Determination of 3-MCPD & Glycidol in Edible Oils & Fats via Automated GERSTEL Multipurpose Sampler Based on AOCS Cd 29a-13 Method

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ABSTRACT

The objective of this note focuses on the fully automated solution of the determination of 3-monochloropropanediol (3-MCPD) and glycidol in edible oils based on the AOCS Cd 29a-13 methods. This method is also applicable for the determination of 2-monocloropropanediol (2-MCPD).

The glycidyl esters first react with acidified sodium bromide to form its 3-monobromopropanediol (3-MBPD) ester form. Following which, 3-MBPD, 2-MCPD and 3-MCPD esters undergo acidic transesterification to release to their respective free forms. Following derivatisation, the derivatives of each these compounds are analysed. The fully automated method is then subsequently applied to refined bleached deodorized palm oil samples to determine the levels of these contaminants in this study. It shows good correlation with the reference manual method under the validation with the statistical t-test – there is no significant difference.

The use of the automation preparation simplifies the analysis of 3-MCPD and glycidol, which can be applied without the need for multiple specialised technicians and yet optimising the sample throughput. In addition, automation also minimises the possibility of any human error during sample preparation and allows shorter analysis time to be attained.

INTRODUCTION

3-monochloropropanediol (3-MCPD), 2-monocloropropanediol (2-MCPD) and glycidol have particularly gained attention recently as contaminants present in the food sample. These compounds are usually formed in food samples whenever high temperatures are applied during processing in the presence of chloride ions. As an example, significant amounts of MCPD fatty acid esters and glycidyl fatty acid esters (GE) are notably formed in the refining process of the edible oil, which can be highlighted in the different stages as outlined in Figure 1, especially so in the final deodorisation step where unwanted odors and bittering agents are removed from the oil sample. They are subsequently released as free forms in the human body.

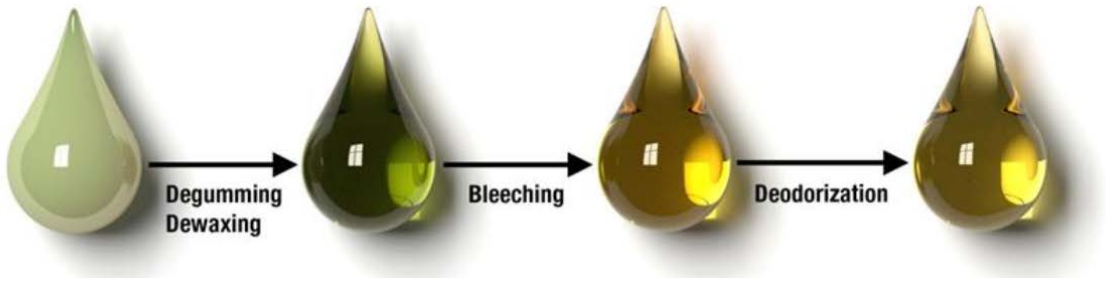

Figure 1. Refining process for production of edible oils

It has been demonstrated that 3-MCPD can cause tumors based on the toxicological studies on rats, and has been labelled as a possible human carcinogen. Even though the effect of 2-MCPD is less well-known, it can be evaluated with the current method should the need arise to study its effects. Glycidol, on the other hand, has already been classified as a probable human carcinogen.

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 AOCS Cd 29a-13.

GE are converted to 3-MBPD monoesters in an acid solution containing a bromide salt. 3-MBPD esters, together with 2- and 3- MCPD esters, are then converted into the free (non-esterified) form in acid methanolic solution. The fatty acid methyl esters generated during the reaction are extracted from the sample; and 2- and 3-MCPD as well as 3-MBPD, are then derivatised with phenylboronic acid prior to GC-MS analysis (see Figure 2).

