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A Novel Approach of Periodate Oxidation Coupled with HPLC-FLD for the Quantitative Determination of 3-Chloro-1,2-propanediol in Water and Vegetable Oil

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Supporting Information

ABSTRACT: A novel approach of periodate oxidation coupled with high-performance liquid chromatography (HPLC)–fluorescence detection (FLD) for the quantitative determination of 3-chloro-1,2-propanediol (3-MCPD) has been established. The essence of this approach lies in the production of chloroacetaldehyde by the oxidization cleavage of 3-MCPD with sodium periodate and the HPLC analysis of chloroacetaldehyde monitored by an FLD detector after fluorescence derivatization with adenine. The experimental parameters relating to the efficiency of the derivative reaction such as concentration of adenine, chloroacetaldehyde reaction temperature, and time were studied. Under the optimized conditions, the proposed method can provide high sensitivity, good linearity (r^2 = 0.999), and repeatability (percent relative standard deviations between 2.57% and 3.44%), the limits of detection and quantification were 0.36 and 1.20 ng/mL, respectively, and the recoveries obtained for water samples were in the range 93.39–97.39%. This method has been successfully applied to the analysis of real water samples. Also this method has been successfully used for the analysis of vegetable oil samples after pretreatment with liquid–liquid extraction; the recoveries obtained by a spiking experiment with soybean oil ranged from 96.27% to 102.42%. In comparison with gas chromatography or gas chromatography–mass spectrometry, the proposed method can provide the advantages of simple instrumental requirement, easy operation, low cost, and high efficiency, thus making this approach another good choice for the sensitive determination of 3-MCPD.

KEYWORDS: *3-chloro-1,2-propanediol (3-MCPD), periodate oxidation, chloroacetaldehyde, high-performance liquid chromatography (HPLC)–fluorescence detector (FLD), water samples, vegetable oil*

INTRODUCTION

3-Chloropropane-1,2-diol (3-MCPD) is a well-known food-processing contaminant. Since its first detection in acid-hydrolyzed vegetable proteins (HVP) and in soy sauces,[1] 3-MCPD could be detected in various kinds of food products such as crackers, bread, toast, and other bakery products, malt, grilled cheese, meat products, fish products, and soups.[2,3] More recently, the occurrences of free and ester-bound 3-MCPD in some edible oils have been reported,[4] thus attracting more attention to this traditional food processing contaminant.

3-MCPD was identified as a genotoxic carcinogen by the European Commission's Scientific Committee for Food, and the UK Food Advisory Committee recommended that 3-MCPD should be reduced in foods and food ingredients to minimum levels of less than 0.01 mg/kg. The European Commission has adopted a regulatory limit of 0.02 mg/kg for 3-MCPD in liquid soy sauce and HVP.[5] A provisional maximum tolerable daily intake (PTDI) of 2 mg/kg body weight per day was recommended for 3-MCPD by JEFCA and SCF.[6,7]

To protect the consumer's health, the development of effective and convenient methodologies to identify and determine 3-MCPD in foods is urgently needed and of great significance. Currently, several analytical techniques have been developed for the quantitative determination of 3-MCPD in foods including gas chromatography (GC) with electron capture detection (ECD), GC combined with mass spectrometry (MS), capillary electrophoresis, and molecular imprinting. Mostly, GC-MS is used as a sensitive approach for analyzing 3-MCPD. Various derivatization schemes employing different derivatizing agents, such as *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA),[8] phenylboronic acid (PBA),[9,10] butaneboronic acid (BBA),[11] 1-trimethylsilylimidazole (TSIM),[12] heptafluorobutyrylimidazole (HFBI), and heptafluorobutyric acid (HFBA),[13,14] have been reported. The GC-MS method had been applied for the analysis of 3-MCPD in a variety of matrices, and the detection limit achieved by these methods was generally in the parts-per-billion range. However, from the industrial applicability and routine analysis point of view, the GC-MS method still encounters some problems such as comparatively high instrumental requirement, expensive measurement cost, and tedious sample preparation (e.g., the extraction of 3-MCPD from aqueous solution). Therefore, a simple and sensitive method for the routine analysis of 3-MCPD in different matrices is very desirable.

Herein, we will present a novel sensitive method for the quantitative determination of 3-MCPD based on periodate oxidation combined with high-performance liquid chromatography (HPLC)–fluorescence detector (FLD). In this assay, 3-

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MCPD in the aqueous solution was first cleaved to produce chloroacetaldehyde with the treatment of sodium periodate and then was fluorescent derivatized with adenine to produce εAde, which could be analyzed with HPLC-FLD. The proposed method showed distinct advantages as follows: First, the sample pretreatments of periodate oxidization and fluorescence derivatization were processed in aqueous solution, and the reaction mixture did not need any further treatment and could be directly injected for HPLC analysis, thus providing the advantages of simple instrumental requirement, easy operation, low cost, and being environmentally friendly. Furthermore, the specific fluorescence derivatization of chloroacetaldehyde with adenine and the fluorescent detection at a specific wavelength will also guarantee the high sensitivity and excellent selectivity of the proposed method.

