Research Article

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

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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 2ng/ml and 5ng/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.

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 chlorpropanol are shown in Figure 1. 3-MCPD is an organic chemical compound which is carcinogenic [13-16], as the most commonly found member of chemical contaminants first found in hydrolyzed vegetable protein since 1978 [17,18]. 3- and 2- MCPD and their esters are formed during the hydrochloric acid hydrolysis of cereal materials, by reaction of the acid with lipids [19]. They are also formed during high temperature food processing operations such as the baking of low-moisture cereal based foods [20,21]. Further reaction of 3-MCPD with acetic acid can produce 1,3-DCP [22,23]. According to the WHO assessment report, the maximum temporary maximum daily tolerable intake (PMTDI) of 3-MCPD was 2μg/kg BW. 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 1mg/kg of 3-MCPD in aHVP [17,24,25]. 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 0.005mg/kg (Figure 1).

Chloropropanols have highly polar and small relatively 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 to determination of chloropropanol fatty acid esters. 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-heptafluorobutylyl diester derived from chloropropanol was used to GC-MS analysis.

In conclusion, 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 chloropropanol esters 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. Cnw-bond macro porous diatomite cartridge (5g, 60ml) was also purchased from CNW technologies (Lot: F5790040). All chemicals were commercially available and analytical grade. Milli-Q water (18.2MΩcm^{-1}) was applied for preparation of all aqueous solutions. Sample of refined corn oil comes from pharmaceutical excipient factory.

Instruments and measurements

The GC-MS experiment was carried out on Agilent GC-MS 5975. Advanced multi-tube vortexer was from Talboys (USA). The Agilent 7890A gas chromatography system was used. A 60m long, 0.32mm ID GC column with 0.5μm particle size stationary phase (DB-5) was used. High purity helium with was carrier gas with 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 split less. 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).

Sample extraction and purification

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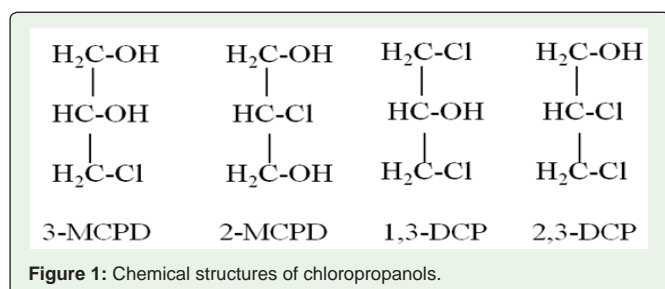

Figure 1: Chemical structures of chloropropanols.

Table 1: The qualitative and quantitative ions of chloropropanol derivative.

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

for 1min. Take upper-layer; add 0.3g sodium sulfate anhydrous to remove water. Prior to GC-MS, the hexane phase was filtered through a 0.45μm filter. The blank solution was prepared as the same way of derivatization. Inject 1μl of above solution into GC-MS, measure the corresponding peak area, and calculate the quality of chloropropanols 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, 0.05, 0.1, 0.2ml, 0.4ml, and 0.8ml) into 10ml colorimetric tube, add 2 ml of n-hexane and mix. Series of standard solutions were prepared as the same way of sample derivatization. The series of solutions are used to construct calibration plots (5, 25, 50, 100, 200, and 400ng/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 chloropropanols into refined corn oil. As chloropropanols of sample were below the detection limits, standard solution was mixed with sample. Weigh 3 oil samples about 0.1g, add 0.16, 0.2, and 0.24 ml of 1mg/ml chloropropanol mixed standard solution, respectively. The extraction and purification were carried out as described in the previous section (See part 3.3).

Results and Discussions

Linearity, LOD and LOQ

The linearity of chloropropanols was performed with six different concentrations of 1,3-DCP, 2,3-DCP, 3-MCPD, and 2-MCPD under the optimal separation conditions and MS detection. 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 5 ~ 400 ng/ml. The calculated results are summarized in Table 2. Good linear calibrations (r2 > 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).

Table 2: Calibration curves, LOD and LOQ for chloropropanols derivative.

Compound	Calibration curve	Correlation coefficient (r²)	LOD (ng/ml)	LOQ (ng/ml)
1,3-DCP	y = 59.15x-311.6	1.000		
2,3-DCP	y = 66.15x-311.8	1.000	2	5
3-MCPD	y = 66.95x-1176	0.998		
2-MCPD	y = 63.07x-1132	0.999		

Table 3: Precision and repeatability of the chloropropanols.

Compound	Concentration (µg/ml)	Precision RSD (%) (n=5)		Repeatability (n=6)
		Intra-day	Inter-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

Precision

The precision of the method was determined by analysis of sample for chloropropanols. The intra-day assay variation was evaluated by analyzing the known concentrations of chloropropanols 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 in 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 chloropropanols in refined corn oil (Table 3).

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 standard chloropropanols were added into the sample, respectively, to evaluate the accuracy of the developed analytical method. The mixtures were extracted and quantified as above 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 results are summarized in Table 4. The method had a satisfactory accuracy with the overall recovery from 98.6 to 108.3 % for the chloropropanols.

Table 4: Recoveries of the chloropropanols.

Compound	Level of standard added (%)	Added amount (ng)	Detected amount (ng)	Recovery (%)	RSD (%)
1,3-DCP	80	160	167.36	104.6	1.2
	100	200	201.04	100.5	1.0
	120	240	241.35	100.6	1.4
2,3-DCP	80	160	173.30	108.3	1.5
	100	200	203.84	101.9	1.2
	120	240	241.11	100.5	1.7
3-MCPD	80	160	173.04	108.2	1.9
	100	200	215.39	107.7	0.8
	120	240	236.52	98.6	0.4
2-MCPD	80	160	158.50	99.1	1.7
	100	200	197.43	98.7	1.3
	120	240	241.61	100.7	2.1

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 3-MCPD, 2-MCPD, 1,3-DCP and 2,3-DCP haven't been detected.

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