

Application News

Food Safety, GCMS-QP2020 NX, Gerstel MPS

Determination of 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters (GE) in Edible Oils by GC/MS (GCMS-QP2020 NX) Coupled with Gerstel MPS Workstation According to AOCS Method Cd 29c-13.

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Introduction

The formation of 2- and 3-monochloropropane-1,2-diol (2- and 3-MCPD) esters and glycidyl esters (GE) during high-temperature edible oil refining is a well-documented concern. These contaminants pose significant health risks, as free MCPD and glycidol are classified as potential human carcinogens.

To mitigate these risks, European Union Regulation (EU) 2023/915 establishes maximum allowable levels for these contaminants in foodstuffs. For most vegetable oils (e.g., coconut, maize, rapeseed, sunflower, soybean, olive), the permissible limit for 3-MCPD esters is 1,250 µg/kg. In contrast, oils from palm kernel are subject to a higher limit of 2,500 µg/kg. The limit for glycidyl esters (expressed as glycidol) is 1,000 µg/kg across all oils and fats.

This application note details the simultaneous analysis of 2-, 3-MCPD and glycidyl esters in accordance with the above regulatory requirements. The AOCS Official Method Cd 29c-13 was used due to its significant advantages for high-throughput labs. Unlike the lengthy hydrolysis (often 16 hours) of methods Cd 29a or 29b, this method uses a rapid alkaline transesterification, offering faster, more efficient sample preparation, particularly when integrated with automated workflows. This work demonstrates the complete, automated procedure using the Gerstel Multi-Purpose Sampler Workstation and a Shimadzu GCMS-QP2020 NX system, a combination that enhances analytical precision, minimizes human error, and improves laboratory productivity.

Experimental

Instrumental and Analytical Conditions

Automated sample preparation was performed using the Gerstel MPS WorkStation (Dual Head version), operated via Maestro software, following Method Cd 29c-13. Detection and quantitation of the target analytes were performed using a Shimadzu GCMS-QP2020 NX single quadrupole GC-MS system (Figure 1). This system is equipped with an end-column backflush system. The analytical conditions are detailed in Table 1.



Figure 1: Full setup of Shimadzu GCMS-QP2020 NX with Gerstel MPS Workstation

Table 1: GCMS-QP2020 NX system & analytical conditions

GC Conditions	System Parameter Setting
Column	SH-I-17Sil MS (30 m, 0.25 mm I.D, 0.25 µm df)
Injection Temperature	250.0 °C
Injection Mode	Splitless (1.00 min)
Injection Volume	1 µL
Flow Control Mode	Constant pressure with end-column backflush
Inlet Pressure Program	130.8 kPa (17.00 min); -400.00 kPa to 30.0 kPa (12.92 min)
Column Oven Temperature	85.0 °C (1.00 min); 6.00 °C/min to 150.0 °C; 12.00 °C/min to 210.0 °C (2.00 min); 30.00 °C/min to 280.0 °C (9.00 min). [Total: 30.17 min]
Carrier Gas	Helium
MS Conditions	System Parameter Setting
Ion Source	EI (230 °C)
Interface Temperature	300 °C
Detector Voltage	Relative to tuning result +0.3 kV
Acquisition Mode	SIM
Ions Monitored (m/z): 3-MCPD	Target : 146.00 References: 196.00, 198.00
Ions Monitored (m/z): 2-MCPD	Target : 196.00 References: 198.00
Ions Monitored (m/z): 3-MCPD-d5	Target : 201.00 References: 149.00, 203.00

Standards and Sample Preparation

The AOCS Official Method Cd 29c-13 provides a streamlined workflow for the analysis of 3-MCPD esters and glycidyl esters (GE) in edible oils, making it ideal for high-throughput laboratories. This method utilizes two complementary assays (Assay A and Assay B) to accurately quantify both 3-MCPD (from esters) and GE content in the sample.

