SHIMADZU
Excellence in Science

Application News

GC-MS GCMS-TQ™8050 NX

SIM and MRM Analysis of 3-MCPD, 3-MCPD Fatty Acid Esters, and Glycidol Fatty Acid Esters in Powdered Milk

T. Ishii

User Benefits

- 3-MCPD, 3-MCPD fatty acid esters, and glycidol fatty acid esters can all be analyzed from a single sample.
- MRM mode is less susceptible to contaminants and gives a more selective analysis.
- The robustness of GCMS-TQ8050 NX enables highly sensitive MRM analysis with reliable results.

■ Introduction

Monochloropropanediols (MCPDs) are compounds that can potentially be generated from oils during food processing. Oils are also known to contain MCPD fatty acid esters, which are MCPDs bound to fatty acids, and glycidol fatty acid esters that form 3-MCPD in the body.

In many countries, efforts are underway to reduce MCPD fatty acid esters and glycidol fatty acid esters in food due to concerns over health hazards. The European Food Safety Agency (EFSA) will also consider setting stricter standards for 3-MPCD fatty acid ester levels in infant milk formula during the 2 years commencing January 1, 2021.

This study used GCMS-TQ8050 NX to perform SIM and MRM analysis of 3-MCPD, 3-MCPD fatty acid esters, and glycidol fatty acid esters in powdered milk. Samples were prepared based on AOAC Official Method 2018.12[1], which has been adopted as an analytical method by the EFSA.

Fig. 1 GCMS-TQ™ 8050 NX

■ Reagent Preparation

• Sodium hydroxide/methanol solution (60 g/L)

6.0 g of sodium hydroxide was dissolved in 100 mL of methanol.

• Sodium bromide/aqueous phosphoric acid (600 g/L)

60 g of sodium bromide was dissolved in 100 mL of aqueous phosphoric acid (6.5 mL/L).

• Phenylboronic acid solution (5.0 mg/mL)

50 mg of phenylboronic acid was dissolved in 10.0 mL of diethyl ether.

• *d*-3-MCPD solution (10 µg/mL)

d-3-MCPD was prepared at 10 µg/mL in methanol.

• Stock solution for calibration curve (10 µg/mL)

3-MCPD was prepared at 10 µg/mL in methanol. For quantitative analysis of fatty acid esters, 3-MCPD-1,2-dioleoyl ester and glycidyl oleate were prepared at 5 µg/mL.

■ Analysis Flow (3-MCPD)

Fig. 2 shows the sample preparation process for 3-MCPD analysis. 2.00 g of powdered milk was weighed, dissolved with 6.0 mL of methanol (MeOH), and subjected to ultrasonic extraction at 65 °C for 15 min. The sample was then centrifuged for 5 min at 3000 rpm and the supernatant was collected. This same extraction process was then performed using a methanol/methyl tert-butyl ether (1:1) solvent mixture and methyl tert-butyl ether, then the supernatant of each was combined and dried with nitrogen.

The dried sample was then dissolved with 4.0 mL of a saturated aqueous solution of sodium sulfate. This was followed by liquid-liquid separation performed twice with 2.5 mL of a hexane/methyl tert-butyl ether (4:1) solvent mixture, separating the layers each time.

The resulting aqueous phase contained 3-MCPD present in its free form in milk powder. Solvent extraction was then performed by adding 2.0 mL of diethyl ether to the water layer and collecting the resulting organic layer. This solvent extraction process was performed three times. Phenylboronic acid solution was added to the diethyl ether layer, the mixture was left to stand at room temperature for 5 minutes and dried completely with nitrogen. Finally, 300 µL of isooctane was added to the dried sample, and 200 µL of the sample was transferred to a vial for analysis.

