SHIMADZU
Excellence in Science

Application News

Gas Chromatograph Tandem Mass Spectrometer GCMS-TQ™8050 NX

Analysis of 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Palm Oil Using GC-MS/MS in Accordance with AOCS Method Cd 29c-13

Elvi Horiyanto[1], Ang May Yen[2], Cynthia Lahey[1]
1 Shimadzu (Asia Pacific) Pte Ltd 2 Shimadzu Malaysia Sdn Bhd

User Benefits

- Sample preparation workflow in accordance with AOCS Cd 29c-13 Method
- Highly sensitive 3-MCPD in palm oil analysis with LOQ of 20 ppb and calculated LOD of 6 ppb
- Backflush system to discharge high boiling substances from the column

■ Introduction

Fatty-acid-bound 3–monochloropropane-1,2-diol or known as ester-bound 3-monochloropropanediol (3-MCPD fatty acid esters) and glycidyl fatty acid ester (GE) are processed contaminants generated mainly during the refining of edible oil.[1] During the oil refining, often high temperature is required which causes 3-MCPD (fatty acid esters) and GE to be formed as by-products.

Both 3-MCPD (fatty acid esters) and GE are possibly carcinogenic which has raised concern to human health if consumed at high levels. European Commission has recently amended Regulation (EC) No. 1881/2006 which regards to the maximum levels of 3-MCPD (fatty acid esters) and GE. The sum of 3-MCPD (fatty acid esters) expressed in 3-MCPD for edible oil should not exceed 1,250 µg/kg for oils and fats produced from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils (composed of refined olive oil and virgin olive oil) and mixtures of oils and fats with oils and fats from this category only.[2] Meanwhile, the amount of GE expressed in glycidol should not exceed 1,000 µg/kg from oils and fats produced from vegetables, fish, and other marine organism.[2] Among these, palm oil has the highest content of 3-MCPD (fatty acid esters) and GE due to the nature of refining process that require high temperature in the deodorization stage.

Following this regulation, AOCS has developed official methods, namely Cd 29a-13, Cd 29b-13 and Cd 29c-13 to quantify 3-MCPD (fatty acid esters) and GE in edible oils and fats. In this application news, an MRM method using Shimadzu GCMS-TQ8050™ NX was established and evaluated for quantitative determination of both 3-MCPD (fatty acid esters) and glycidol esters in palm oil expressed in their free-forms following Cd 29c-13 method. Furthermore, reproducibility of the method was also evaluated.

■ Experimental

Instrumental and Analytical Conditions

A triple quadrupole GC-MS/MS system, GCMS-TQ8050™ NX, as shown in Figure 1, (Shimadzu Corporation, Japan) with backflush system was employed in this work. The details of the instrumentation and analytical conditions for an MRM method are shown in Table 1. An MRM method was created using the *Shimadzu MRM Optimization Tool and Smart Database*. The details of the MRM transitions, collision energy (CE) values of the target compounds analyzed are shown in Table 2.

Figure 1: Shimadzu GCMS-TQ8050 NX with AOC-20i+s Plus liquid auto-sampler

Table 1. GC-MS/MS instrumentation & analytical conditions

Instrumentation	
GC-MS/MS system	GCMS-TQ8050 NX
Auto Injector	AOC-20i+s Plus
Column	SH-I-17Sil MS (P/N 221-75916-30) (30 m x 0.25 mmID x 0.25 µm df)
Additional Accessories	Shimadzu Backflush System
Software	GCMSsolution
Gas Chromatograph	
Carrier Gas	Helium
Injection Condition	250 °C, Splitless Mode
Sampling Time	1 min
Flow Control Mode	Constant Pressure
Injection Pressure Program	130.80 kPa (17.20 min)* → -400 kPa/min to 30 kPa (12.70 min)
Total Flow	50 mL/min
Purge Flow	3 mL/min
Oven Temp. Program	85 °C (1 min) → 6 °C/min to 150 °C → 12 °C/min to 210 °C (2 min) → 30 °C/min to 280 °C (9 min)
APC1 Pressure (Backflush)	30 kPa (17.20 min)* → 400 kPa/min to 130 kPa (12.65 min)
Mass Spectrometer	
Ion Source Temp.	230 °C
Interface Temp.	300 °C
Solvent Cut Time	10 min

*The time must be modified accordingly when retention times of the target compounds shift. The injection and APC1 pressure programs were set to facilitate backflush.