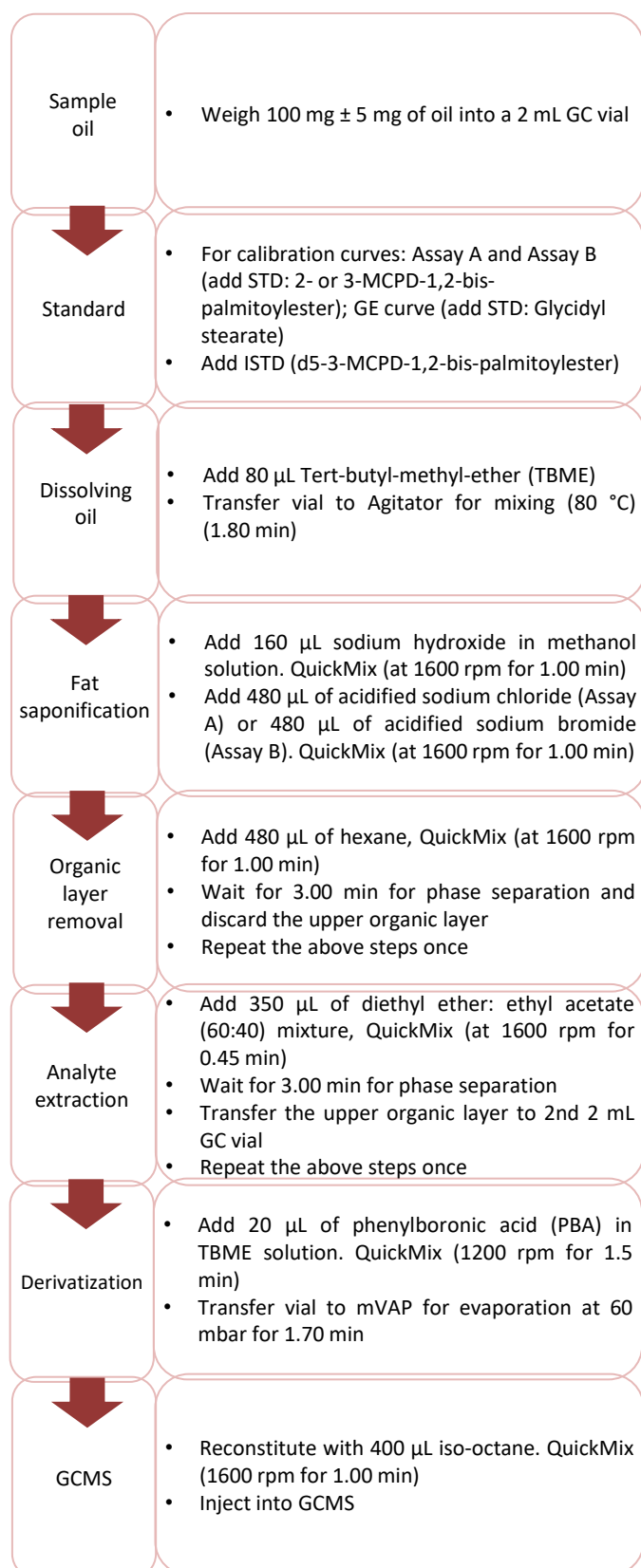


Figure 2: Fully automated standard and sample preparation procedures

Figure 2 illustrates the automated sample preparation workflow performed using the Gerstel MPS WorkStation. In Assay A, the sample is treated with chloride ions, converting glycidol (released from GE) into 3-MCPD. This assay determines the total 3-MCPD content, which includes 3-MCPD from its esters and 3-MCPD generated from glycidyl esters. Assay B, performed without chloride ions, quantifies only the 2-MCPD and 3-MCPD derived from its esters. Following sample preparation, derivatization is carried out, and the samples are analyzed via GCMS.

