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Analysis of 3-monochloropropanediol, 3-MCPD fatty acid ester and Glycidyl Ester in Infant Formula based on AOAC Official Methods 2018.12

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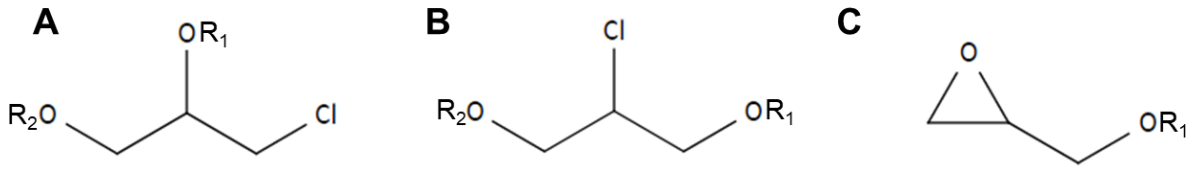
1. Introduction

Monochloropropanediols (MCPDs) are unintentionally produced from edible oils and infant formula. MCPDs are generated from digesting vegetable protein with hydrochloric acid and be formed by heating process. It has been reported long term intake of MCPDs affects negative impacts to the kidney and androgenesis.

MCPDs fatty acid esters, glycidol fatty acid esters are also monitored in purified edible oils. In animal experiments, most of MCPDs fatty acid esters and glycidol fatty acid esters are decomposed in the intestinal tract and absorbed as MCPDs. EFSA investigated and evaluated the intake of MCPDs, MCPDs fatty acid esters and glycidol fatty acid esters. EFSA concluded it needs to be researched in detail, but efforts to reduce them are required.

We reported GCMS quantification method of 3-MCPD, 3-MCPD fatty acid esters and glycidol fatty acid esters.



	A	B	C
R_1, R_2 = H	3-MCPD	2-MCPD	Glycidol
R_1, R_2 = H, Fatty acid	3-MCPD monoester	2-MCPD monoester	Glycidol ester
R_1, R_2 = Fatty acid	3-MCPD diester	2-MCPD diester	-

Figure 1 Chemical structures of MCPDs and Glycidol

2. Methods and Materials

2.1. Sample Preparation

Figure 2 shows the pretreatment procedure. Weigh 2.00 g of powdered milk, add 6.0 mL of methanol, and perform ultrasonic extraction at 65℃ for 15 minutes. Centrifuge at 3000 rpm for 5 minutes and separate the supernatant. Add MeOH-MtBE（1+1, v/v） to residue, extract in the same way and separate the supernatant. Combine supernatants and evaporate under the nitrogen stream. Perform third extraction to residue with MtBE in the same way and evaporate. Combine the extracts and add 4 mL saturated sodium sulphate solution and add 2.5 mL (×2) of an isohexane–tBME (4 + 1, v/v) mixture to separate the polar and nonpolar contents.
Aqueous phase includes free MCPDs. Carry out MCPDs with extraction with 2.0 mL (×3) of a diethyl ether to aqueous phase. Add phenyl boronic acid (5 mg/ml) to ether layer and leave at room temperature for 5 minutes or more. Evaporate ether under the N2 stream at 65 ℃. Finally, dissolve residue with 300 µL isooctane and take 200 µL as analysis sample.

Oils and fats including MCPDs fatty acid esters and glycidol fatty acid esters migrate to organic layer. Evaporate organic solvent, dissolve the oil with 3.0 mL MtBE. Add 1.4 mL NaOH/MeOH solution (0.6g/mL) and leave it 15-18 hours at -25℃. After the saponification, neutralize with 2.4 mL NaBr/phosphorus acid solution. Evaporate organic layer formed by addition of acidic solution. Through this process glycidol reacts with NaBr to produce 3-MBPD. Add hexane and discard the upper layer in order to remove the remaining oils. The lower layer is extracted and derivatized under the same conditions as the aqueous layer.

