

Simultaneous Determination of Chloropropanol Fatty Acid Esters in Refined Corn Oil Using GC-MS

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Citation: Jin G, Wang C, Wu W, Mo Q (2018) Simultaneous Determination of Chloropropanol Fatty Acid Esters in Refined Corn Oil Using GC-MS. Int J Anal Bioanal Tech: IJABT-101. DOI: 10.29011/AABST-101. 000001

Received Date: 22 December, 2017; **Accepted Date:** 05 January, 2018; **Published Date:** 15 January, 2018

Abstract

A Gas Chromatography Mass Spectrometry (GC-MS) method was developed for the simultaneous determination of 3-chloropropane-1,2-diol fatty acid esters (3-MCPDEs), 2-chloropropane-1,3-diol fatty acid esters (2-MCPDEs), 1,3-dichloro-2-propanol fatty acid esters (1,3-DCPES) and 2,3-dichloro-1-propanol fatty acid esters (2,3-DCPES) in refined corn oil. The analytes were extracted by solid-phase extraction and were eluted with ethyl acetate. The detection was performed by selected ion monitoring mode for the target compounds. The procedure showed good linearity and precision. The limit of detection and quantification were less than 0.03 ng/ml and 0.1 ng/ml, respectively. The recoveries of chloropropanol fatty acid esters were in the range of 98.6 % ~ 108.3 %. The method has been successfully applied to determine these compounds in refined corn oil.

Keywords: Chloropropanol Fatty Acid Esters; GC-MS; Refined Corn Oil

Introduction

The oil refining process was introduced to improve quality and safety. The process was optimized to reduce not only free fatty acids, natural flavor and color present in the crude oil but also the levels of minor contaminants such as poly aromatic hydrocarbons and pesticide residues [1-3]. In the process of refining, oil can be hydrolyzed and chlorinated to form chloropropanol esters under certain conditions [4]. The food contaminants chloropropanol and fatty acid esters have attracted considerable attention in the past few years due to their toxic properties and their occurrence in numerous foods [5-11]. In general, the chloropropanol includes of 3-monochloropropane-1,2-diol (3-chloropropane-1,2-diol, 3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD), 1,3-dichloro-2-propanol (1,3-DCP) and 2,3-dichloro-1-propanol (2,3-DCP) [12].

The chemical structures of chloropropanol are shown in Figure 1. 3-MCPD is an organic chemical compound which is carcinogenic [13-14], as the most commonly found member of chemical contaminants first found in hydrolyzed vegetable protein since 1978 [15-16]. 3- and 2-MCPD and their esters are formed during the hydrochloric acid hydrolysis of cereal materials, by reaction of the acid with lipids [17]. They are also formed during high temperature food processing operations such as the baking of low-moisture cereal based foods [18-19]. Further reaction of 3-MCPD with acetic acid can produce 1,3-DCP [20-21]. According to the WHO assessment report, the maximum temporary maximum daily tolerable intake (PMTDI) of 3-MCPD was 2 µg/kg BW [22]. The European Union (EU) has set a maximum concentration of 0.02 mg/kg of 3-MCPD in acid hydrolyzed vegetable protein (aHVP), and the Food and Drug Administration (FDA) sets a guidance limit of 1 mg/kg of 3-MCPD in aHVP. 1,3-DCP is not an approved food additive and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has set a limit at 0.005 mg/kg.

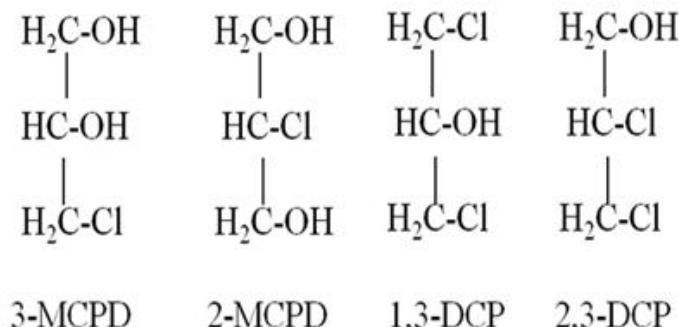


Figure 1: Chemical structures of Chloropropanol.

Chloropropanols have highly polar and relatively small molecular weight. After derivatization, it can improve the volatility and detection sensitivity, and increase the relative molecular mass of analyte, which is very important for mass spectrometry analysis. This article explains the N-heptafluorobutyrylimidazole as derivatization reagent. The relative molecular mass of chloropropanols has been improved greatly after derivatization, the GC-MS analysis can obtain higher mass to charge ratio of characteristic ion, the specificity is improved, and the sensitivity is obviously improved. N-hexane is generally used in the derived medium. Due to the rapid response of N-heptafluorobutyrylimidazole to water, the water will affect the derivatization. In order to reduce the influence of moisture during derivatization, the extract must be dehydrated with sodium sulfate anhydrous. In addition, sodium chloride solution was added to eliminate excessive derivatization reagents. N-heptafluorobutyl diester derived from chloropropanol was used to GC-MS analysis.