Table 2: The calibration ranges prepared for 2-MCPD, 3-MCPD, and GE

	Concentration (µg/g)		
	3-MCPD (Assay A & B)	2-MCPD (Assay B)	GE (Assay A)
STD 1	0.058	0.056	0.073
STD 2	0.115	0.112	0.145
STD 3	0.211	0.205	0.266
STD 4	0.518	0.502	0.652
STD 5	1.151	1.116	1.450
STD 6	2.014	1.952	2.537
STD 7	5.179	5.020	6.524
STD 8	9.591	7.438	12.081

As outlined in Method Cd 29c-13, each oil sample and standard is analyzed using both Assay A and Assay B. GC-MS measurements are quantified using calibration curves generated for each assay. The GE calibration curve is generated using Assay A data. The results from these two assays are subsequently used to estimate the glycidol content. The calculation steps are as follows:

$$\text{Assay A } (\mu\text{g/g}) = \frac{\text{Measured 3-MCPD } (\mu\text{g}) \text{ from Assay A curve}}{\text{Sample Weight (g)}}$$

$$\text{Assay B } (\mu\text{g/g}) = \frac{\text{Measured 3-MCPD } (\mu\text{g}) \text{ from Assay B curve}}{\text{Sample Weight (g)}}$$

The GE conversion factor is derived from the slope of the glycidol ester calibration curve:

$$\text{GE Conversion Factor} = \frac{1}{\text{Slope of GE calibration curve}}$$

The glycidol content in the sample is then calculated using the following equation:

$$\text{Glycidol } (\mu\text{g/g}) = \frac{\text{Assay A } (\mu\text{g/g}) - \text{Assay B } (\mu\text{g/g})}{\text{GE Conversion Factor}}$$

For 2-MCPD and 3-MCPD, the content in the sample can be directly calculated from the 2-MCPD and 3-MCPD calibration curve in Assay B.

Result and Discussion

Detection and Mass Chromatograms

Separation and quantitation of 2-MCPD, 3-MCPD, and its internal standard, 3-MCPD-d5, were successfully achieved. The corresponding mass chromatograms are displayed in **Figure 3**.

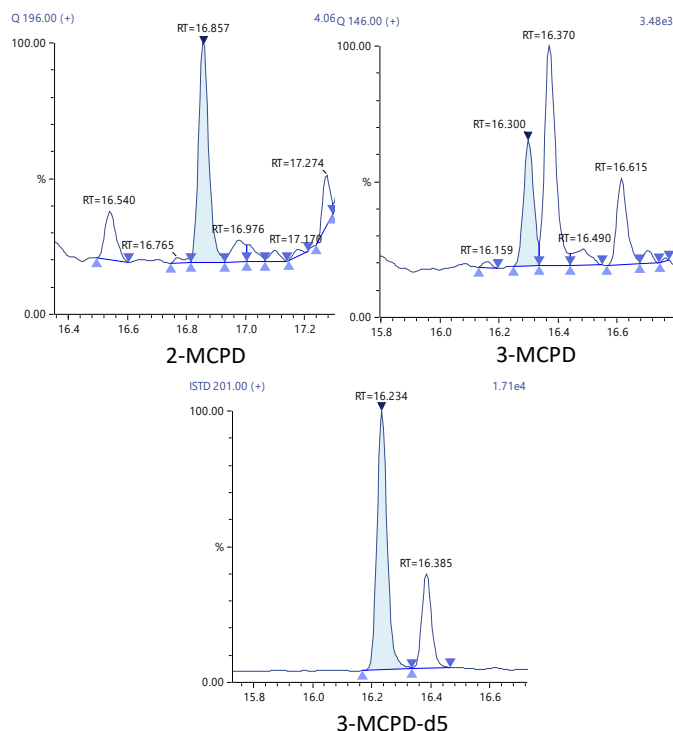


Figure 3: Mass chromatograms of 2-MCPD, 3-MCPD, and 3-MCPD-d5 at the lowest calibration standard.

Linearity and Limit of Quantitation (LOQ)

Calibration standards for Assays A and B including glycidyl esters (GE) curve were prepared using a refined olive oil matrix to mitigate matrix-related effects. All three calibration curves demonstrated excellent linearity, with coefficients of determination (R^2) greater than 0.999. Representative calibration plots for Assays A and B including GE curve are presented in **Figure 4**, while **Table 3** summarizes the percent accuracy at each calibration point. Calibration accuracy was within the acceptable range of $\pm 15\%$ across all levels.