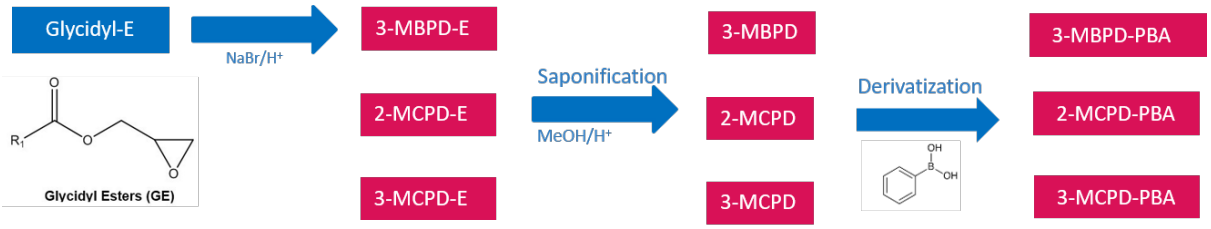

Figure 2. Overview of the mechanism in AOCS Method Cd 29a-13

EXPERIMENTAL

The automated sample preparation is performed on a Gerstel MultiPurpose Sampler (MPS robotic, DualHead version). The configuration is illustrated as in Figure 3. One of the key modules of the solution includes the GERSTEL Quickmix, which allows the vigorous shaking during the liquid-liquid extraction steps. The work presented here also incorporates an automated evaporation step (with the use of mVAP) as highlighted in the official AOCS method. A sonicator bath is also included in this automated platform to allow the derivatisation of the 3-MCPD and 3-MBPD at room temperature. The sample is then injected and transferred to the column. For

separation and detection, a 7890 GC coupled to a 5977 MSD is used (Agilent Technologies).

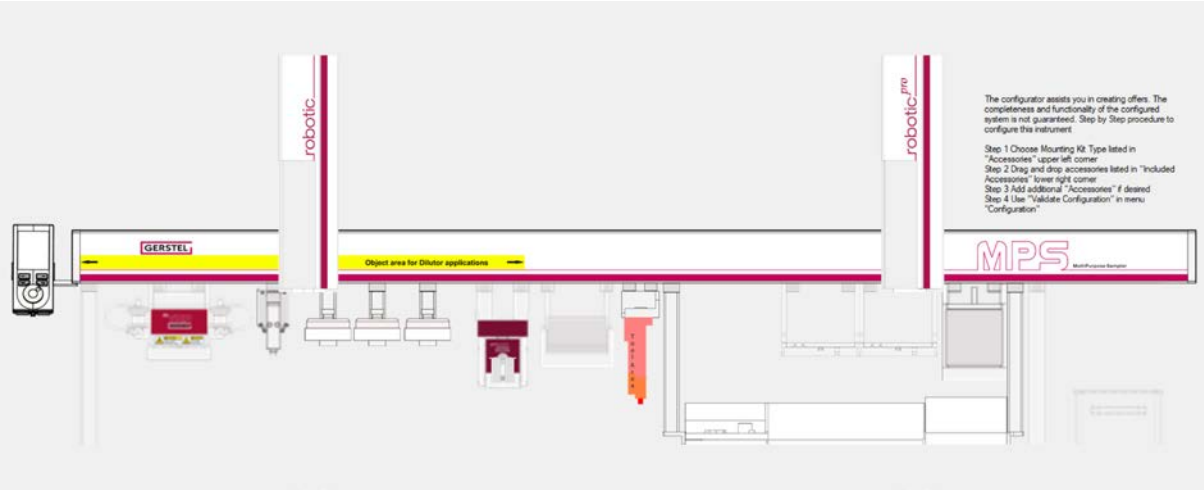

Figure 3. Overview of the configuration for AOCS Method Cd 29a-13

Materials

Tetrahydrofuran, Methanol, n-Heptane, Acetone, Toluene, Water, Sulfuric acid, Sodium hydrogen carbonate, Sodium sulfate, Phenylboronic acid, Sodium bromide, 1,2-Dipalmitoyl-3-chloropropanediol (PP-3-MCPD), Pentadeuterated 1,2-dipalmitoyl-3-chloropropanediol (PP-3MCPD-d5), Glycidyl palmitate (Gly-P), Pentadeuterated glycidyl palmitate (Gly-P-d5)

Sample Preparation

100 mg of oil/fat was weighed in the 10 mL screw cap vial and placed onto the MPS. After adding 50 µL of internal standard solutions and 2 mL of tetrahydrofuran, the sample was shaken vigorously in the Gerstel QuickMix module. For the conversion of the GE to 3-MBPD ester, 30 µL of acid aqueous solution of sodium bromide was added and the sample was shaken vigorously and incubated at 50°C. The reaction was then quenched by the addition of 3 mL of 0.6% aqueous solution of sodium hydrogen carbonate. In order to separate the oil/fat from the water phase, 2 mL of n-heptane was added and the sample was once again shaked vigorously. Upon separation of the two phases, the upper layer was then transferred to an empty 10 mL screw cap vial and evaporated to dryness with the use of mVAP. The residue was re-dissolved in 1 mL of tetrahydrofuran.