The proposed method has been successfully applied for the determination of 3-MCPD in real water samples and edible oils. To the best of our knowledge, so far there is no publication on the sensitive determination of 3-MCPD by using HPLC-FLD. Our study will initiate this work and will provide another choice for the routine analysis of 3-MCPD.

MATERIALS AND METHODS

Materials. 3-MCPD standard, chloroacetaldehyde diethyl acetal (99.0%), glycerol (99.5%), and adenine (99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium periodate, sodium dihydrogen phosphate, disodium hydrogen phosphate, and hydrochloride solution were obtained from Aladdin Chemical Reagent Co. (Shanghai, China). Methanol (HPLC grade) was obtained from Tedia (Fairfield, OH, USA). Wahaha bottled water (Wahaha Group, Hangzhou, China) was obtained from a local supermarket. Tap water was collected in the lab. Vegetable oils such as soybean oil, corn oil, and rice bran oil were obtained from a local supermarket. Spectrophotometric grade purified water from J&K Scientific Ltd. (Beijing, China) was used for the overall measurements.

Preparation of 3-MCPD Standard Solution. A 100.0 μg/mL stock solution of 3-MCPD was prepared in saturated sodium chloride solution (to suppress the hydrolysis of substituted chloride and to avoid its decomposition) and was stored at 4 °C in the dark. For the preparation of calibration standard solution, the stock solution was diluted to the concentration range of 0.008−12.0 μg/mL with purified water.

Periodate Oxidation of 3-MCPD. The periodate oxidation of 3-MCPD solution was performed as follows: 0.25 mL of sodium periodate solution (70 mM) was added to 1.5 mL of 3-MCPD standard solution. After 0.5 min of vortex-mixing, the mixture was allowed to stand in the dark at room temperature for 30 min, and subsequently the excess sodium periodate was deactivated by the addition of 0.25 mL of sodium sulfite (140 mM) and incubated for 15 min at room temperature. Meanwhile, 1.5 mL of purified water and glycerol solution (2.4 μg/mL) were also processed with the same procedure for comparison purposes.

Preparation of Chloroacetaldehyde Solution. The preparation of chloroacetaldehyde was performed according to the procedure reported by Virta;[15] in brief, 5.0 mL of chloroacetaldehyde diethyl acetal (32 mmol) was mixed with 1 M HCl (15 mL) and ethanol (5.0 mL) and then incubated for 2 h at 70 °C with magnetic stirring. The final solution was diluted to the required concentration with 0.1 M phosphate buffer solution (PBS, pH = 4.5) containing 1 M sodium chloride and stored at 5 °C before use.

Fluorescence Derivatization. Adenine was selected as the fluorescence derivatization reagent, and the derivatization procedure was performed according to the report of Huang[16] with slight modification. Typically, 0.5 mL of 3.0 mg/mL adenine (dissolved in 0.1 M PBS solution, pH 4.5) was evenly mixed with the periodate-oxidized 3-MCPD solution (2.0 mL) in a screw-capped test tube and then was incubated for 3 h under 80 °C with mild magnetic stirring. During and after heating, the samples were protected from light by covering with aluminum foil. After being cooled to room temperature, the sample was highly centrifuged and an appropriate portion (e.g., 1 mL) was transferred to amber HPLC vials and was directly autoinjected for HPLC analysis. The samples could be stored at 4 °C prior to HPLC analysis for at least 48 h without noticeable degradation. To optimize the derivatization conditions, a series of single-factor experiments for the parameters such as the concentration of adenine, incubation temperature, and incubation time were conducted using the procedure described above. For comparison, the chloroacetaldehyde solution hydrolyzed by chloroacetaldehyde diethyl acetal was also processed with the same procedure.

Chromatographic Conditions. A Waters Breeze 2 HPLC system, consisting of 1525 binary HPLC pumps, a 2475 multi-wavelength fluorescence detector, and a 2707 autosampler, was used for the HPLC-FLD analysis. The system control, data acquisition, and processing were carried out with Waters Breeze 2 software. All of the samples in this study were chromatographed on a Luna C18 (2) column (5 μm, 150 × 4.60 mm, Phenomenex, Torrance, CA, USA) using 15% methanol in water as the mobile phase at a flow rate of 0.5 mL/min. The injection volume was 20 μL, and the fluorescence detection was performed at the optimized excitation with emission wavelengths at 311 and 405 nm, respectively. The slits were set to 4 nm for both excitation and emission.

Calibration Curve. In order to evaluate the linearity of the proposed method, a series of freshly prepared 3-MCPD standard solutions in the concentration range 0.008−12.0 μg/mL were analyzed with the previous HPLC-FLD procedures, and the integrated peak area of εAde was used for the quantification of 3-MCPD. The linearity was evaluated by plotting the peak area of εAde versus the concentration of 3-MCPD standard solution. The calibration curves were calculated by the equation $Y = aX + b$ using weighted least-squares regression. A correlation of more than 0.99 was desired for all the calibration curves. The limit of detection (LOD) and limit of quantitation (LOQ) were individually defined as the 3-MCPD concentration that gives a peak height three times (S/N = 3) and 10 times (S/N = 10) the level of baseline noise.

