Determination of 3-MCPD Diesters and Monoesters in Vegetable Oils and Fats

Application Note

Food

Authors

Adriana P. Arisseto,
Priscila F. C. Marcolino, and
Eduardo Vicente
Instituto de Tecnologia de Alimentos (ITAL),
Campinas-SP, Brazil

Kumi S. Ozawa
Agilent Technologies,
Barueri-SP, Brazil

Abstract

Esters of 3-monochloropropane-1,2-diol (3-MCPD) are contaminants formed in several processed foods and food ingredients, especially during the refining process of vegetable oils and fats. This application note describes a procedure based on solid phase extraction (SPE) using silica cartridges Agilent HF Mega BE-SI for the determination of 3-MCPD diesters and monoesters to identify the different species of the compounds when indirect analytical methods are used for their quantification. This procedure showed good linearity and no matrix effects. The limit of detection (LOD), and limit of quantification (LOQ) were set at 0.1 and 0.2 mg/kg, respectively. Recovery rates ranged from 74 to 98% and coefficients of variation were between 6.9 and 11.5% for repeatability and between 6.8 and 16.2% for within-laboratory reproducibility. The application of the method to samples of vegetable oils and fats showed that most of the 3-MCPD esters are in the form of diesters.

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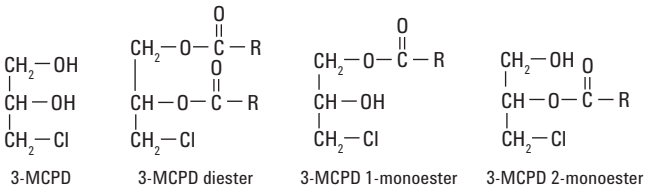
Introduction

Chloropropanols are a well-known group of contaminants found in several processed foods and food ingredients. These compounds are formed through chemical reactions between lipids and chloride ions when subjected to high temperatures, and can be found both in free and bound form, the latter being called chloropropanol esters. Although free chloropropanols were identified in hydrolyzed vegetable protein approximately 30 years ago [1], the occurrence of bound forms, especially 3-monochloropropane-1,2-diol (3-MCPD) esters, was reported only recently and at concentrations much higher than those of their free forms [2]. Particular attention has been addressed to the occurrence of these compounds in refined vegetable oils and fats, in which very high concentrations may be formed during the refining process [3]. The chemical structures of 3-MCPD and its esters are shown in Figure 1.

The presence of 3-MCPD esters in foods raised an immediate concern in terms of public health due to the possibility of these compounds undergo hydrolysis by enzymes in the gastrointestinal tract, and represent an additional source of exposure to 3-MCPD previously not considered [4-7]. This concern is justified considering that 3-MCPD has shown nephrotoxic properties as well as having an ability to affect male fertility and induce cancer in experiments with animals. It has been classified as a possible human carcinogen (group 2B) [8]. For these reasons, this discovery has been considered a priority issue in relation to food safety.

Preliminary studies about intake assessment, considering the levels of 3-MCPD esters found in foods and, assuming that 100% of these esters are hydrolyzed during the digestion, showed that the exposure to free 3-MCPD may exceed the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight (bw) currently established for this compound, suggesting a potential health risk [4]. However, the conditions of hydrolysis and absorption of 3-MCPD esters in the body should be well-known in order to allow a more reliable evaluation of these risks.

Experiments recently conducted in rodents have shown that the relative concentration of 3-MCPD metabolites excreted in urine following the administration of 3-MCPD-dipalmitoyl approached 86% on average compared to the concentration of the same excreted metabolites after oral administration of equimolar doses of 3-MCPD. This can indicate the *in vivo* hydrolysis of the diester as the high bioavailability of 3-MCPD from these compounds after oral administration [9].



3-MCPD = 3-monochloropropane-1,2-diol

Figure 1. Chemical structures of 3-MCPD and its esters (R = alquil).

Therefore, for risk assessment purposes, this evidence suggests that the complete hydrolysis of 3-MCPD diesters should be considered and, thus, the determination of the relationship between the concentrations of monoesters and diesters is highly recommended.