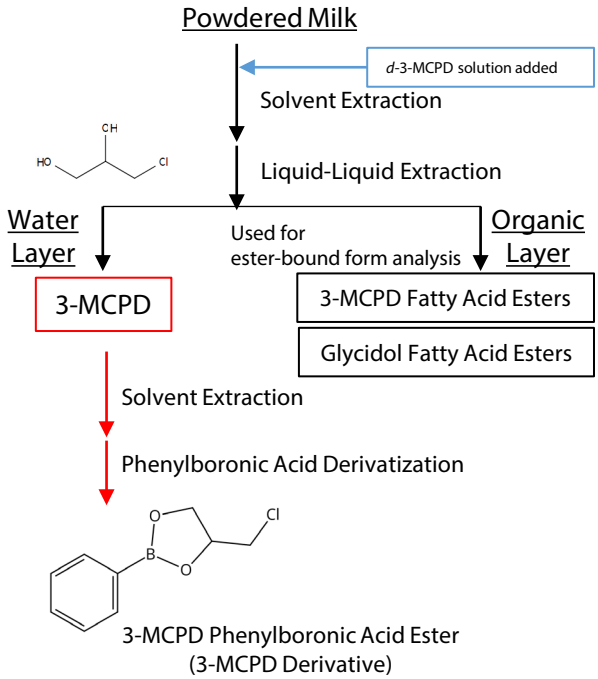

Fig. 2 Sample Preparation Flow for 3-MCPD Analysis

■ Analysis Conditions

Model:	GCMS-TQ8050 NX
GC	
Injection Unit Temp.:	250 °C
Injection Method:	Split
Split Ratio:	20
Carrier Gas:	He
Carrier Gas Control:	Linear velocity (50 cm/s)
Column:	SH-5MS (30 m × 0.25 mm, I.D. 0.25 µm)
Column Temp.:	50 °C (20 min) - 10 °C /min - 300 °C (3 min)
MS (Electron Ionization, MRM)	
Ion Source Temp.:	200 °C
Interface Temp.:	300 °C
Tuning Mode:	Standard
Measurement Mode:	MRM
d-3-MCPD	150.0 > 93.0 (CE 12 V)
3-MCPD	147.0 > 91.1 (CE 12 V)
3-MBPD	242.0 > 147.2 (CE 15 V)
Event Time:	0.3 sec
MS (Electron Ionization, SIM)	
Measurement Mode:	Scan/SIM Simultaneous Measurement
	Scan (*m/z* 50 to 500)
d-3-MCPD	201.0, 150.0, 93.0
3-MCPD	147.0, 146.0, 91.0
3-MBPD	242.0, 147.0, 91.0
Event Time:	SIM (0.3 sec), Scan (0.1 sec)

■ 3-MCPD Reference Standard Analysis

The results obtained from quantitative analysis of 3-MCPD are as follows. Standard samples of 3-MCPD comparable to levels in powdered milk (50, 100, 500, and 1000 ppb) were derivatized with phenylboronic acid and analyzed. Results were corrected using a *d*-3-MCPD internal standard. The SIM chromatogram of the lowest concentration (50 ppb) sample is shown in Fig. 3. The S/N ratio in SIM analysis of 50 ppb was 125.8, and the S/N ratio in MRM analysis of 50 ppb was 275.7, showing sufficient sensitivity was achieved with both methods.

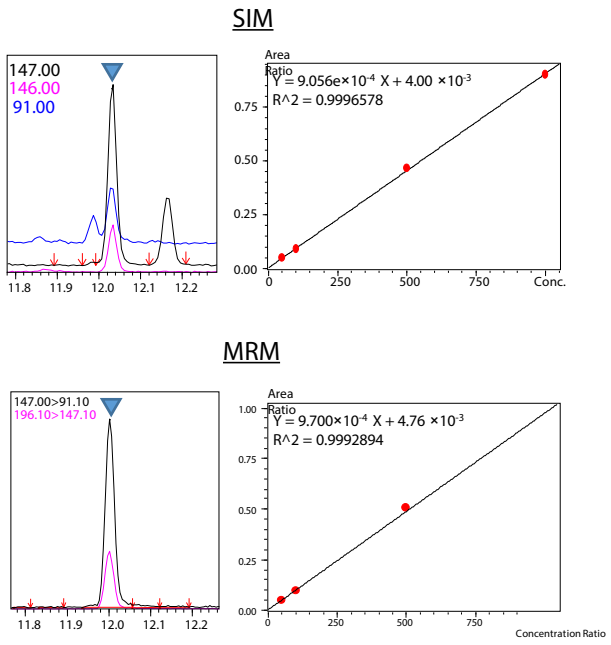

Fig. 3 Calibration Curves and SIM and MRM Chromatograms of 3-MCPD (50 ppb)

■ Results from Analysis of 3-MCPD in Powdered Milk

The results obtained from quantitative analysis of 3-MCPD in powdered milk are as follows. 3-MCPD was detected at around 15 ppb. The results obtained from SIM and MRM analysis are shown separately in Fig. 4.