Analytical Features. To evaluate the proposed method, accuracy was determined at three different concentrations of quality controls (QC) (0.04, 0.1, and 0.2 μg/mL) each in five replicates. Similarly, precision was measured using five determinations per concentration for all QC samples. Intra- and interassay precision was measured by determinations at a particular day and also at five consecutive days.

The analytical recovery of this method was assessed by the ratio of found concentration and fortified concentration of 3-MCPD measured by a standard spiking experiment of two simulated samples. In brief, three different volumes (50, 100, 200 μL) of 3-MCPD standard solutions (10 μg/mL) were spiked to 2 mL of the simulated samples. After vigorously vortexing for 2 min, 1 mL of this mixture was subsequently periodate oxidized, fluorescence derivatized, and analyzed with the HPLC-FLD procedure. The fortified concentration of 3-MCPD was equal to the difference of concentration before and after spiking, and the found concentration was calculated by the regression equation with target peak area.

Analysis of 3-MCPD in Vegetable Oil. Three different kinds of commercial vegetable oil samples, namely, corn oil, rice bran oil, and soybean oil, were analyzed with the proposed method coupled with liquid−liquid extraction (LLE). The detailed procedure was as follows: 10 mL of oil sample was weighted into a 50 mL screw-capped glass tube and was dissolved in 20 mL of heptane. The solution was then extracted twice with 1.5 mL of NaCl solution (0.01 g/mL). Each extraction was performed by agitation in a rotary shaker for 10 min. After phase separation, the extracts were combined, 2.0 mL of which was subjected to analysis with the HPLC-FLD procedure as described above. For recovery studies, the soybean oil samples were spiked with 3-MCPD standard before the corresponding extraction procedure. A representative 100 mL of oil sample was weighed and fortified homogeneously with different volumes (0.02, 0.04, and 0.10 mL) of 3-MCPD solution in ethyl acetate (0.1 mg/mL) to obtain concen-

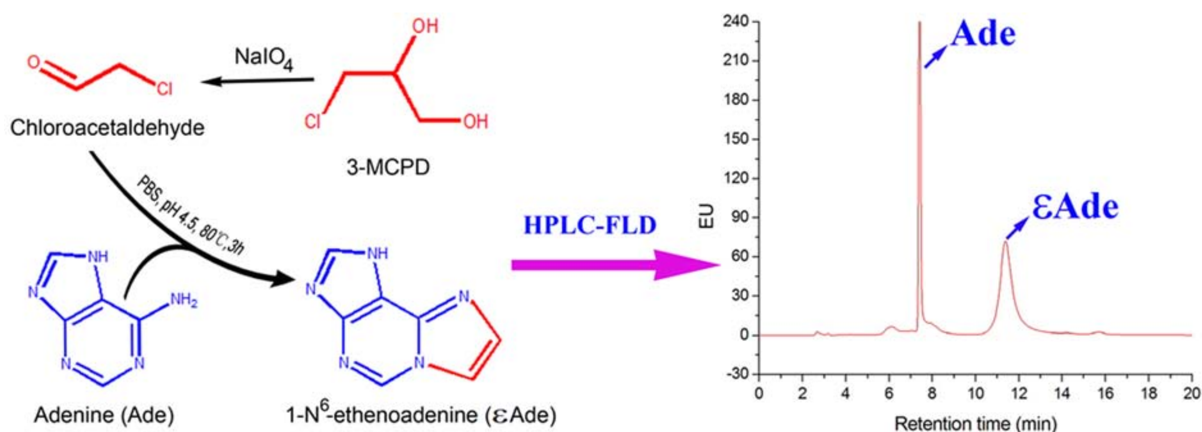

Figure 1. Analysis of 3-MCPD by periodate oxidation coupled with HPLC-FLD.