The proposed analytical approaches for determining 3-MCPD esters involve both indirect analysis, in which the total concentration of the compounds is measured as free 3-MCPD obtained after a hydrolysis/methanolysis procedure, and direct analysis, in which the different species of 3-MCPD esters are identified individually. Indirect methods have shown good application for routine tests due to the high sensitivity and the need of a reduced number of analytical standards. However, these procedures do not provide information related to the concentrations of diesters and monoesters. Based on this limitation, this application note proposes the use of SPE on silica cartridges for the separation of the different types of 3-MCPD esters before the transesterification step employed in the indirect determination of the compounds.

Experimental

Reagents and solvents

The following reagents and solvents were used in the analysis: tetrahydrofuran (analytical grade, Sigma-Aldrich), methanol, and acetonitrile (HPLC grade, Tedia); hexane, acetone, petroleum ether, ethanol, sulfuric acid, sodium bicarbonate, and ammonium sulfate (analytical grade; Synth); acid phenylboronic (97% purity, Sigma-Aldrich), diethyl ether (HPLC grade, Malinkrodt); ethyl acetate (HPLC grade, JT Baker), dichloromethane (HPLC grade, Carlo Erba). Water was purified by reverse osmosis.

Standards

3-MCPD-dipalmitoyl (PP-3-MCPD), 3-MCPD-dipalmitoyl-d5 (PP-3-MCPD-d5), 3-MCPD-1-monooleate (1-O-3-MCPD), and 3-MCPD-1-monooleate-d5 (1-O-3-MCPD-d5) were purchased from Toronto Research Chemicals. The stock solutions were prepared at a concentration of 0.5 mg/mL by dissolving the standards in tetrahydrofuran.

Procedure to determine the total amount of 3-MCPD esters

The total amount of 3-MCPD esters (corresponding to the sum of diesters and monoesters) was determined according to Zelinková *et al.* (2006) [3] and Hrncirik *et al.* (2011) [10], with some modifications. The sample (100 mg) was weighed into a 10-mL screwcap tube and dissolved in 1 mL of PP-3-MCPD-d5 0.5 µg/mL (prepared in THF). After adding 1.8 mL of sulfuric acid in methanol (1.8%, v/v), the tube was sealed and incubated in a water bath at 40 °C for 16 hours for transesterification and release of 3-MCPD bound to fatty acids. The reaction was stopped by adding 0.5 mL of a saturated aqueous solution of sodium bicarbonate, and the organic solvents were evaporated under a steam of N_2. The fatty acid methyl esters (FAMEs) were separated by adding 2 mL of ammonium sulfate 20% followed by liquid-liquid extraction with hexane (2 × 2 mL). An aliquot of 250 µL of the derivatizing solution (1 g of phenylboronic acid dissolved in 4 mL of acetone:water, 19:1, v/v) was added to the aqueous extract. The tube was sealed, shaken for 15 seconds on a vortex mixer, and heated in a water bath at 90 °C for 20 minutes. After cooling at room temperature, 2 mL of hexane were added and the tube was vortexed for 30 seconds to extract the derivatized 3-MCPD. The supernatant (hexane) was transferred to a vial, stored in the freezer for at least 4 hours and filtered through cotton before injection into the chromatographic system.

Procedure to determine 3-MCPD diesters and monoesters

For the analysis of 3-MCPD diesters and monoesters, the sample (100 mg) was weighed into a gas chromatography vial and diluted with 50 µL of hexane. After the addition of 50 µL of PP-3-MCPD-d5 and 1-O-3-MCPD-d5, both prepared in hexane at a concentration of 10 µg/mL, the sample was applied on a preconditioned silica cartridge HF Mega BE-SI 1 mg/6 mL (p/n 14256008). For the elution of the compounds, three procedures were evaluated (Table 1). The extracts resulting from the elution of diesters and monoesters were collected separately in 10-mL screwcap tubes and evaporated under a steam of N_2. The residue was dissolved in 1 mL of tetrahydrofuran. Transesterification and derivatization were performed as described above.