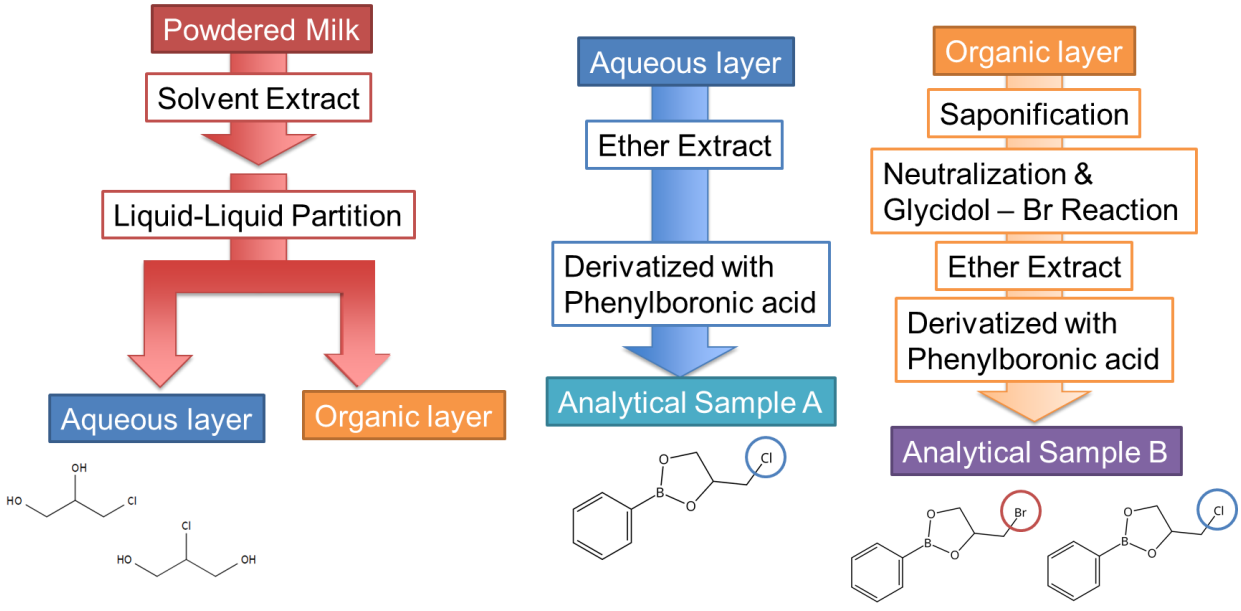


Figure 2 Pretreatment procedure

2. Methods and Materials

2.2. AOAC Official Method Condition

We tried two type methods. One of them is AOAC Official Methods 18. The feature is that stationary phase 50% diphenyl, 50% dimethylpolysiloxane is used for column, and PTV is used for inlet.

GCMS conditions (GCMS QP-2020NX)

GC
Column: Rxi-17 (30 m×0.25 mm I.D., 0.25 µm)
Injection Mode: splitless
Injection Port Temperature : 85℃ - (250℃/min) - 165℃(10 min) – (250℃/min) - 320℃(8 min)
Control Mode: Column Flow (1.25 mL/min)
Purge Flowrate: 3.0 mL/min
GC Oven Program: 85℃ - (6℃/min) - 150℃ - (12℃/min) - 180℃ - (25℃/min) - 280℃
MS
Ionization: EI
Interface Temperature: 200℃
Ion Source Temperature: 280℃
Measurement Mode: SIM

SIM monitor ion:	*d*-3-MCPD	150.0, 201.0
	3-MCPD	147.0, 146.0, 196.0,198.0
	3-MBPD	242.0, 240.0

2.3. Original SIM&MRM Method Condition

It based on edible oil analysis method. In this method, 5%-phenyl-methylpolysiloxane column is used. The column is used that has a high heat-resistant and is resistant to column deterioration due to matrix.

