the saponification step is the main difference between the two. In assay A, the saponification reaction is stopped by adding an acidic sodium chloride solution. Under these reaction conditions, free Glycidol is converted to 3-MCPD and the combined amounts of 3-MCPD and Glycidol are determined as 3-MCPD. In assay B, the quenching reagent is a chlorine free acidic salt solution. In this case free Glycidol is not converted to 3-MCPD. The 3-MCPD amount in both samples is determined by GC/MS after derivatization with Phenylboronic acid. The amount of Glycidol in the edible oil sample is determined as the difference between the 3-MCPD amounts found in assays A and B, appropriately corrected using the conversion factor.

Experimental

Instrumentation. The automated sample preparation was performed on a GERSTEL MultiPurpose Sampler (MPS robotic, DualHead version). One key module of the solution is the GERSTEL QuickMix, which performs the vigorous shaking required during the liquid/liquid extraction steps. Furthermore the method ISO 18363-1, which is equal to the AOCS Cd 29c-13 method and the DGF C-VI 18 (10) method, requires evaporative concentration of the samples during derivatization. This step is automated using the GERSTEL mVAP and provides the significant added benefit of removing excess derivatization reagent, which could otherwise build up in the GC/MS system and influence system stability. The sample was injected via a Cooled Injection System CIS 4 (GERSTEL) and transferred to the column (Restek Rxi-17 Sil ms, 30 m,

Figure 2. GERSTEL MPS Workstation used for automated sample preparation of edible oils prior to GC/MS determination of 3-MCPD and Glycidol.

d_i = 0.25 mm, d_f = 0.25 µm) using programmed temperature vaporization. For separation and detection a 7890 GC coupled to a 5977 MSD was used (both Agilent Technologies).

Materials. 3-MCPD-d5-1,2-bis-palmitoylester, 3-MCPD-1,2-bis-palmitoylester, 3-MCPD, Glycidyl stearate, Sodium hydroxide in Methanol- Solution, Acetic NaBr-Solution (600 g/L), Acetic NaCl-Solution (600 g/L), Phenylboronic acid, MTBE/EtAc-Solution (3/2 v/v), Hexane, Isooctane, Water, Acetone, Toluene

Sample Preparation. One analysis is comprised of two assays (A and B). For each assay, 100 mg of oil are weighed in 4 mL screw cap vials and placed onto the MPS. After adding 250 µL of MTBE and 100 µL ISTD-solution, the sample is shaken vigorously in the GERSTEL QuickMix module. For the saponification of MCPD- and Glycidyl esters, 350 µL of a MeOH/KOH solution is added. The sample is shaken slowly for 10 minutes. The assay A reaction is quenched with 600 µL of acidic NaCl Solution while a chlorine free NaBr solution is used for assay B to avoid the formation of additional MCPD from Glycidol.

The following preparation steps are similar for both assays. After the addition of 600 µL Hexane, the sample is vigorously shaken and incubated for 10 minutes. The sample is again shaken vigorously and the organic Hexane layer is dispensed to waste. This step is repeated twice to remove matrix. Free 3-MCPD is extracted by 600 µL MTBE/EtAc (3/2 v/v). The extract is collected in a new 2 mL vial pre- filled with Sodium sulfate as drying agent. After adding 30 µL of Phenylboronic acid the sample is evaporated to dryness in the GERSTEL mVap module. The Phenylboronic acid derivates are redissolved in Isooctane and transferred to a new vial with µ-vial insert ready for injection. The fact that Phenylboronic acid is not very soluble in Isooctane helps reduce the amount of derivatization agent injected. The evaporation step is therefore used both to increase the sensitivity of the analysis and also to remove excess Phenylboronic acid in order to protect the MSD.

Analysis conditions.

MPS:	3 µL injection volume
PTV:	baffled liner, deactivated solvent vent 40°C (0 min); 12°C/s; 300°C (5 min)
Column:	30 m Rxi-17 sil ms (Restek) d_i = 0.25 mm d_f = 0.25 µm
Pneumatics:	He, constant flow = 1 mL/min
Oven:	50°C (2 min); 10°C/min; 200°C (0 min) 20°C/min; 300°C (5 min)
MSD:	Selected ion monitoring SIM 3-MCPD: 196/198/147 amu 3-MCPD-d5: 201/203/150 amu