Table 2. MRM transitions

	Target Transition	CE	Ref 1 Transition	CE	Ref 2 Transition	CE
3-MCPD-d5	201.0 > 150.2	10	150.0 > 93.1	15	201.0 > 93.2	30
3-MCPD	196.0 > 147.1	9	196.0 > 91.1	24		

Standards and Sample Preparation

The sample preparation in Cd 29c-13 method can be performed much faster than those of Cd 29a-13 and Cd 29b-13 methods. Hence, it is a preferred method when fast result is required. In Cd 29c-13, two different assays were described, namely Assay A (ester cleavage in the presence of chloride ion) and Assay B (ester cleavage in the absence of chloride ion). In Assay A, free glycidol was converted into free 3-MCPD; therefore, the final amount of 3-MCPD is a sum of both original free 3-MCPD and converted free glycidol. For Assay B, the free glycidol was not converted into free 3-MCPD. Hence, Assay B contained both 3-MCPD and glycidol. Both Assay A and Assay B were derivatized before analysis to GCMS. Assay B was used to calculate the amount of 3-MCPD. Meanwhile the differential measurement of Assay A and Assay B multiplied by the transformation factor of glycidol to 3-MCPD will determine the amount of glycidol esters. Refer to Figure 2 for the analysis flowchart. In addition, Table 3 summarizes the sample preparation assays to be performed for each standard and unknown sample, while Figure 3 describes the workflow of sample preparation in accordance with Cd 29c-13 method.

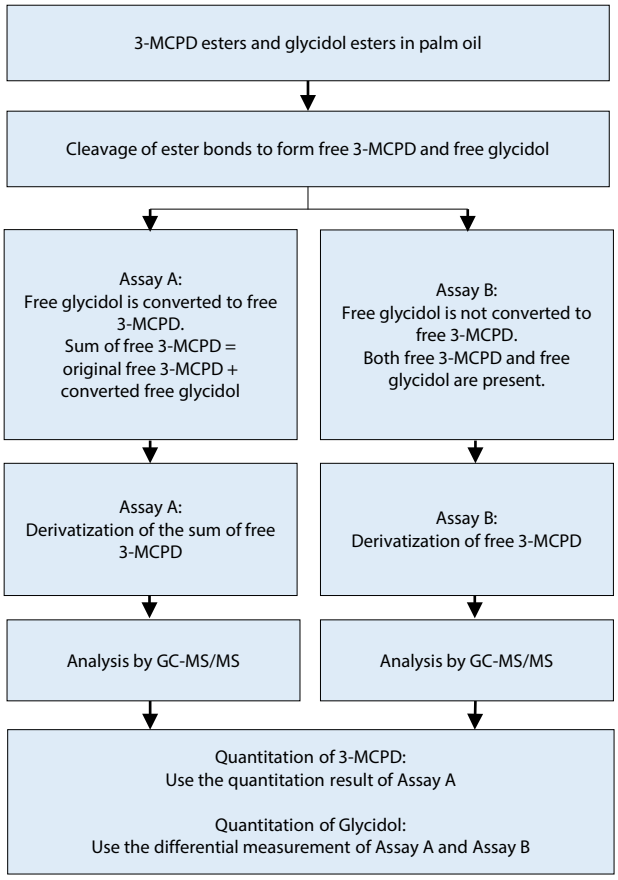

Figure 1. Analysis Flowchart

Table 3. Summary of the sample preparation assays to be performed for each standard and unknown sample