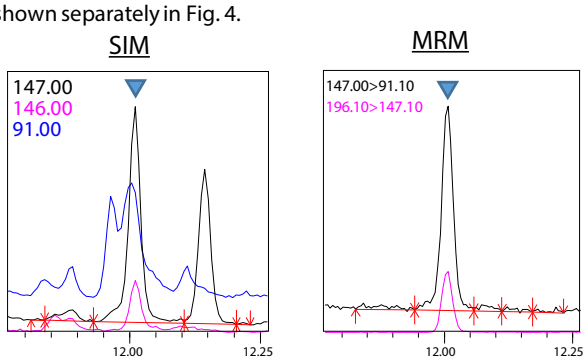

Fig. 4 SIM and MRM Chromatograms of 3-MCPD in Powdered Milk

■ Preparation of 3-MCPD Fatty Acid Ester and Glycidol Fatty Acid Ester Samples

The fatty acid ester-bound analytes present in powdered milk are contained in the organic layer. After drying the organic phase with nitrogen, 3.0 mL of methyl tert-butyl ether was added to dissolve oils. Next, 1.4 mL of sodium hydroxide/methanol solution was added and the mixture was left to stand at -25 °C for 15 to 18 hours. 2.4 mL of sodium bromide/aqueous phosphoric acid was then added to neutralize the mixture. After mixing thoroughly, the sample separated into two layers and the organic upper layer was removed by blowing nitrogen. During this process, glycidol and sodium bromide reacted to form 3-MBPD. To remove the remaining oils, hexane was added and the upper layer was discarded. This process of adding hexane and discarding the upper layer was performed three times. At this point, MCPD and MBPD derived from fatty acid esters had migrated to the remaining lower layer, where they were derivatized under the same conditions as the water phase mentioned earlier before being used for analysis.

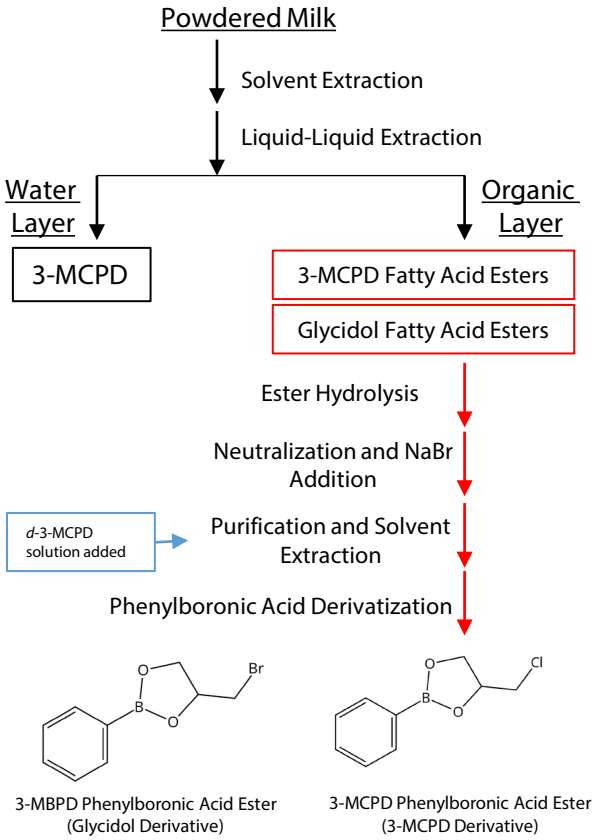

Fig. 5 Sample Preparation Flow for MCPD Fatty Acid Ester Analysis

■ Analysis of 3-MCPD Fatty Acid Ester and Glycidol Fatty Acid Ester Reference Standards

The results obtained from quantitative analysis of a 3-MCPD fatty acid ester and glycidol fatty acid ester reference standard are shown below. Standard samples comparable to levels in powdered milk (10, 50, 100, and 500 ppb) were derivatized with phenylboronic acid and analyzed. Results were corrected using a *d*-3-MCPD internal standard. The SIM chromatogram for the lowest concentration (10 ppb) sample is shown in Fig. 6.