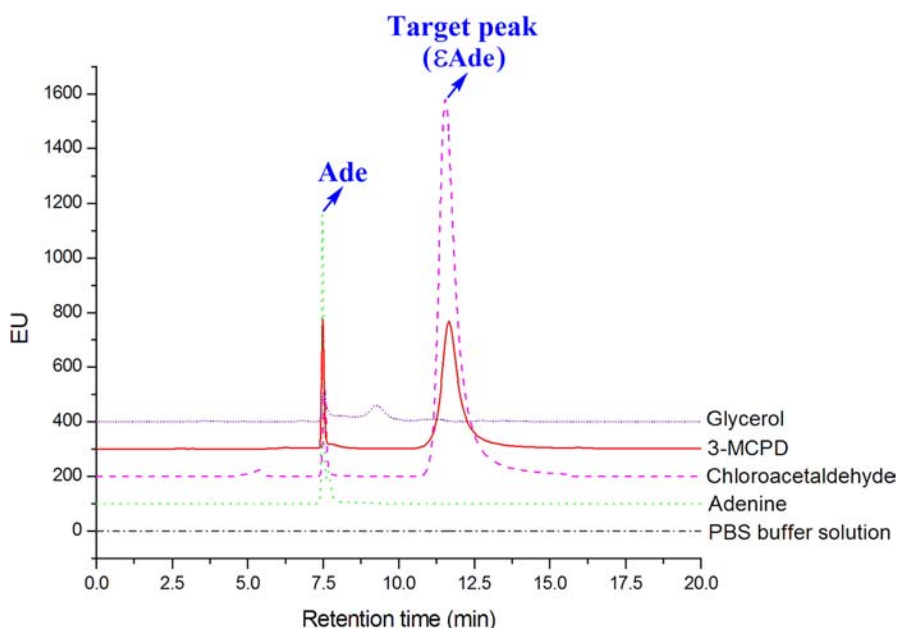

Figure 2. Comparison of the chromatograms of five different samples. 3-MCPD and glycerol solutions (2.4 μg/mL) were processed with periodate oxidation and fluorescence derivatization; chloroacetaldehyde (6.0 μg/mL) was directly derivatized with adenine fluorescent reagent. Adenine denotes a blank adenine fluorescent reagent incubated for 3 h at 80 °C; PBS buffer solution denotes a PBS solution (20 mM, pH 4.5) employed in the derivative reaction.

trations of 20, 40, and 100 ng/mL. The recovery was calculated by the ratio of found concentration to the added concentration of 3-MCPD.

Statistical Analysis. The linear regression of standard curves was completed with Origin software (version 6.0, OriginLab, Northampton, MA, USA). The analysis of variance (ANOVA) for linear regression was performed using the *F*-test, with $p < 0.05$ indicating the difference was statistically significant.

■ RESULTS AND DISCUSSION

Principle of This Assay. Periodate oxidation, also called the Malaprade reaction, first found in 1928 by Malaprade,[17] has been widely used in the quantitative determination of geological, environmental, and biological samples, which was reviewed in detail by Vlessidis[18] recently. As is well known, vicinal 1,2-diols can be cleaved into aldehyde by the oxidization of sodium periodate; for example, 3-MCPD can be cleaved into chloroacetaldehyde and formaldehyde. In this study, periodate oxidation was adopted for the pretreatment of 3-MCPD, and the produced chloroacetaldehyde was fluorescence derivatized with adenine. The resultant solution was then analyzed by HPLC-FLD for the indirect quantitative determination of 3-MCPD. The detailed principle of this approach is presented in Figure 1. In order to optimize the detection wavelength and to obtain a good sensitivity and low LOD for this assay, the excitation and emission spectra of adenine and εAde, the fluorescence derivatization product of chloroacetaldehyde, are compared in Figure S-1. The result showed that the maximum excitation and emission wavelengths of εAde have individually shifted 15 and 25 nm bathochromically in comparison with adenine, so the detection wavelength was set at λ_{ex} 311 nm, λ_{em} 405 nm in accordance with εAde's fluorescent features.

Chloroacetaldehyde has great nephrotoxicity and neurotoxicity with the possible mechanisms of glutathione depletion and lipid peroxidation and, moreover, binding with DNA to form etheno adducts.[19] According to the previous reports, chloroacetaldehyde can react with the adenine, cytosine, and guanosine derivative moieties of nucleosides and nucleotides,[20] with the possible mechanism discovered by Chichibabin,[21] which can be briefly described as alkylation of the endocyclic

nitrogen atom, ring-closing reaction of the exocyclic amino group with the aldehyde group, and the intermediate's dehydration. The obtained "etheno derivatives" of adenine-containing compounds showed much more intensive fluorescent properties than that of the cytosine and guanosine analogues (quantum yields 0.6 vs 0.003), and the yields were much higher than the latter ones (90% vs 25% and 45%). Therefore, the chloroacetaldehyde reaction of adenine-containing compounds had been widely used in the fields of biochemistry and molecular biology.[22,23] In this paper, adenine instead of adenosine was chosen as the derivative reagent since the presence of a carbohydrate group of adenosine will lead to the decrease of the derivative product's hydrophobicity, which will further affect its retention and lower the selectivity and accuracy of the proposed method.

According to a previous report,[24] the effect of pH on the reaction rate of fluorescence derivatization showed a bell-shaped profile with a maximum at pH 4.5 for the adenine and its derivatives. Therefore, we also performed the fluorescence derivatization under the conditions of pH 4.5.