Sample type	Target compounds	Assay(s) to be performed	Purpose
Std	3-MCPD	Assay B	To plot 3-MCPD calibration curve
Std	Glycidol	Assay A	To plot glycidol calibration curve expressed in 3-MCPD for transformation factor determination
Unk	3-MCPD & Glycidol	Assay A & B	To quantify 3-MCPD and glycidol

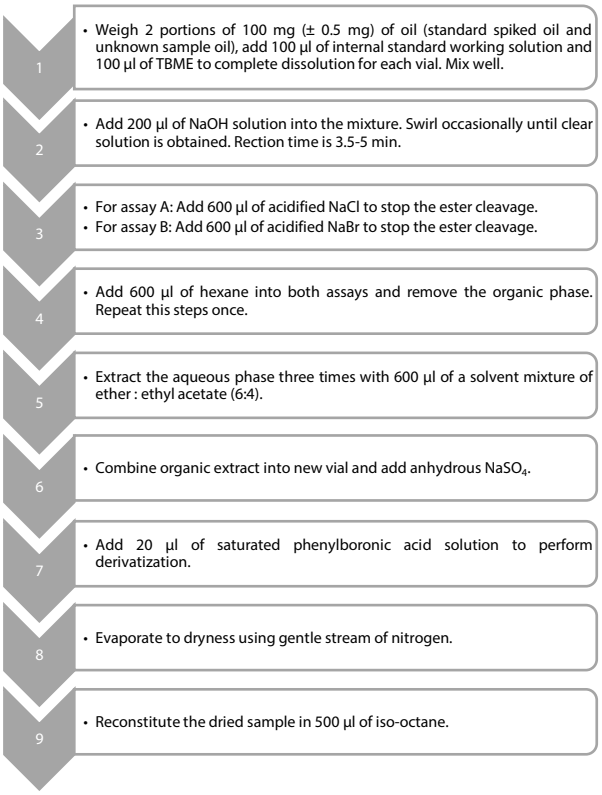

Figure 3. Workflow of sample preparation according with AOCS method Cd 29c-13

In Cd 29c-13 method, only one-point calibration standard was specified. To improve the accuracy, modification to prepare 5-point calibration levels was carried out. For the preparation of 3-MCPD internal standard calibration solutions, oil blank sample was spiked with ester bound 3-MCPD (for this application news we used rac-1,2-Bis-palmitoyl-3-chloropropanediol). The ester was spiked in such a way that after the ester cleavage step, the known amount of 3-MCPD in the calibration solutions are 0.02, 0.05, 0.25, 0.5 and 1.0 µg/g. The internal standard used in this application news was rac-1,2-Bis-palmitoyl-3-chloropropanediol-d5 which was added in the first step of sample preparation described in Figure 3 such that 0.25 µg/g of 3-MCPD-d5 are in the final solutions. The 3-MCPD spiked oil was subjected to the workflow in Figure 2, according to Assay B sample preparation and injected into the GC-MS/MS for analysis.

In order to quantitate glycidol amount in sample oil, glycidol to 3-MCPD transformation factor has to be determined. Oil blank sample was spiked with glycidyl fatty acid ester (for this application news we used glycidyl palmitate). The known amount of glycidol in the calibration solutions are 0.5, 1.0, and 2.0 µg/g after the ester cleavage step. The ester bound glycidol spiked oils were subjected to the workflow in Figure 2, according to Assay A sample preparation and injected into the GC-MS/MS for analysis.

To determine the reproducibility, 0.02 µg/g of both 3-MCPD and glycidol spiked oil were subjected to both Assay A and B sample preparation. Samples were aliquot into 4 portions to be analysed in 4 different days within 2 weeks.