Results and Discussion

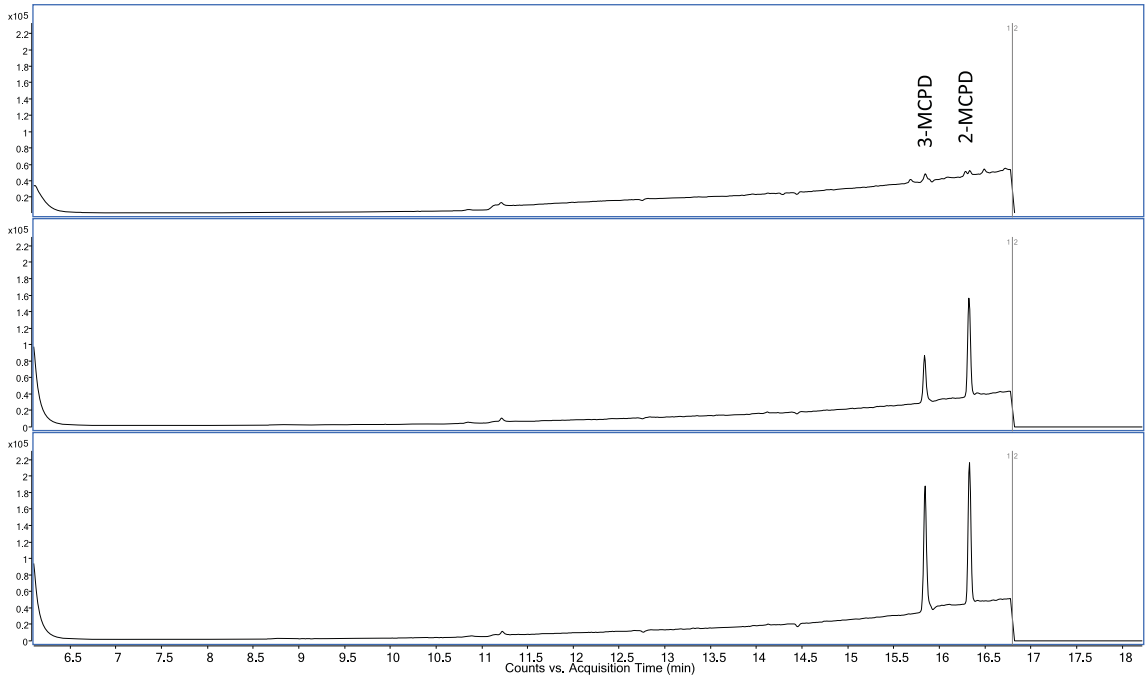

Figure 3. SIM-Chromatogram m/z 198 : Top: Virgin olive oil used as blank oil. Middle: Edible oil sample assay B (3-MCPD). Bottom: Edible oil sample assay A (3-MCPD + Glycidol).

The first step for determination of 3-MCPD and Glycidol based on the indirect ISO 18363-1, AOCS Cd 29c-13, or DGF C-VI 18 (10) methods is to evaluate the efficiency of the conversion from Glycidol to 3-MCPD following the method used for Assay A. Figure 4 shows the amount of 3-MCPD formed as a function of the amount of Glycidol (in the form of Glycidyl stearate) in a spiked blank oil at five different levels. A linear regression of the type y = mx + b is performed, the reciprocal slope (1 / m) provides the conversion factor (t).

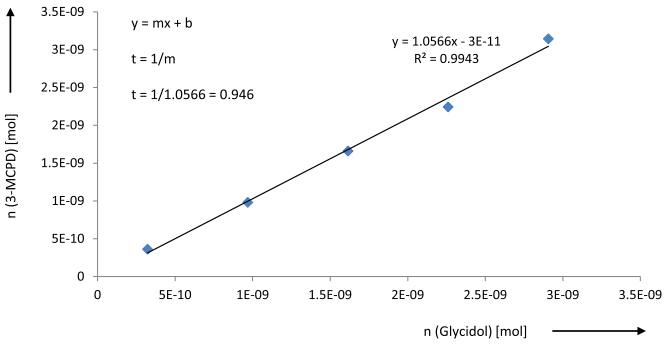

Figure 4. The amount of 3-MCPD formed as a function of the amount of Glycidol at five different levels. A linear regression of the type y = mx + b is performed, the reciprocal value of the slope (1 / m) provides the conversion factor (t).

The linearity of the method was verified by analyzing virgin olive oil spiked at five different levels. This was performed for both assays. In figure 5, the excellent linearity (R^2> 0.9998) achieved for both assays from 0.12 – 1.9 mg/kg is shown.