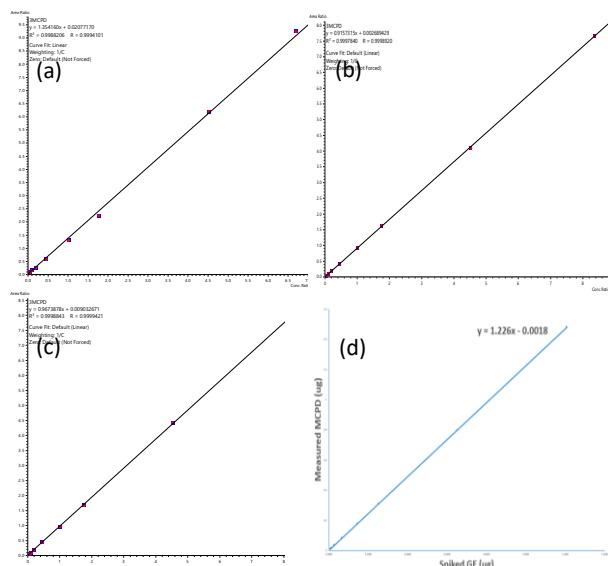


Figure 4: Calibration curve for (a) 2-MCPD Assay B, (b) 3-MCPD Assay B, (c) 3-MCPD Assay A, (d) GE curve

Table 3: % Accuracy for each level of calibration standard for 2-MCPD (Assay B) and 3-MCPD (Assay A & B)

Standard	% Accuracy		
	2-MCPD (Assay B)	3-MCPD (Assay B)	3-MCPD (Assay A)
1	115.22	89.52	103.79
2	107.06	102.78	95.12
3	95.63	108.91	102.41
4	89.73	97.54	100.5
5	94.50	100.61	98.93
6	93.73	101.49	98.26
7	103.29	99.06	101.16
8	100.84	100.09	99.82

The limit of quantitation (LOQ) for 2-MCPD and 3-MCPD was calculated at an absolute amount of 0.005 μg , corresponding to 0.05 $\mu\text{g/g}$ relative to the sample weight. The automated method was validated by ten replicate injections ($n = 10$), each yielding a signal-to-noise (S/N) ratio of 94.68 and 16.68, as well as an average accuracy of 112.75 % and 97.35 % respectively (**Table 4**). These findings confirm that 0.05 $\mu\text{g/g}$ is a suitable level for the lowest calibration standard for both compounds.

Table 4a: LOQ for 2-MCPD (Assay B) ($n=10$)

	Measured concentration (μg)	% Accuracy	S/N
Rep 1	0.00645	115.18	135.16
Rep 2	0.00614	109.64	82.72
Rep 3	0.00635	113.39	94.05
Rep 4	0.00625	111.61	79.61
Rep 5	0.00655	116.96	147.69
Rep 6	0.0061	108.93	69.57
Rep 7	0.00611	109.11	66.18
Rep 8	0.00652	116.43	107.81
Rep 9	0.00634	113.21	86.33
Rep 10	0.00633	113.04	77.72
Average ($n=10$)	0.00631	112.75	94.68

Table 4b: LOQ for 3-MCPD (Assay B) ($n=10$)

	Measured concentration (μg)	% Accuracy	S/N
Rep 1	0.00582	101.09	20.14
Rep 2	0.00565	98.12	17.81
Rep 3	0.00566	98.3	19.04
Rep 4	0.00563	97.79	17.89
Rep 5	0.00549	95.39	17.55
Rep 6	0.00572	99.46	17.06
Rep 7	0.00577	100.33	15.42
Rep 8	0.00529	92.02	14.84
Rep 9	0.00558	97.03	12.2
Rep 10	0.00541	93.99	14.88
Average ($n=10$)	0.00560	97.35	16.68

Accuracy and Precision

To assess the performance of the automated analytical workflow, a FAPAS-certified quality control (QC) material with known 3-MCPD and GE values was used. The material, obtained from Fera Science Ltd., had certified concentrations of 3-MCPD at 1.013 µg/g and GE at 0.911 µg/g. This QC sample was analyzed five times per day (intraday) over three non-consecutive days (interday). **Table 5** presents the intraday results, while **Table 6** summarizes the interday data (n=15).