Table 1. Conditions Tested for the Separation of 3-MCPD Diesters and Monoesters using Solid Phase Extraction (SPE)

Parameters	Procedure 1	Procedure 2	Procedure 3
Cartridges	Silica 1 mg / 6 mL (Agilent)	Silica 1 mg / 6 mL (Agilent)	Silica 1 mg / 6 mL (Agilent) + C18 500 mg / 6 mL (Agilent)
Conditioning	4 mL petroleum ether	4 mL hexane	4 mL hexane (silica) 5 mL methanol (C18)
Diesters elution	12 mL petroleum ether:diethyl ether (96:4)	4 mL hexane + 8 mL hexane:dichloromethane: diethyl ether (89:10:1)	SPE 1) Silica: 4 mL hexane + 8 mL hexane:dichloromethane: diethyl ether (89:10:1) SPE 2) C18: 50 mL ethanol:acetonitrile: methanol (5:30:65)
Washing	3 mL petroleum ether:diethyl ether (60:40)	2 mL hexane: ethyl acetate (85:15)	–
Monoesters elution	5 mL petroleum ether:diethyl ether (60:40) + 2 mL diethyl ether	15 mL hexane: ethyl acetate (85:15)	–

The elution conditions used in Procedure 1 were adapted from Zelinková *et al.* (2009) [11]. Procedure 2 was based on the method reported by Seefelder *et al.* (2007) [12]. Procedure 3 was based on the method described by Yamazaki *et al.* (2013) [13] and determines only 3-MCPD diesters.

Considering the tested conditions, the best results were obtained using Procedure 2. The elution of the compounds was performed without using a vacuum pump, at a flow rate of approximately 0.7 mL/min. The established method was applied only to samples with total amount of 3-MCPD esters above 0.2 mg/kg.

Chromatographic analysis

The experiments were conducted on an Agilent 7890A Gas Chromatograph coupled to an Agilent 5975C Series GC/MSD System. An aliquot of 1 µL of the extract was introduced into the injector operating at 180 °C in splitless mode. The separation was carried out on a capillary column VF-1ms, 30 m × 0.25 mm, 0.25 µm (p/n CP8912) using helium as carrier gas at a flow rate of 1.2 mL/min. The following temperature program was used in the oven: 60 °C (held for 1 minute), 6 °C/min to 190 °C, 20 °C/min to 280 °C (held for 30 minutes). Detection was performed by Selected Ion Monitoring (SIM) after positive electron impact ionization (70 eV). The following ions were monitored: *m/z* 147, 91, and 196 for 3-MCPD and *m/z* 150, 93, and 201 for the internal standard 3-MCPD-d5.

Method validation

The method was in-house validated in relation to linearity, selectivity, LOD, LOQ, trueness (recovery), and precision (repeatability and within-laboratory reproducibility) according to the guidelines laid down by the Instituto Nacional de Metrologia, Normalização e Qualidade Industrial [14]. These experiments were conducted with a sample of extra virgin olive oil, for which the presence of 3-MCPD esters was not detected.

Results and Discussion

This method for the separation of 3-MCPD monoesters and diesters was initially based on the procedure described by Zelinková *et al*. (2009) [11], in which the compounds were separated in a chromatographic column of silica gel. In this study, we decided to investigate the feasibility of SPE on silica cartridges to optimize the time of analysis and reduce the volume of solvents. For that, three procedures were tested for the elution of the compounds (Table 1).

The data obtained during the in-house validation of the procedure that demonstrated the best preliminary results (Procedure 2) are shown in Tables 2 and 3. The method showed good linearity in the investigated range and no matrix effects. LOD and LOQ can be considered satisfactory for the determination of low concentrations of the compounds. Recovery ranged from 74 to 98% and coefficients of variation (CV) for repeatability and within-laboratory reproducibility are considered acceptable, since the HORRAT values (CV obtained in the experiment divided by the CV calculated from the Horwitz equation at the concentration of interest) were below 2 (not shown). The chromatograms obtained for the sample of extra virgin olive oil not spiked (blank sample), and spiked at 0.2 mg/kg PP-3-MCPD and 1-O-3-MCPD, and 1 mg/kg of their respective deuterated internal standards are illustrated in Figure 2.