Chromatograms. In order to investigate the separation of the final fluorescence derivatization reaction mixture and identify the fluorescent product of εAde, five different solutions such as blank PBS buffer solution, blank incubated adenine fluorescent derivative solution, and three fluorescence derivatization reaction mixtures produced by 3-MCPD, chloroacetaldehyde, and glycerol were chromatographed with the procedure described in the Materials and Methods section. Figure 2 shows the comparison of their chromatograms. It is obvious that the peak of εAde only appeared in the chromatograms of 3-MCPD and chloroacetaldehyde solutions, whose chemical structure could be further identified with mass spectral data (Figure S-2). As shown in Figure 2, the fluorescent product of εAde can be well separated with adenine, and their peak shapes were symmetrical, with retention times of 11.70 and 7.50 min, respectively, which is helpful for the improvement of the accuracy of this assay. In the profiling of glycerol, no peak occurred at the retention time of 11.70 min, which was mainly due to the different periodate oxidization product of formaldehyde and formic acid for glycerol,[25] and thus implied that the proposed method had a very good selectivity and specificity.

Optimization of the Parameters. To achieve high sensitivity and a low LOD for the proposed method, two important parameters, fluorescence derivation time and concentration of adenine, were investigated as follows.

Effect of Fluorescence Derivation Time. As described by Huang,[16] the chloroacetaldehyde reaction is a nonenzymatic reaction and can proceed at room temperature (24 °C) at a very low rate, and the increase of temperature can accelerate the reaction dramatically. However, an extremely high temperature (e.g., 100 °C) for the incubation will cause the degradation of the fluorescent product. Therefore, we have examined the time course for the formation of εAde at 80 and 90 °C in the presence of 3.0 mg/mL adenine and 8.0 μg/mL 3-MCPD oxidization cleaved solution. The result showed that the yield of εAde gradually increased with the reaction time and reached a plateau after 3 h incubation time (Figure 3). On the contrary, the yield below 90 °C was slightly higher than that at 80 °C, but somehow a little degradation occurred in the succeeding 1 h. So heating at 80 °C for 3 h was chosen as the optimum derivatization conditions for all subsequent experiments, with the consideration that batch incubation in the practical determination can largely improve the detection efficiency and compensate for the side effect of longer incubation time.

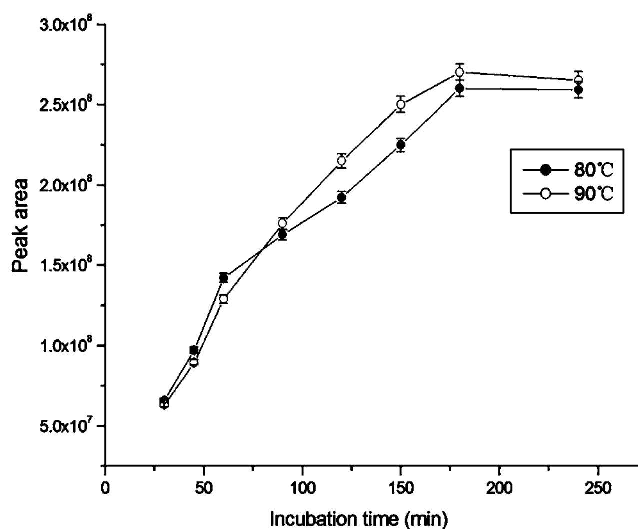

Figure 3. Dependence of the peak area of εAde on incubation time under the reaction temperature of 80 °C (●) and 90 °C (○). The concentration of 3-MCPD solution was 8.0 μg/mL. The derivatization and HPLC analysis were performed with the procedure described in the Materials and Methods section. Each point represents the average value of triplicate determinations.

Effect of Adenine Concentration. Previous reports usually focused on the measurement of a low concentration of adenosine after derivatization with a large excess of chloroacetaldehyde so as to obtain a high sensitivity and low LOD and LOQ. Instead, in this study, we will use an excess amount of adenine to fluorescence derivatize chloroacetaldehyde for the sensitive and indirect analysis of 3-MCPD in the original sample. To optimize the concentration of adenine, a 9.0 μg/mL standard chloroacetaldehyde solution and a 10.0 μg/mL 3-MCPD periodate-oxidized solution were reacted with a serials of adenine solutions with a concentration ranging from 0.10 to 6.0 mg/mL. The results are shown in Figure 4 and indicate that the response was basically linear, increasing with the concentration of adenine below 3.0 mg/mL. After that, it still increased but no longer linearly. In theory, the higher adenine concentration will lead to a higher sensitivity; however, the solubility of adenine in the reaction buffer solution practically limits the selection of adenine concentration. Accordingly, 3.0 mg/mL was suggested as a preferred adenine concentration as a compromise to achieve higher sensitivity and better reproducibility (which was easily affected by the solubility of adenine). In addition, the results also showed that the peak area ratio of chloroacetaldehyde and 3-MCPD remained at around 1.56 (RSD = 2.13%), which thus implied the oxidation cleavage of 3-MCPD was very complete, and the conversion rate reached 98.96%, the effect of the periodate oxidation step for this assay being negligible.