For the saponification of 3-MCPD- and 3-MBPD esters, 1.8 mL of MeOH/H_2SO_4 was added. The sample was shaken vigorously and incubated in the cool stack tray at 40°C for 16 hours. After incubation, the reaction was quenched by the addition of 0.5 mL of sodium hydrogen carbonate saturated solution. The sample was shaken once again and evaporate till 1 ml of the mixture was left in the vial. 2 mL of sodium sulfate solution and 2 mL of n-heptane were added and the mixture was shaken and two

layers of phases were formed. The upper phase which contained fatty acid methyl esters was discarded and the extraction was repeated again with n-heptane. 250 µL of phenylboronic acid solution was added to the final solution and then brought to incubation for 5 min in an ultrasonic bath at room temperature. The phenylboronic derivatives of 2- and 3-MCPD as well as 3-MBPD were next extracted with the addition 1 mL of n-heptane, shaking of the mixture and transferring the upper phase to an empty customised 10 mL vial (see Figure 4).

The extract was evaporated under vacuum until 400 µL of n-heptane solvent remained in the vial before injection into the system. The 3-MCPD and glycidol amounts in the sample were then determined by the GC-MS system (see Table 1 for analysis conditions).

Figure 4: Customised 10 mL sample vial

Table 1: Analysis conditions

MPS:	1 µL injection volume
Injection mode:	Pulsed Splitless at 250°C
Column:	Supelco Equity-1, 30 m length × 0.25 mm i.d. × 1.0 µm film Thickness
Pneumatics:	He, constant flow = =0.8 ml /min
Oven:	80°C (1 min), from 80°C to 170°C at 10°C/min, from 170°C to 200°C at 3°C/min, from 200°C to 300°C at 15°C/min, 15 min at 300°C
MSD:	Transfer line temperature: 300°C Ion source temperature: 230°C Quadrupole temperature: 150°C Ionisation mode: EI, SIM mode Parameters for SIM mode: (i) phenylboronic derivative of 3-MCPD (*m/z*) 147 (quantifier ion); 196, 198 (qualifier ions); (ii) phenylboronic derivative of 3-MCPD-d5 (*m/z*) 150 (quantifier ion for 3-MCPD); 201 (quantifier ion for -MCPD); 203 (qualifier ion); (iii) phenylboronic derivative of 3-MBPD (*m/z*) 147 (quantifier ion); 240 (qualifier ion); (iv) phenylboronic derivative of 3-MBPD-d5 (*m/z*) 150 (quantifier ion); 245 (qualifier ion).

RESULTS AND DISCUSSION

The linearity of the method was assessed by analysing blank olive oil samples spiked at eight different levels. It could be observed that excellent linearities of R^2 values of 0.9992 and 0.9999 were obtained for both 3-MCPD and Glycidol, as shown in Figures 5 and 6, respectively.

