Fully Automated Determination of 3-MCPD, 2-MCPD and Glycidol in Edible Oils by GC/MS Based on the Commonly Used Methods ISO 18363-1, AOCS Cd 29(a,b,c)-13, and DGF C-VI 18

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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, in particular during deodorization process. At least some of the above-mentioned substances are classified as potential human carcinogen/genotoxic, 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 poster describes a solution for fully automated determination of 3-MCPD and Glycidol in edible oils based on the reliable indirect methods AOCS Cd 29(a&c)-13. The version "b" can be as well automated on several steps, with the exclusion of cooled saponification. With particular regards to version "c", which is deeply described in this work, the edible oil sample is divided into two parts (assays A and B). Both are saponified using a Sodium methoxide methanolic 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 even using a single quadrupole mass spectrometer, instead of a triple Quad. 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.

Keywords

3-MCPD, Glycidol, edible oil, lab automation, AOCS Cd 29c-13

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.

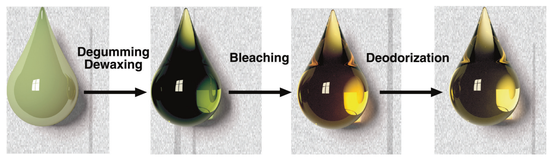

Figure 1. Refining process for production of edible oils.

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.

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 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 (2&3)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, all the aforementioned esters are split completely into related free (2&3)MCPD&glycidol. For all these reasons, the indirect methods are currently being favored. All these indirect methods basically work according to the same principle: all esters are coverted into free MCPD and Glycidol, which are derivatized and determined by GC/MS.

Experimental[1]

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 AOCS Cd 29(a,b,c)-13 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, dj = 0.25 mm, df = 0.25 µm) using programmed temperature vaporization. For separation and detection a 7890B GC coupled to a 5977B MSD was used (both Agilent Technologies).

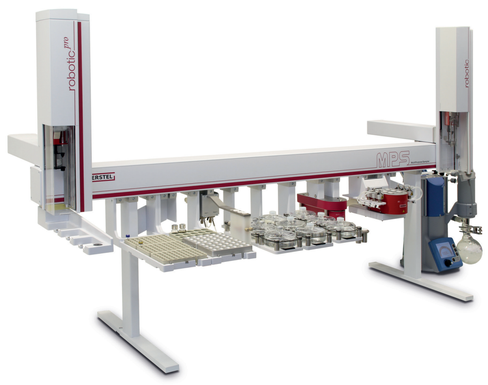

Figure 2. GERSTEL MPS Workstation used for automated sample preparation of edible oils prior to GC/MS determination of 3-MCPD and Glycidol.

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 consists 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 tray. 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 4 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.

The subsequent preparation steps are similar for both assays. After the addition of 600 µl Hexane, the sample is vigorously shaken and incubated for 10 minutes; is then dispensed the organic Hexane layer is dispensed to waste. This step is repeated twice to remove matrix. Free MCPD&-Glycidol are extracted twice with 600 µl MTBE/EtAc (3/2 v/v) and 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 re-dissolved in isooctane and transferred to a new vial with ready for injection. The fact that Phenylboronic acid is not very soluble in isooctane helps to reduce the amount of derivatization agent injected. The evaporation step is therefore used both to increase the sensitivity of the analysis and to remove excess Phenylboronic acid in order to protect the MSD.

[1]Related to Method "c"

Analysis conditions		
MPS	3 µL injection volume	
CIS	baffled liner, deactivated solvent vent 40ºC; 12ºC/s; 300ºC (5 min)	
Column:	30 m Rxi-17 sil ms (Restek)	
	d_1= 0,25 mm	d_1= 0,25 µm
Pneumatics:	He, constant tlow = 1.0 ml	
Oven:	50ºC (2 min); 10ºC/min; 200ºC (0 min) 20ºC/min; 300ºC (5 min)	
MSD:	Selected ion monitoring SIM:	
	3-MCPD	196/198/147 amu
	3-MCPD-d5:	201/203/150 amu