Analytical Features. Under the optimum conditions, various concentrations of 3-MCPD standard solution were analyzed with the proposed method, the calibration curve was constructed by comparing the peak areas against the analyte concentration, the result showed that a very satisfactory linear relationship between the amount of 3-MCPD and the integrated peak area was obtained over a wide range of 3-

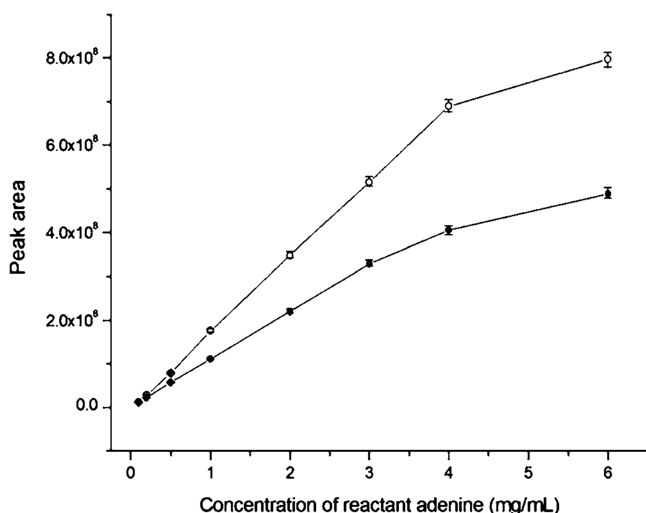

Figure 4. Effect of adenine concentration on the peak area of εAde. The 9.0 μg/mL chloroacetaldehyde (○) and 10.0 μg/mL 3-MCPD (●) solutions were derivatized with different concentrations of adenine under the conditions described above. Each point represents the average value of triplicate determinations.

MCPD concentrations (from 0.008 to 12.0 μg/mL), the linear regression equation can be expressed as eq 1:

$$Y = (2.8036 \pm 0.0217) \times 10^{7} \times X + (1.5110 \pm 0.5726) \times 10^{4} \quad (1)$$

where $n = 12$, $r^2 = 0.999$, SD = 0.8347×10^4, and $p < 0.0001$. The LOD and LOQ calculated with S/N values of 3 and 10 were 0.36 ng/mL and 1.20 ng/mL, respectively, which was similar to that of the GC-MS/MS method reported by Carro[26] and a little lower than that of the GC-ECD method by Matthew.[30] In addition, several other selected methods for the analysis of 3-MCPD in various matrices are also summarized in Table 1. The LOD data showed that the proposed method could provide an excellent sensitivity even without any extraction and enrichment measures. By the way, the upper limit of the calibration was set at only 40 μg/mL. No higher concentrations have been investigated due to the requirement of trace determination of 3-MCPD in real samples. If a higher concentration sample needs to be analyzed, it can be diluted to the linear range first.

The intraday and interday precision and accuracy of QC samples are summarized in Table 2. Intraday precision ranged from 2.9% to 4.1% with an accuracy ranging from 97.5% to 100.5%, and interday precision ranged from 3.1% to 5.7% with an accuracy ranging from 97.6% to 99.5%. These data indicated that the present method was accurate, precise, and reproducible for the quantification of 3-MCPD in aqueous solution with a concentration of 0.04 to 0.20 μg/mL.

The recovery of this method was assessed by a standard spiking experiment with two different simulated samples. The statistical results listed in Table 3 showed that the recovery was higher than 93% for all measurements, and the average recovery was 95.36%, which indicates the accuracy of this method is excellent. Meanwhile, the statistical values of the relative standard deviation (RSD) obtained by five replicate measurements were all less than 3.44%, and the lowest RSD was only

Table 1. Comparison of the Proposed Method and Literature Reported Methods for the Analysis of 3-MCPD in Various Matrices[a]

matrix	pretreatment	clean up	derivatization	detection	LOD for 3-MCPD (μg/kg)	reference
water	0.07 M sodium periodate		adenine	HPLC-FLD	0.36	this paper
aqueous solutions			BBA	GC-ECD	100	11
solvents			BSTFA	GC-FID	5000	8
bakery food	ethyl acetate extraction	pressurized liquid extraction	BSTFA	GC-MS	1.7	26
soya sauce	saturated NaCl solution	Extrelut	4-heptanone	GC-MS scan	1.2	27
various foods	saturated NaCl solution	aluminum oxide	HFBA	GC-MS SIM	1	14
blood, urine	dilution,acidification (enzymatic pretreatment)	silica gel (60 mesh)	HFBA	GC-MS NCI SIM	2	28
soya sauce	5 M NaCl solution	silica gel (60 mesh)	HFBA	GC-MS SIM	5	29
water	ethyl acetate extraction		HFBA	GC-ECD	0.73	30
model systems	hexane extraction	ASE	HFBI	GC-MS	5	31, 32
various foods	5 M NaCl solution	Extrelut, two-stage extraction	HFBI	PTV-LV GC-MS/MS	0.044 ng/mL	33
HVP, soya sauce	dilution 1:10	HS-SPME	PBA	GC-MS SIM	3.87	9
various foods	fat extraction, interesterification		PBA	GC-MS SIM	3	10
various foods	20% NaCl solution	LLE with MTBE	PBA	GC-MS SIM	1−6	34
soya sauce	NaCl addition	HS-SPME	MSTFA	GC-MS SIM	4.62	35
edible oil	diluted with hexane	dispersive microextraction	TSIM	GC-MS	1.1	36
seasoning	no data	no data	TSIM	GC-MS SIM	0.14	12