Table 2. Linearity, Selectivity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Analite	Linearity	Selectivity	LOD (mg/kg)	LOQ (mg/kg)
PP-3-MCPD	0 – 4 mg/kg $y = 2.980x + 0.023$ $R^2 = 0.999$	No matrix effects	0.1	0.2
1-O-3-MCPD	0 – 4 mg/kg $y = 2.775x – 0.007$ $R^2 = 0.999$	No matrix effects	0.1	0.2

Table 3. Recovery and Precision

Analite	Spike level (mg/kg)	R (%)	CV_r (%)	CV_R (%)
PP-3-MCPD	0.2	77	11.5	13.3
	1.0	89	9.7	9.9
	4.0	74	7.0	6.8
1-O-3-MCPD	0.2	98	10.8	16.2
	1.0	90	7.3	14.4
	4.0	83	6.9	12.1

R = recovery (12 replicates for each spike level); CV_r = coefficient of variation under repeatability conditions (same day, six replicates for each spike level); CV_R = coefficient of variation under within-laboratory reproducibility conditions (same laboratory, different days, 12 replicates for each spike level).

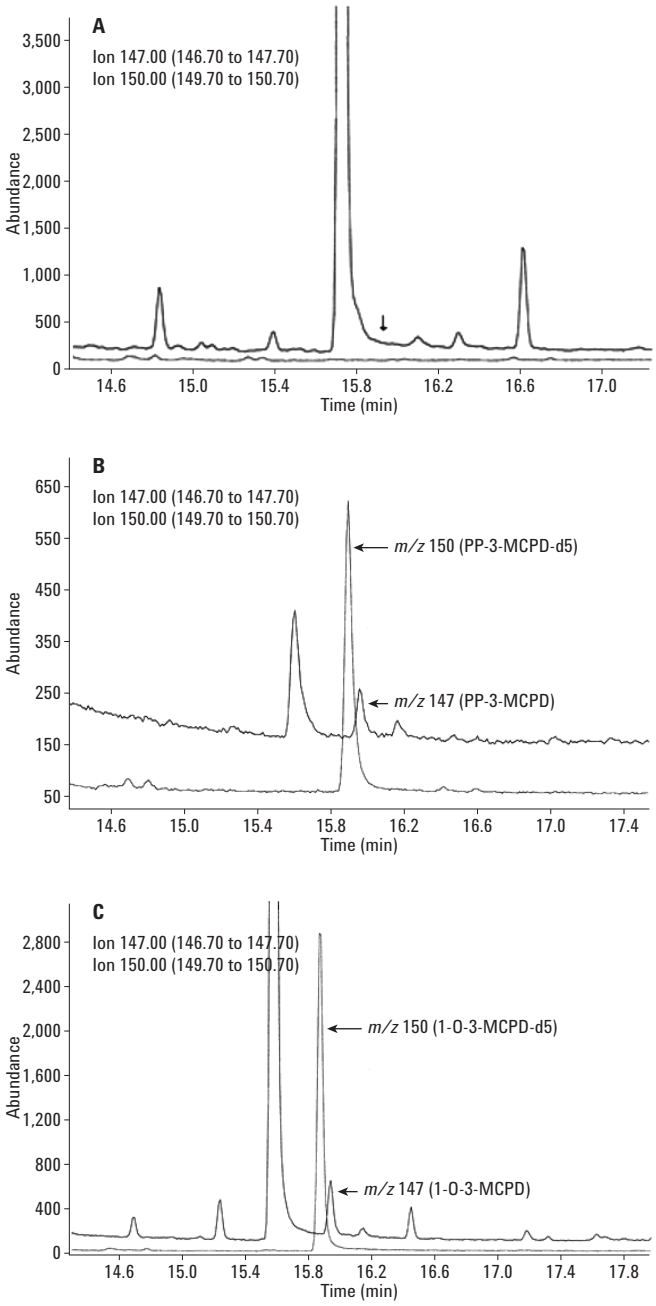


Figure 2. Ion chromatograms obtained for a sample of extra virgin olive oil, without the addition of the standards (A) and spiked at 0.2 mg/kg PP-3-MCPD (B) and 1-O-3-MCPD (C).