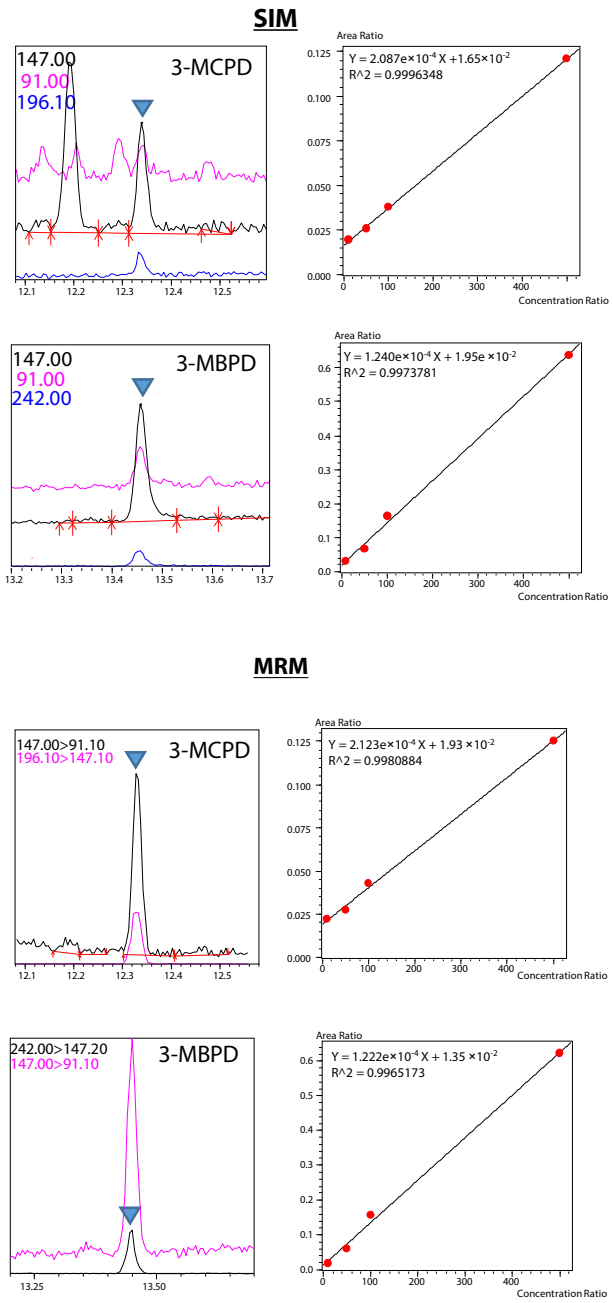

Fig. 6 Calibration Curves and SIM and MRM Chromatograms of MCPD Fatty Acid Ester (10 ppb)

■ Analysis Results for Actual Samples

Example SIM and MRM chromatograms obtained upon analyzing MCPD fatty acid esters in actual samples are shown below. Both methods gave a highly selective analysis.

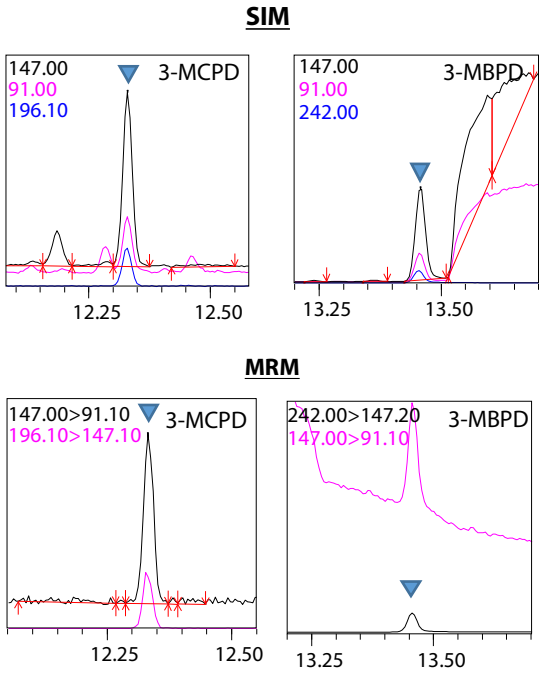

Fig. 7 SIM and MRM Chromatograms of MCPD Fatty Acid Esters in Powdered Milk

■ Conclusion

Adequate sensitivity was obtained by both SIM and MRM analysis after sample preparation based on the AOAC Official Method. Although adequate sensitivity can be obtained by SIM analysis, MRM analysis is less affected by contaminating components from the food matrix. *d*-3-MCPD was used to correct the amounts of ester-bound analytes in this study, but using *d*-3-MCPD-1,2-dioleoyl ester and *d*-glycidyl oleate is recommended.

Please refer to the reference below for details of the GCMS conditions in the AOAC Official Method. Other than 3-MCPD, 2-MCPD can also be quantified with the same sample preparation conditions.

■ Reference

1) AOAC Official Method 2018.12 "*2-Monochloropropanediol (2-MCPD), 3-Monochloropropanediol (3-MCPD), and Glycidol in Infant and Adult/Pediatric Nutritional Formula*"

GCMS-TQ is a trademark of Shimadzu Corporation in Japan and other countries.

01-00289-EN First Edition: Jan. 2022

SHIMADZU
Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedure.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
See http://www.shimadzu.com/about/trademarks/index.html for details.
Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.
The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2022