[a]Abbreviations: ASE, accelerated solvent extraction; BBA, *n*-butylboronic acid; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; GC-ECD, gas chromatography with electron capture detection; GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography−mass spectrometry; GC-MS/MS, gas chromatography−tandem mass spectrometry; HFBA, heptafluorobutyric anhydride; HFBI, heptafluorobutyrylimidazole; HS-SPME, headspace solid-phase microextraction; HVP, acid-hydrolyzed vegetable protein; LLE, liquid liquid extraction; LOD, limit of detection; 3-MCPD, 3-chloro-1,2-propanediol; MS, mass spectrometry; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; MTBE, methyl *tert*-butyl ether; NaCl, sodium chloride; NCI, negative chemical ionization; PBA, phenylboronic acid; SIM, selected ion monitoring; TSIM, 1-trimethylsilylimidazole. Data updated from ref 37.

Table 2. Intraday and Interday Repeatability and Accuracy

	intraday (n = 5)			interday (n = 5)		
QC concentration (μg/mL)	concentration found (μg/mL)	RSD (%)	accuracy (%)	concentration found (μg/mL)	RSD (%)	accuracy (%)
0.040	0.039 ± 0.002	4.1	97.5	0.039 ± 0.002	5.7	97.6
0.100	0.099 ± 0.003	3.3	99.0	0.098 ± 0.004	3.6	98.0
0.200	0.201 ± 0.006	2.9	100.5	0.199 ± 0.006	3.1	99.5

Table 3. Evaluation of the Accuracy and Precision of the Proposed Method by a 3-MCPD Spiking Experiment

sample	3-MCPD added (μL)	fortified concentration of 3-MCPD (μg/mL)	found concentration of 3-MCPD (μg/mL)	recovery (%)[c]	RSD (%)[d]
simulated sample 1[a]	50	0.235	0.221	94.04	2.85
	100	0.459	0.447	97.39	2.59
	200	0.876	0.847	96.69	3.44
simulated sample 2[b]	50	0.242	0.226	93.39	3.37
	100	0.472	0.444	94.07	2.57
	200	0.902	0.871	96.56	3.21

[a]Simulated sample 1 refers to a 1:3 dilution of 1.12 μg/mL 3-MCPD standard solution in tap water. [b]Simulated sample 2 refers to a 1:3 dilution of 0.25 μg/mL 3-MCPD standard solution in Wahaha bottled water. [c]Recovery (%) was calculated with the ratio of found to fortified concentration of 3-MCPD. [d]Relative standard deviation (RSD) or coefficient of variation was the percentage of standard deviation over mean of found concentration of 3-MCPD.

2.57%. All these data show that the precision of this method is also very good.

Application of the Method to Real Water Samples. In practical measurements, 3-MCPD usually needs to be extracted into the aqueous phase for further quatitative analyzing due to its hydrophilic nature (e.g., the determination of 3-MCPD in the edible oil), and moreover, the determination of 3-MCPD in environmental water was also of great significance.[38] So herein we applied the proposed method for the detection of 3-MCPD in real water samples. In order to evaluate its applicability, three different kinds of samples including tap water and Wahaha bottled water before and after 3-MCPD spiking were analyzed with the procedures as described above. A representative chromatogram of the tested samples is presented in Figure 5. The results showed that the chromatogram was very "clean" (the interference peak is well separated and the peak area is very low), which thus implied that the proposed method had a good selectivity and was very suitable for testing water samples. The residue concentration of 3-MCPD in the tap water was found to be 2.6 ng/mL, and no residue was detected in the Wahaha bottled water sample.

Application of the Method to Vegetable Oil Samples. In order to study the application potential of this method in food samples, we have preliminarily adopted this approach for the determination of free 3-MCPD in edible oils. Three different kinds of vegetable oil samples (soybean oil, rice bran oil, and corn oil) were preconditioned with LLE, and the obtained extracts were subjected to HPLC-FLD detection. A representative chromatogram is shown in Figure 6. The results illustrate that, although the composition of oil was very complicated, the interference originating from the matrix was very small, and its effect on the identification and quantification of target peak can be neglected. In addition, the accuracy and precision of this method for the analysis of oil samples have been evaluated by the spiking experiment with soybean oil. The recovery of 20, 40, and 100 ng/mL of 3-MCPD standard addition was individually 96.27%, 99.34%, and 102.42%, and the corresponding relative standard deviations (RSD) (n = 5) were 3.87%, 3.51%, and 2.96%. All of these results indicate that the proposed method is very suitable for the determination of free 3-MCPD in vegetable oil. The measured residue concentration of 3-MCPD in the corn oil, rice bran oil, and