This method was applied to 61 samples of vegetable oils and fats, of domestic and industrial use, and the results are shown in Tables 4 and 5. The level of 3-MCPD diesters (Table 4) varied from not detected to 3.52 mg/kg (45 samples with results above the LOQ). The % of diesters calculated in relation to the total concentration of esters of the positive samples (above LOQ) shows that most of the compounds are present in this form, which was also observed by other authors [3, 11-13]. The levels of 3-MCPD monoesters (Table 5) ranged from not detected to 2.63 mg/kg (23 samples with results above the LOQ) and, in general, the data confirms that such compounds are present in lower concentrations. This is considered highly relevant from a toxicological point of view, since studies recently conducted in rodents showed a significant bioavailability of 3-MCPD from 3-MCPD-dipalmitoyl after oral administration [9].

Table 4. Levels of 3-MCPD Diesters in Oils and Fats (N = 61)

		3-MCPD diesters (mg/kg)		
Sample	**N**	**N > LOQ**	**Min–max**	**%***
Soybean	6	4	nd–1.04	80–94
Maize	3	2	< 0.20–1.04	90–93
Sunflower	6	0	nd–< 0.20	–
Peanut	1	1	0.25	83
Sesame	2	2	0.39–0.40	69–82
Canola	4	1	nd–0.22	75
Mixed (canola, maize and sunflower)	1	0	< 0.20	–
Olive**	1	1	2.46	48
Dendê	4	1	< 0.20–0.24	74
Palm	13	13	0.24–2.44	53–96
Palm (fat)	5	5	0.57–2.43	35–93
Palm (olein)	1	1	0.89	68
Shortening	2	2	2.64–3.52	84–91
Hydrogenated vegetable fat	5	5	0.21–0.56	59–96
Mixed fats	7	7	0.27–0.30	35–69

N = number of samples; LOQ = 0.2 mg/kg; nd = results below the LOD (0.1 mg/kg). *% calculated in relation to the total amount of esters, considering only the samples with results above the LOQ. ** Sample containing olive oil and pomace oil.

Table 5. Levels of 3-MCPD Monoesters in Oils and Fats (N = 61)

		3-MCPD monoesters (mg/kg)		
Sample	**N**	**N > LOQ**	**Min–max**	**%***
Soybean	6	1	nd–0.24	20
Maize	3	0	nd–< 0.20	–
Sunflower	6	0	nd–< 0.20	–
Peanut	1	0	nd	–
Sesame	2	0	nd–< 0.20	–
Canola	4	0	nd–< 0.20	–
Mixed (canola, maize and sunflower)	1	0	< 0.20	–
Olive**	1	1	2.63	52
Dendê	4	0	nd–< 0.20	–
Palm	13	11	< 0.20–0.82	13–47
Palm (fat)	5	3	< 0.20–1.37	7–65
Palm (olein)	1	1	0.41	32
Shortening	2	2	0.35–0.50	9–16
Hydrogenated vegetable fat	5	0	nd–< 0.20	–
Mixed fats	7	4	< 0.20–0.51	51–65

N = number of samples; LOQ = 0.2 mg/kg; nd = results below the LOD (0.1 mg/kg). *% calculated in relation to the total amount of esters, considering only the samples with results above the LOQ. ** Sample containing olive oil and pomace oil.

Due to the high occurrence of nonquantifiable results of 3-MCPD monoesters, it is considered more appropriate to calculate the levels of these compounds by difference, taking into account the total concentration of esters and diesters. Thus, only the fraction corresponding to the elution of 3-MCPD diesters is sufficient to obtain the required information.

Conclusions

This study demonstrates that the separation of 3-MCPD diesters and monoesters can be performed successfully employing the technique of SPE on silica cartridges Agilent HF Mega BE-SI, presenting advantages over the use of a glass chromatographic column such as the optimization of the time of analysis and reduced volume of solvents. The validation results indicated that the procedure may be applied with appropriate confidence level for the determination of these compounds in oils and fats. The analysis of 61 samples showed that most of 3-MCPD esters were present as diesters, which is highly relevant from a toxicological point of view.

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Acknowledgement

São Paulo Research Foundation (FAPESP), grant 2011/08936-0.

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Printed in the USA
October 31, 2013
5991-3406EN

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