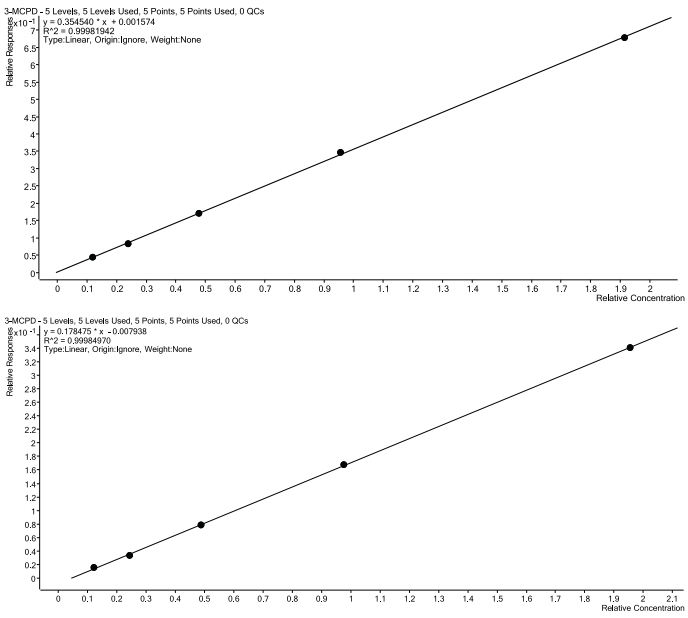

Figure 5. Linearity study for 3-MCPD assay B (top) and Glycidyl assay A (bottom), 0.12-1.9 mg/kg each.

Three different edible vegetable oil samples were analyzed and the results compared with the provided reference values. These kind of edible oils do not produce large amounts of 3-MCPD- and Glycidyl esters. Therefore they are in the low level range for

3-MCPD and Glycidol contamination. Table 1 shows the results from assay B, listing the amount of 3-MCPD determined in three different edible oil samples as well as the reference values.

Table 1. 3-MCPD amount found in three different edible oils in mg/kg.

3-MCPD	Amount [mg/kg]	
	Reference	Automated
Oil 1	0.77	0.68
Oil 2	0.68	0.63
Oil 3	0.27	0.29

For a given edible oil sample, the difference between the results for assays A and B multiplied by the previously determined conversion factor is used to calculate the amount of Glycidol in the sample. In table 2, the amounts obtained using this method are listed along with reference values.

Table 2. Glycidol amount found in three different edible oils in mg/kg.

Glycidol	Amount [mg/kg]	
	Reference	Automated
Oil 1	0.14	0.18
Oil 2	0.44	0.48
Oil 3	0.11	0.09

To demonstrate the good repeatability of the automated sample preparation method, five samples of the same edible oil were analyzed undergoing individual sample preparation and analysis. Table 3 shows the repeatability based on the entire sample preparation procedure and the subsequent GC/MS analysis.

Table 3. Repeatability for 3-MCPD and Glycidol (n=5 samples).

#	Amount [mg/kg]	
	3-MCPD	Glycidol
1	0.72	0.54
2	0.63	0.53
3	0.66	0.49
4	0.69	0.50
5	0.68	0.57
Mean	0.68	0.52
SD	0.03	0.03
RSD %	5.00	6.44

For 3-MCPD, a relative standard deviation of 5 % was calculated. The relative standard deviation for the amount of Glycidol is 6.44 %.

Conclusions

In this work, we have shown that method ISO 18363-1 can be automated using the GERSTEL MPS and that the results obtained correlate well with reference data. This method is similar to two other frequently used methods: AOCS Cd 29c-13 and DGS C-VI 18 (10). The excellent relative standard deviations achieved for the complete process including GC/MS analysis speak in favor of the presented automation solution.

The work presented here involves an automated evaporation step as prescribed in the abovementioned official methods. This ensures that for most matrices, the required limits of detection can be reached using a single quadropole mass spectrometer (MSD). A further important aspect of the evaporation step is that it removes excess derivatization reagent, which could otherwise build up in the GC/MS system and influence system stability.

Outlook

The described automation steps are not limited to the presented method. Such methods have alredy been tested for derivatization methods like the recently presented 3 in 1 approach, and can be adapted for that method with similar performance. The presented method has the advantage of being able to analyze a sample for Glycidol, 3-Monochloropropanediol (3-MCPD) and additionally 2-Monochloropropanediol (2-MCPD), all in a single run.

In addition to extracting and determining MCPD and Glycidol esters, the described automation platform can also extract and determine PAHs from edible oils using automated solid phase extraction combined with GC/MS determination.

Literature

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[2] DGF C-VI 18 (10)
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[4] ISO 18363-1:2015 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

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