Shimadzu Backflush System

To minimize column contamination of high boiling substances from the oil matrix, Shimadzu Backflush System was employed in the instrument setup. The backflush system discharged these substances from the column by reversing the carrier gas flow and flushing them out of the injection port after target compounds were detected.

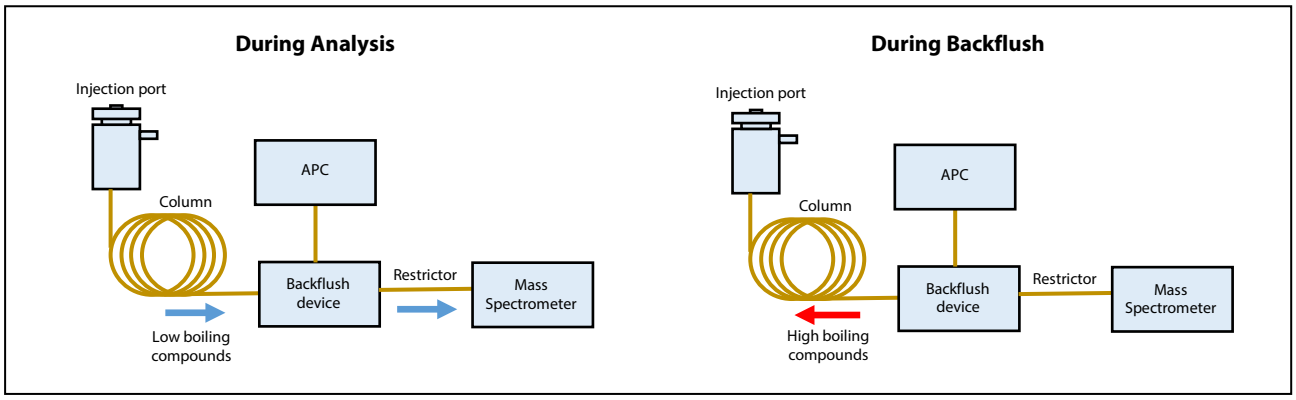

Figure 4. Schematic Diagram of Shimadzu Backflush System

The backflush device was connected to 3 parts: the end of the column, an advanced pressure controller (APC) and the detector (mass spectrometer) via a restrictor. During the analysis, the pressure of the injection port was set to be higher than that of the APC. After all target compounds eluted from the column, backflush was activated by increasing the APC pressure while lowering the injection port pressure. This allowed the carrier gas to reverse the flow. Figure 4 shows a schematic diagram of Shimadzu Backflush System.

■ Analysis Results Using GC-MS/MS

Detection and Mass Chromatogram

The 3-MCPD and 3-MCPD-d5 were detected and separated using SH-I-17Sil MS column. The mass chromatograms of 3-MCPD Standard and 3-MCPD-d5 internal standard are shown in Figure 5. Glycidol was converted and expressed as 3-MCPD in this analysis. Therefore, there was no glycidol peak detected in the chromatogram.

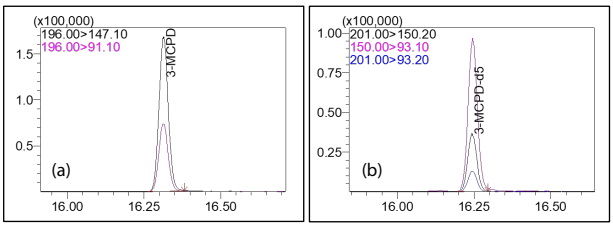

Figure 5. Mass chromatogram of (a) 3-MCPD standard and (b) 3-MCPD-d5 internal standard

Calibration Curve and Linearity of 3-MCPD

Matrix-matched internal standard calibration curve was set up using the spiked oil with Assay B preparation. The calibration points of 3-MCPD internal standard calibration curve prepared were 1.0, 0.5, 0.25, 0.05, 0.02 µg/g. Figure 6 shows the internal standard calibration curve of 3-MCPD, demonstrating excellent linearity with R^2 of 0.9997.