A further sample purification procedure was introduced in this article to obtain sufficient removal of co-existing interferences that might disturb the quantitative and stable detection by GC-MS. A reliable GC-MS method for the quantification of MCPDs in refined corn oil is described in this research.

Experimental

Reagents and Chemicals

3-MCPD, 2-MCPD, 1,3-DCP, 2,3-DCP and N-heptafluorobutyrylimidazole were purchased from ANPEL laboratory technologies (Shanghai) Inc. Cnwbond macroporous diatomite cartridge (5g, 60ml) was also purchased from CNW technologies (Lot: F5790040). All chemicals were commercially available and analytical grade. Milli-Q water ($18.2\text{M}\Omega\text{ cm}^{-1}$) was applied for preparation of all aqueous solutions. 9 batches samples of refined corn oil come from different pharmaceutical and excipient factories

Instruments and Measurements

The GC-MS experiment was carried out on Agilent GC-MS5975. Advanced multi-tube vortexer was from Talboys (USA). The Agilent 7890Agas chromatography system was used. A 60m long, 0.32 mm ID GC column with 0.5 μm particle size stationary phase (DB-5) was used. High purity helium was used as carrier gas at a constant flow of 1ml/min. The oven temperature was held constant at 60°C for 1 min and then ramped to 90°C at 2°C/min, and then ramped to 270°C at 40°C/min to keep 10 min. The injector temperature was 250°C, and mode was splitless. The transfer line temperature between gas chromatograph and mass spectrometer was set to 280°C. Electron impact ion source (EI) was chosen as the ionization method. EI-MS analysis was performed in the positive ion mode. Electron impact ionization at 70eV was applied maintaining ion source temperature at 230°C. MS scan mode is Selected Ion Monitor (SIM) i.e. single ion monitor. The quantitative and qualitative ions are as follows (Table 1).

Compound	quantitative ions (m/z)	qualitative ions(m/z)
3-MCPD	253	275,289,291
2-MCPD	253	75,289,291
1,3-DCP	75	77,275,277
2,3-DCP	75	77,111,253

Table 1: The qualitative and quantitative ions of MCPDs.

Sample Extraction and Purification

About 0.1 g of refined corn oil sample was weighed accurately into a screw-capped 10 ml glass tube wherein 0.5 ml of methyl tert-butyl ether - ethyl acetate (8:2) and 1 ml of 0.5 mol/L sodium methoxide methanol solution were added. The mixture was shaken for 30 s and incubated for 4 min. And 100 μL of acetic acid was added to stop reaction. Then 3 ml of 20% sodium bromide and 3 ml of n-hexane were added, then shaken for 30 s. Allow to stand for 1 min. Discard the upper n-hexane, extract with 3 ml for n-hexane again. Take lower layer solution into Cnwbond cartridge, balance for 10 min. 20 ml of ethyl acetate was then applied to the cartridge, and the eluent was collected. Then 4 g of sodium sulfate anhydrous was added into the eluent, stand for 30 min, then filter. The filtrate was evaporated to dryness using a nitrogen stream. The dried residues were carefully dissolved in 2 ml n-hexane for derivatization. 0.04 ml of N-heptafluorobutyrylimidazole was added, then vortexed for 20 min at 70. Allow to stand at room temperature. Add 2 ml of 20 % sodium chloride solution, vortexed for 1 min. Take upper layer, add 0.3 g sodium sulfate anhydrous to remove water. Prior to GC-MS, the hexane phase was filtered through a 0.45 μm filter. Series of standard solutions and blank solution was prepared as the same way of derivatization. Inject 1

μl of above solution into GC-MS, measure the corresponding peak area, and calculate the quality of MCPDs according to the standard curve.

Calibration Curve

Precisely weigh proper 3-MCPD, 2-MCPD, 1,3-DCP and 2,3-DCP to prepare 1 mg/L mixed standard stock solution. Take the standard stock solution of MCPDs (0.01 ml, 0.05 ml, 0.1 ml, 0.2 ml, 0.4 ml, and 0.8 ml) into 10 ml colorimetric tube, add 2 ml of n-hexane and mix. The series of solutions are used to construct calibration plots (5, 25, 50, 100, 200, and 400 ng/ml). The calibration curve was generated from plots using the chromatographic peak area for each analyte in the extracted ion chromatogram.