Results and Discussion

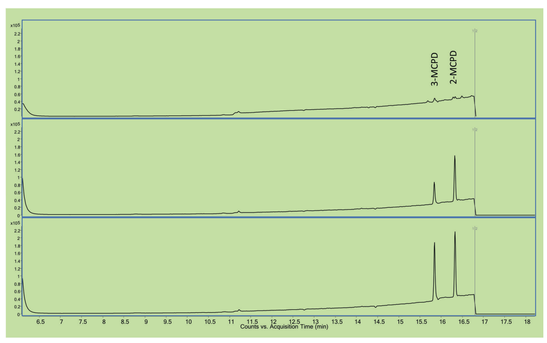

Figure 3. SIM-Chromatogram m/z 198: Top: Virgin olive oil used as blank oil. Middle: Edible oil sample assay B (3-MCPD). Bottom: Edible oil sample assay A (3-MCPD + Glycidol).

The first step for determination of 3-MCPD and Glycidol based on the indirect AOCS Cd 29c-13 method is to evaluate the efficiency of the conversion from Glycidol to 3-MCPD following the reaction path 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).

Using the same hardware, it is possible to automate also the version "a", while version "b" can be partially automated (the cooled saponification needs to be carried out offline).

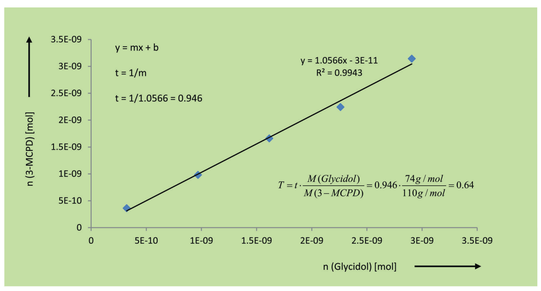

Figure 4. The amount of 3-MCPD formed as a function of the amount of Glycidol at five different levels. A linear regression of the type y = mx + b is performed, the reciprocal value of the slope (1/m) provides the conversion factor {t}.

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.

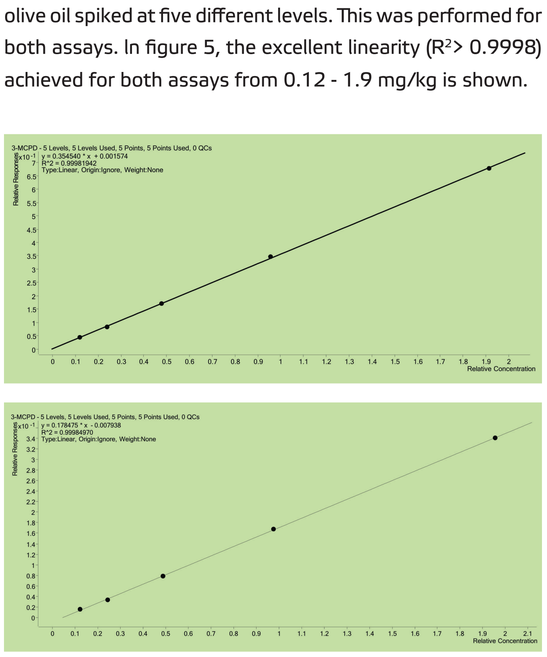

Figure 5. Linearity study for 3-MCPD assay B {top} and Glycidyl assay A {bottom}, 0.12-1.9 mg/kg each.

Three different edible vegetable oil samples were analyzed and the results compared with the provided reference values. These kinds 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	0.12
Oil 2	0.44	0.31
Oil 3	0.11	0.06

To demonstrate the good repeatability of the automated sample preparation method, five samples of the same edible oil were analyzed. 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	0.34
2	0.63	0.34
3	0.66	0.31
4	0.69	0.32
5	0.68	0.37
Mean	0.68	0.33
SD	0.03	0.02
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 AOCS Cd 29c-13 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: ISO 18363-1and 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 quadrupole 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.

References

[1] http://www.bfr.bund.de/de/fragen und antworten zur kontamination van lebensmitteln mit 3 mcpd 2 mcpd und glycidyl fettsaeureestern-10 538.html

[2] DGF C-VI 18 (10)

[3] AOCS Official Methods Cd 29(a&b&c)-13

[4] ISO 18363-1 :2015