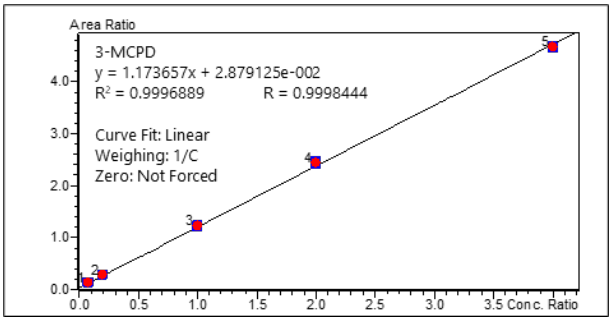

Figure 6. Internal standard calibration curve of 3-MCPD.

Glycidol Transformation Factor Determination

The transformation factor (t) is a factor that quantifies the amount of 3-MCPD converted from glycidol. Matrix-matched glycidol calibration curve was set up to determine the transformation factor. The matrix was spiked with 0.5, 1.0, and 2.0 µg/g of glycidol and subjected to sample preparation of Assay A.

Based on the calibration curve, transformation factor of glycidol was calculated according to the formula below from AOC Method 29c-13

$$t = \frac{1}{m}$$

Where t = transformation factor of glycidol to 3-MCPD; m = gradient of the calibration curve

Figure 7 shows the matrix-matched glycidol calibration curve (converted to 3-MCPD by Assay A sample preparation). The gradient (m) of calibration curve was 1.2048; hence, the glycidol to 3-MCPD transformation factor (t) was determined to be 0.83.

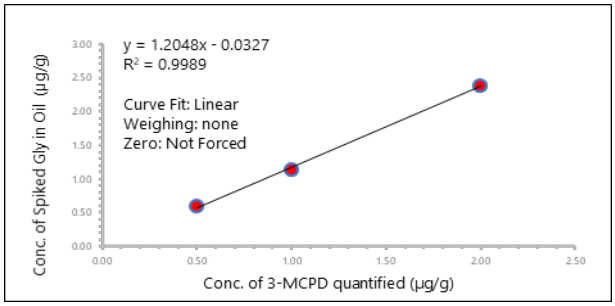

Figure 7. Matrix-matched glycidol calibration curve for glycidol transformation factor determination (prepared using Assay A sample preparation)

Quantitation of Glycidol

Glycidol was quantitated by subjecting an oil sample to sample preparation of both Assay A & B. The concentration of glycidol was calculated using the equation below according to the AOCS Method 29c-13:

$$[Gly] = \left([3-MCPD]_{Assay\ A} - [3-MCPD]_{Assay\ B}\right) \times t$$

Where [Gly] = Concentration of glycidol;
$[3\text{-MCPD}]_{Assay\ A}$ = Concentration of 3-MCPD in assay A;
$[3\text{-MCPD}]_{Assay\ B}$ = Concentration of 3-MCPD in assay B;
t = transformation factor of glycidol to 3-MCPD

Recovery

The recovery of 3-MCPD and glycidol were determined by analyzing known concentrations of 3-MCPD and glycidol standard sample spiked in the oil and quantitated using the 5-point internal standard calibration curve. The recovery for 0.02 µg/g of 3-MCPD and glycidol were between 94 - 107% and 105 - 118%, respectively. The quantitation and recovery results of 3-MCPD and glycidol are presented in Table 4.