GCMS conditions (GCMS TQ-8050 NX)

GC
Column: SH-5MS (30 m×0.25 mm I.D., 0.25 µm)
Injection Mode: Split
Injection Port Temperature : 250℃
Control Mode: Linear velocity (50 cm/s)
Purge Flowrate: 3.0 mL/min
GC Oven Program: 50℃(1 min) – 10℃/min - 300℃(3 min)
MS (SIM)
Ionization: EI
Interface Temperature: 300℃
Ion Source Temperature: 200℃
Measurement Mode: FASST(Scan/SIM)
Scan Range: 50 - 500 amu

SIM monitor ion:	*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

MS (MRM)

MRM trandition :	*d*-3-MCPD	150.0>93.0
	3-MCPD	147.0>91.1
	3-MBPD	147.0>91.1

3. Result

3.1. Quantitative Analysis by AOAC Official Method

This method tends to detect sharp peaks. However, if the cleanup is not enough or if a large amount of phenylboronic acid is mixed in, the column liquid phase will deteriorate. For 3-MBPD, 10 ppb may not be detected depending on the state of the column liquid phase. In each case, it was possible to quantify the concentration contained in powdered milk.

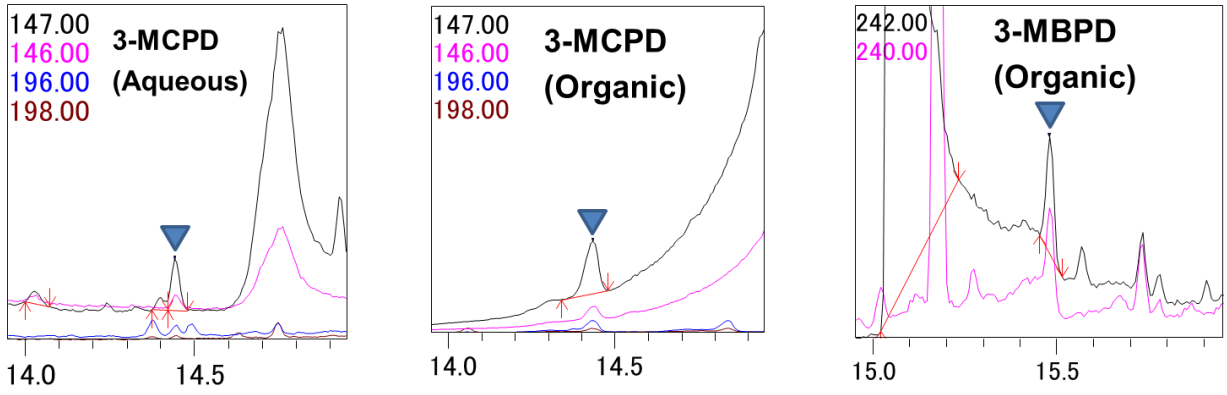


Figure 3 SIM Chromatogram of 3-MCPD and 3-MBPD in AOAC Official Method

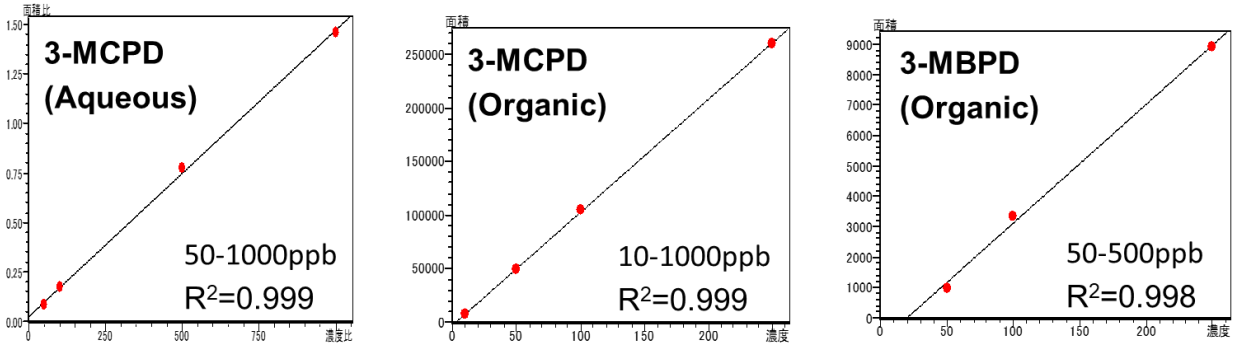