Recovery Tests

The recovery tests were performed by spiking known amounts of MCPDs into refined corn oil. As MCPDs were below the detection limits, standard solution was mixed with samples. Weigh 3 oil samples about 0.1g, add 0.16, 0.2, and 0.24 ml of 1 mg/ml MCPDs mixed standard solution, respectively. The extraction and purification were carried out as described in the previous section. (Sample Extraction and Purification)

Results and Discussions

Linearity, LOD and LOQ

Under the optimal separation and MS detection conditions, the linearity of MCPDs was performed with six different concentrations of 1,3-DCP, 2,3-DCP, 3-MCPD, and 2-MCPD, respectively. Each concentration was analyzed in triplicate. Calibration curves were constructed by plotting the integrated peak areas (Y) versus the corresponding concentrations of the injected standard solutions (X) in the range of 10 ~ 800 ng. The calculated results are summarized in Table 2. Good linear calibrations ($r^2 > 0.998$) for all the analytes were achieved in a relatively wide concentration range. The limits of detection (LOD) and quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively (Table 2).

Compound	Calibration curve	Correlation coefficient (r^2)	LOD (ng/ml)	LOQ (ng/ml)
1,3-DCP	$y = 59.15x - 311.6$	1	0.03	0.1
2,3-DCP	$y = 66.15x - 311.8$	1		
3-MCPD	$y = 66.95x - 1176$	0.998		
2-MCPD	$y = 63.07x - 1132$	0.999		

Linearity was obtained with six different concentrations of chloropropanol fatty acid esters.
S/N is the abbreviation of “signal-to-noise ratio”.
LOD (limit of detection) was estimated based on S/N=3.
LOQ (limit of quantification) was estimated based on S/N=10.

Table 2: Calibration curves, LOD and LOQ for MCPDs obtained with GC-MS method.

Precision

The precision of the method was determined by analysis of sample for MCPDs. The intra-day assay variation was evaluated by analyzing the known concentrations of MCPDs in five replicates during a single day, while inter-day variation was evaluated in duplicated on three consecutive days, respectively. To confirm the repeatability, six independently prepared solutions were analyzed. The results of precision and repeatability are summarized (Table 3). The intra- and inter-day variations were less than 5.3%, indicating that satisfactory precision and stability of the samples were achieved. Furthermore, the analytical method developed a good repeatability with RSD less than 2.5% (n = 6) for MCPDs in refined corn oil.

Compound	Concentration(μg/ml)	Precision RSD (%) (n=5)	Inter-day	Repeatability (n=6)
		Intra-day		RSD (%)
1,3-DCP	0.4	1.1	2.1	1.8

2,3-DCP		2.1	1.0	2.5
3-MCPD		0.1	1.5	2.1
2-MCPD		0.4	5.3	1.4

Table 3: Precision and repeatability of the MCPDs.

Recovery

Accuracy of the method was determined by performing the recovery experiments. Known amount of the standard at 80%, 100%, and 120% levels were added to the samples. 160ng, 200ng, and 240ng MPCDs standards were added into the sample (batch no.1703001), respectively, to evaluate the accuracy of the developed analytical method. The mixtures were extracted and quantified as method. Then the quantity of each component was subsequently calculated from the corresponding calibration curves. Three replicate samples of each concentration level were prepared. The method had a satisfactory accuracy with the overall recovery from 98.6% to 108.3% for the MPCDs.

Sample Analysis

The proposed GC-MS method was applied to simultaneously determine of four major MCPDs in refined corn oil. Each sample was determined in triplicate. The results show that only 1,3-DCP exists in the samples with the range of 0~0.2 ng. 2,3-DCP, 3-MCPD and 2-MCPD have been not detected.

Aim and Conclusion

A Gas Chromatography Mass Spectrometry (GC-MS) method was developed for the simultaneous determination of 3-chloropropane-1,2-diol fatty acid esters (3-MCPDEs), 2-chloropropane-1,3-diol fatty acid esters (2-MCPDEs), 1,3-dichloro-2-propanol fatty acid esters (1,3-DCPEs) and 2,3-dichloro-1-propanol fatty acid esters (2,3-DCPEs) in refined corn oil, in which solid phase extraction was used as the sample clean-up procedure and the analyses of MCPD and DCP were respectively performed by derivatization. The method showed good sensitivity, linearity, and recovery, and it was successfully applied to determine the amounts of these compounds in refined corn oil samples. Further, we believe this method will be useful in development an effective safety study of corn oil.

Acknowledgements

This work was financially supported by the 2016 National Drug Evaluation Special Foundation of China and Guangdong Medical Science Research Fund Project (No. A2016363).

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