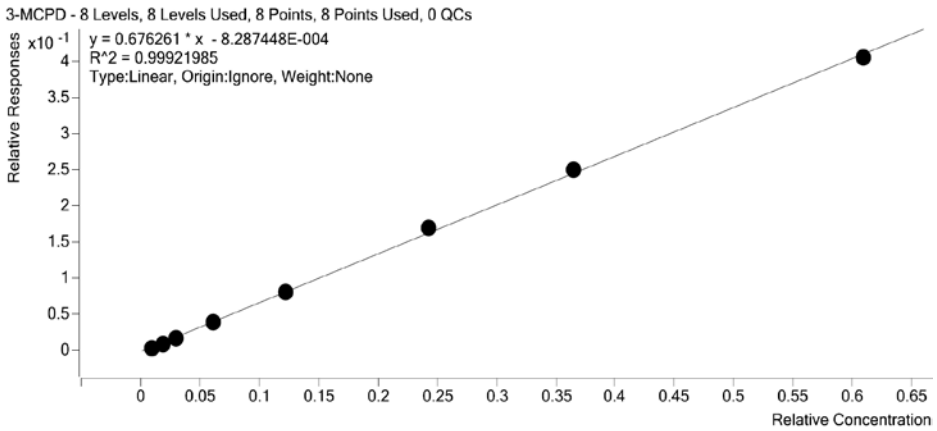

Figure 5. Calibration curve of 3-MCPD

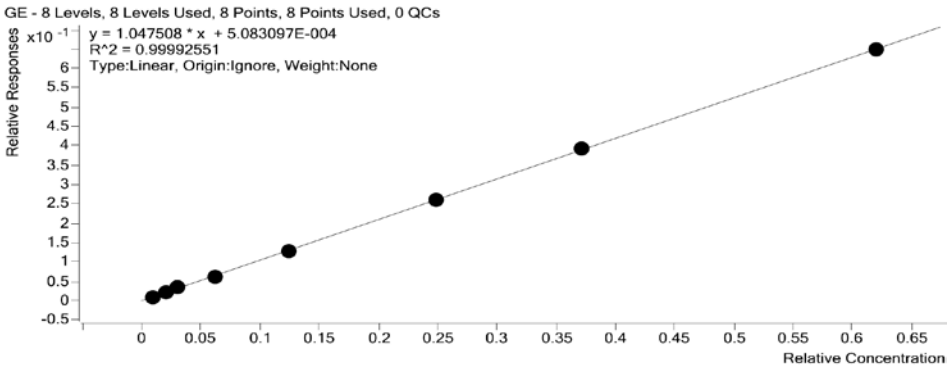

Figure 6. Calibration curve of Glycidol

The subsequent method validation parameters were evaluated with blank olive oil samples. Eight replicates, spiked with 0.07 mg/kg of 3-MCPD and glycidol each, were ran and the standard deviations (SD) were calculated. The limits of detection (LODs) were determined by 3 times of SD, and the LODs were calculated as 0.037 mg/kg and 0.040 mg/kg, respectively (see Table 2).

Table 2: LODs for 3-MCPD and Glycidol

	3-MCPD (mg/kg)	Glycidol (mg/kg)
Spiked amount	0.07	0.07
1	0.0617	0.0458
2	0.0740	0.0740
3	0.0492	0.0779
4	0.0844	0.0560
5	0.0813	0.0612
6	0.0571	0.0562
7	0.0726	0.0418
8	0.0620	0.0736
Mean	0.068	0.061
Standard Deviation	0.012	0.013
LOD	0.037	0.040

As for the recoveries, known amounts of 3-MCPD and glycidol were spiked into the blank olive oil samples. Good recoveries of 96.0% and 87.8% were obtained for 3-MCPD and glycidol, respectively (see Table 3).

Table 3: Recovery results obtained

	3-MCPD (mg/kg)	Glycidol (mg/kg)
Spiked Amount	2.00	2.22
1	111.5	87.8
2	98.0	101.8
3	87.5	77.9
4	97.0	86.5
5	103.5	90.1
6	86.0	88.7
7	88.5	82.0
Mean	96.0	87.8

To demonstrate the good repeatability of the automated method, known amounts of 3-MCPD and glycidol were spiked into refined bleached deodorized palm oil samples and sample preparation were conducted. For 3-MCPD, a relative standard deviation (RSD) of 9.30 % was calculated while for glycidol, it was 12.76%. Table 4 shows the repeatability based on the entire sample preparation and the GC-MS analysis.

Table 4: Repeatability results obtained for 3-MCPD and Glycidol

	3-MCPD (mg/kg)	Glycidol (mg/kg)
Known amount	0.07	0.28
1	0.0645	0.2160
2	0.0569	0.2490
3	0.0665	0.2610
4	0.0672	0.2520
5	0.0764	0.2160
6	0.0718	0.2080
7	0.0592	0.1820
8	0.0633	0.1940
9	0.0708	0.1990
Mean	0.066	0.220
SD	0.006	0.028
RSD	9.30	12.76

To further evaluate the automated and manual method, different palm oil samples were analysed (see Table 5). In order to decide whether the differences between those two methods can be accounted, a statistical test (significance test) were employed to evaluate the experimental results for the samples tested; T-test paired two samples for the means was carried out to identify if there is significant difference between the manual and automated methods. It was observed that for both 3-MCPD and Glycidol, there were no significant difference since the calculated t-value is smaller than t-critical, hence indicating the successful transfer of the manual method AOCS Cd29a-13 to the automated platform.

Table 5: Real palm oil samples analysis

	Concentration of 3-MCPD (mg/kg)		Concentration of Glycidol (mg/kg)	
	Automated	Manual	Automated	Manual
Palm Oil 1	1.71	1.70	0.11	0.12
Palm Oil 2	0.27	0.30	0.17	0.17
Palm Oil 3	1.89	1.80	0.20	0.22

CONCLUSIONS

In this work, the method is automated with strict adherence to the AOCS method A, especially with the incorporation of the automated evaporation and ultrasonication steps as described in the official methods. The evaporation step also removes the excess derivatisation reagent, which could otherwise accumulate in the GC-MS system and affect the system stability. The automated results obtained correlate well with the reference manual method as proven by the use of the statistical t-test – there is no significant difference. In addition, the present method also allows the analysis of Glycidol, 3-MCPD and 2-MCPD in a single run, where needed. Good relative standard deviations were achieved for the complete sample preparation and analysis workflow.

ACKNOWLEDGEMENTS

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