Table 4. Summary of quantitation and recovery of 3-MCPD and glycidol, spiked with 0.02 µg/g each

	[3-MCPD] (µg/g)	Recovery of 3-MCPD (%)	[Gly] (µg/g)	Recovery of Glycidol (%)
Replicate 1	0.02053	102	0.02131	106
Replicate 2	0.01897	94	0.02283	114
Replicate 3	0.02065	102	0.02212	110
Replicate 4	0.02057	102	0.02371	118
Replicate 5	0.02007	99	0.02366	118
Replicate 6	0.02159	107	0.02097	105
Replicate 7	0.01916	95	0.02132	106

Limit of Quantitation (LOQ)

Limit of quantitation (LOQ) is defined to be a concentration which has a signal-to-noise ratio (S/N) of at least more than 10. The LOQ of 3-MCPD was determined experimentally to be 0.02 µg/g (20 ppb). Table 5 shows the peak area, area ratio, and signal-to-noise ratio of 3-MCPD at its LOQ. This LOQ shows that 3-MCPD can be confidently quantitated at a much lower concentration than the maximum limit of 1.25 µg/g in the category for oil and fats from palm kernel placed on the market for final consumer[2], which gives assurance of Shimadzu GCMS-TQ8050 NX sensitivity, should the maximum limit become more stringent in the future.

Table 5. Peak area, area ratio and S/N of 3-MCPD and 3-MCPD-d5 at 0.02 µg/g of spiked oil sample

	Peak Area	Peak Area Ratio	S/N
3-MCPD	6011	0.119	59.25
3-MCPD-d5	50334		

Calculated Limit of Detection (Cal. LOD)

Limit of detection (LOD) is defined to be a concentration which has a signal-to-noise ratio (S/N) of at least more than 3. The ccalculated limit of detection (Cal. LOD) is determined to be 0.3 times of the experimental LOQ. The Cal. LOD of 3-MCPD was determined to be 0.006 µg/g (6 ppb).

Inter-day Repeatability

Analysis method precision was determined by analyzing 7 consecutive analysis of 0.02 µg/g of 3-MCPD and glycidol spiked oil for both Assay A and B. They were analyzed over 4 different days in a span of two weeks. Table 6 shows the concentration %RSD for both 3-MCPD and glycidol were <5% within the 4 different days.

Table 6. Concentration %RSD of 3-MCPD and glycidol spiked with of 0.02 µg/g within 2 weeks

	Conc. %RSD of 3-MCPD (n=7)	Conc. %RSD of Glycidol (n=7)
Day 1	2.94	4.93
Day 6	4.22	2.67
Day 8	3.08	4.89
Day 13	3.04	2.73

■ Conclusion

This application news illustrates the development of an MRM method using Shimadzu GCMS-TQ8050 NX with Shimadzu Backflush System for the analysis of 3-monochloropropane-1,2-diol and glycidol in palm oil in accordance with AOCS Method 29c-13. The result showed that this method had excellent linearity with R^2 value of 0.9997 for 3-MCPD. It also demonstrated trace level detection and quantification of 3-MCPD and glycidol in palm oil, as low as 0.006 µg/g for cal. LOD and 0.02 µg/g for LOQ. In addition, the optimized MRM method showed great concentration repeatability of <5% (n=7) and recoveries of within ±20% for both 3-MCPD and glycidol at their LOQ.

■ References

1. May 2016. European Food Safety Authority. Process contaminants in vegetable oils and foods. https://www.efsa.europa.eu/en/press/news/process-contaminants-vegetable-oils-and-foods
2. 23 Sep 2020. Official Journal of the European Union. Commission Regulation (EU) 2020/1322 amending Regulation (EC) No 1881/2006 as regards maximum levels of 3-monochloropropanediol (3-MCPD), 3-MCPD fatty acid esters and glycidyl fatty acid esters in certain foods.

■ Acknowledgement

We would like to thank Jade Chan Ting Shuen and Nur Nabiilah binte Azlan who participated this work during their internship at Shimadzu Asia Pacific.

<Related Applications>

- SIM and MRM Analysis of 3-MCPD, 3-MCPD Fatty Acid Esters, and Glycidol Fatty Acid Esters in Powdered Milk, Application News No.01-00289-EN

04-AD-0281-EN First Edition: Mar 2023

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU (Asia Pacific) Pte. Ltd,
www.shimadzu.com.sg