Figure 4 Calibration curves of 3-MCPD and 3-MBPD in AOAC Official Method

3. Result

3.2. Quantitative Analysis by Original Method (SIM&MRM)

Figure 5 shows the results and calibration curve of infant formula in the MRM analysis. 3-MCPD that migrates to the water layer could be detected with sufficient sensitivity even with a small content of 14 ppb. The content of fatty acid esters derivatized products in powdered milk could also be quantified.

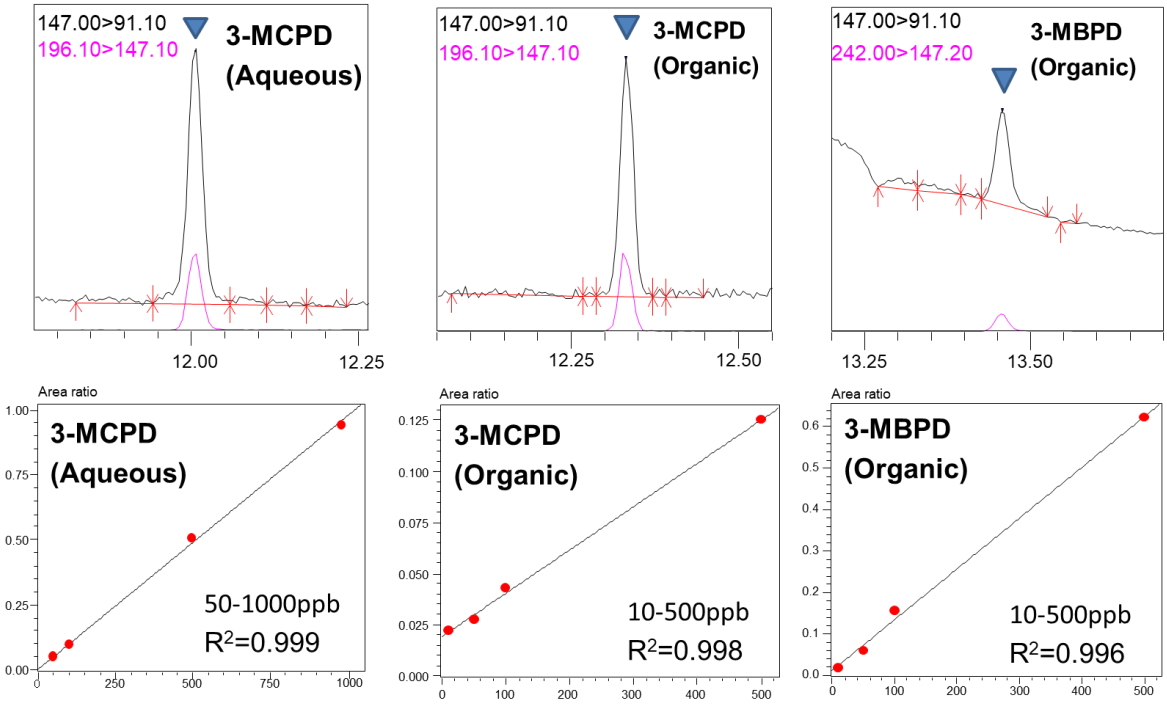


Figure 5 MRM Chromatogram and Calibration curves of 3-MCPD and 3-MBPD in Original Method

4. Conclusion

You can get sharper peaks when using SH-5MS. However, inadequate cleanup or the inclusion of large amounts of derivatizing reagents may damage the column liquid phase and affect robustness. PTV has the advantage of changing the vaporization timing of impurities, but it can accelerate the contamination of inlets during cooling.With a slightly polar column, the peaks will be broad, but sufficient sensitivity can be obtained. If there is concern about peak duplication with contamination, it can be resolved by performing MS separation with MRM.

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