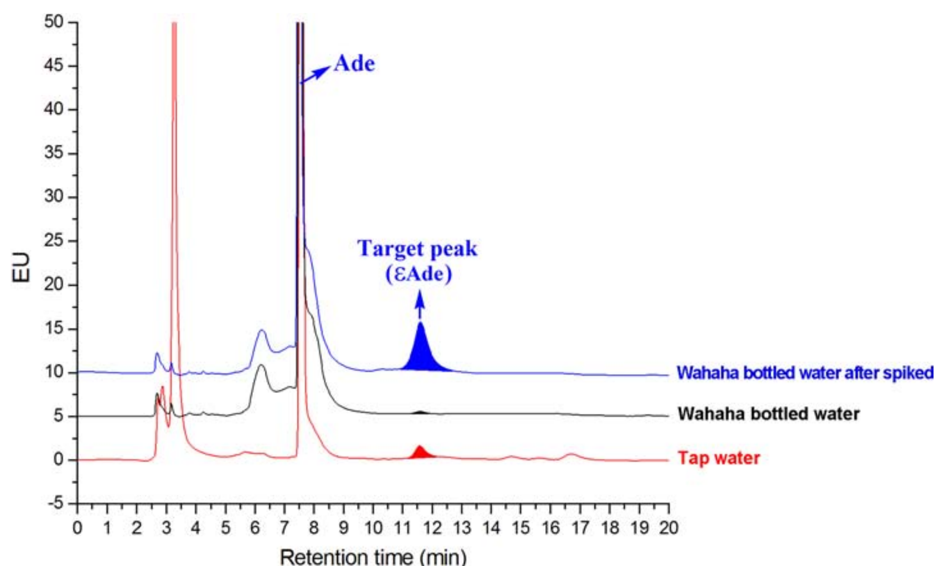

Figure 5. Chromatograms of tap water, Wahaha bottled water, and Wahaha bottled water spiked with 3-MCPD standard solution. Chromatographic conditions and FLD detector settings are described in the Materials and Methods section.

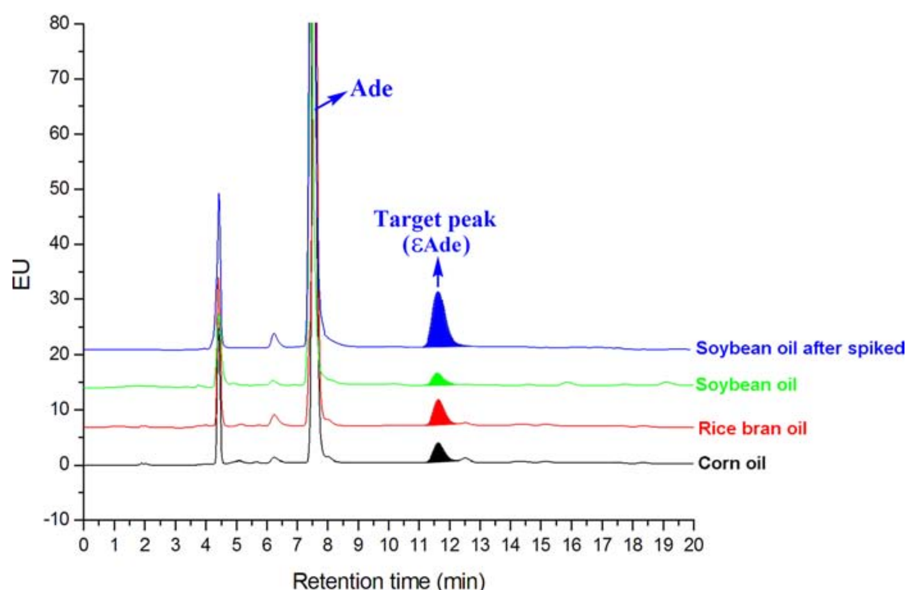

Figure 6. Chromatograms of corn oil, rice bran oil, soybean oil, and soybean oil after 40 ng/mL 3-MCPD standard spiking. Chromatographic conditions and FLD detector settings are described in the Materials and Methods section.

soybean oil is 13.22, 19.18, and 7.67 ng/mL, respectively; the contaminant level coincides with a previous report.[39]

In summary, a novel approach for the quantitative determination of 3-MCPD based on periodate oxidation coupled with HPLC-FLD has been established in this paper. Under the optimized conditions, the proposed method can provide high sensitivity, good linearity (r^2 = 0.999), and repeatability (percent relative standard deviations between 2.57% and 3.44%). The LOD and LOQ were 0.36 ng/mL and 1.20 ng/mL, respectively, and recoveries obtained for water samples were in the range 93.39−97.39%. Furthermore, the proposed method has been successfully used for free 3-MCPD in vegetable oil samples. The accuracy and repeatability have not been affected by the matrix. The recoveries obtained by the spiking experiment with soybean oil ranged from 96.27% to 102.42%. In comparison with gas chromatography or GC-MS, the proposed method can provide the advantages of simple instrumental requirement, easy operation, low cost, and high efficiency. If combined with other analytical technologies, i.e., online solid-phase microextraction and magnetic solid-phase extraction, this approach might provide a much better sensitivity and lower LOD, and thus will provide another ideal choice for routine analysis of 3-MCPD.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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