Table 5: Intraday accuracy and precision for QC sample analysis (n=5)

Day 1	Assay B MCPD (µg/g)	Accuracy (%)	GE (µg/g)	Accuracy (%)
Rep 1	1.141	112.6	1.010	110.9
Rep 2	1.146	113.2	1.018	111.8
Rep 3	1.144	112.9	0.977	107.2
Rep 4	1.138	112.3	0.993	109.0
Rep 5	1.126	111.1	1.048	115.0
Average	1.139	112.4	1.009	110.8
SD	0.008	0.791	0.027	2.953
%RSD	0.7	0.7	2.7	2.7

Day 2	Assay B MCPD (µg/g)	Accuracy (%)	GE (µg/g)	Accuracy (%)
Rep 1	1.139	112.4	1.006	110.4
Rep 2	1.145	113.0	1.025	112.6
Rep 3	1.139	112.4	1.020	112.0
Rep 4	1.156	114.1	1.028	112.8
Rep 5	1.166	115.1	1.008	110.6
Average	1.149	113.4	1.017	111.7
SD	0.012	1.177	0.010	1.104
%RSD	1.0	1.0	1.0	1.0

Day 3	Assay B MCPD (µg/g)	Accuracy (%)	GE (µg/g)	Accuracy (%)
Rep 1	1.124	110.9	1.027	112.7
Rep 2	1.161	114.6	0.998	109.6
Rep 3	1.140	112.5	1.023	112.3
Rep 4	1.157	114.2	0.995	109.2
Rep 5	1.162	114.7	0.983	107.9
Average	1.149	113.4	1.005	110.3
SD	0.017	1.629	0.019	2.087
%RSD	1.4	1.4	1.9	1.9

Table 6: Interday accuracy and precision for QC sample analysis (n=15)

Interday n=15	Assay B MCPD (µg/g)	Accuracy (%)	GE (µg/g)	Accuracy (%)
Average	1.145	113.1	1.011	110.9
SD	0.013	1.247	0.019	2.102
%RSD	1.1	1.1	1.9	1.9

For intraday evaluation, five replicate analyses were performed each day. The results demonstrated high accuracy and low variability for both 3-MCPD and GE quantitation. Across all three days, the measured 3-MCPD accuracy ranged from 110.9 % to 115.1 %, with relative standard deviations (%RSD) between 0.7 % and 1.4 %. For GE, accuracy values ranged from 107.2 % to 115.0 %, and %RSD values were between 1.0 % and 2.7 %, indicating excellent repeatability.

Interday precision was determined from 15 measurements over the three-day period (n = 15). The average measured value for 3-MCPD was 1.145 µg/g, corresponding to 113.1 % accuracy with an %RSD of 1.1 %. For GE, the average concentration was 1.011 µg/g with 110.9 % accuracy and an %RSD of 1.9 % (**Table 6**). The results confirm the robustness and reproducibility of the method under routine laboratory conditions.

Conclusions

The combination of the GCMS-QP2020 NX coupled with Gerstel MPS WorkStation, provides a fully automated and reliable solution for analyzing 2-MCPD, 3-MCPD, and glycidyl esters in edible oils, following AOCS Method Cd 29c-13. The method offers high sensitivity (LOQ: 0.05 µg/g) and excellent performance in linearity, accuracy, and precision. Analysis of a certified QC sample confirmed the system's reliability, supporting its use in routine quality control and regulatory testing.

References

- Lucas, D., Hoffmann, A., & Gil, C. (2023). 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) (Application Note No. 191). GERSTEL GmbH & Co. KG.
- Pasquale Ioime; Elena Piva; Michele Pozzebon; Jennifer P. Pascali; (2021). Automated sample preparation and analysis by gas chromatography tandem mass spectrometry (GC-MS/MS) for the determination of 3- and 2-monochloropropanediol (MCPD) esters and glycidol esters in edible oils .
- American Oil Chemists' Society. (2013). AOCS Official Method Cd 29c-13: Determination of Bound 3-MCPD and Glycidyl Esters in Edible Oils and Fats by GC/MS (Indirect Method). AOCS Press.