Determination of 3-chloropropanediol in soy sauce samples by liquid phase extraction coupled with microwave-assisted derivatization and high performance liquid chromatography-ultraviolet detection

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Abstract**—***3-Chloro-1,2-propanediol (3-chloropropanediol) is a well-known food processing contaminant found in a wide range of foods and ingredients and there has been recent concern about the levels of carcinogenic 3-chloropropanediol (3-MCPD) in some soy sauces. This paper reports on the development of an analytical method for the fast determination of 3-MCPD at trace level in commercial soy sauce using novel liquid phase extraction (LPE)/cleanup coupled with microwave-assisted derivatization (MAD) method followed by high performance liquid chromatography-ultraviolet (HPLC-UV) detection. In this method, 3-MCPD was first isolated from soy sauce sample matrix by LPE/cleanup with Extrude NT3 column cartridges and the isolated (eluent) solution was subjected to MAD with acetophenone to form 2-methyl-2-phenyl-4-(chloromethyl)-1,3-dioxolane under microwave irradiation using a specially modified domestic microwave oven, then the derivatizeddioxolane was directly analyzed with a HPLC-UV system. The optimum conditions for MAD such as the ratio of reagents, acidic catalyst, microwave irradiation power and time, as well as the chromatographic conditions were thoroughly investigated. Experimental results indicated that maximum derivatization can be achieved in 10 min under microwave irradiation at 362 watts when compared to 18 hours by conventional refluxing reaction. The proposed method provided a simple and rapid analytical procedure for 3-MCPD analysis in soy sauce with the detection limit of 80 ng mL-1. The relative standard deviations were all below 3.0 % (n = 7). Application was illustrated by the analysis of commercial sauce sample obtained from a local traditional store in central Taiwan.*

Keywords—3-Chloropropanediol,liquid phase extraction,microwave-assisted derivatization,sample preparation, soy sauce.

I. INTRODUCTION

Chlorinated propanols are toxic compounds, often found in various foods containing protein hydrolysates, like seasonings and savoury food products. 3-chloro-1,2-propanediol (3-MCPD) is a well known contaminant, which was first identified in 1982 in various foods [1-3]. It has been classified as non-genotoxic carcinogen but its carcinogenic potential in rodents has been controversial. 3-MCPD presented in certain oriental soy sauces and oyster sauces has triggered considerable public health and consumer safety concerns in recent years [4]. Its occurrence in hydrolyzed vegetable proteins (HVP) is related to the production process that uses acid hydrolysis and as a result of the high temperature chlorination of lipids present in the protein starting materials [5-6]. It is a suspected carcinogen in assays [7]. Because of its toxicity, several countries had set maximum levels for 3-MCPD in food products. In China and Canada, 3-MCPD is found to contain up to 177.5 mg L-1 in soy sauces. The maximum permission limit of 1 mg L-1 for 3-MCPD in HVP and soy sauces has been set in USA and Japan, and the same limit has been stipulated in Taiwan [8-12].

Most analytical methods for determining 3-MCPD are based on gas chromatography (GC) with different detectors such as flame ionization detector [13], electron capture detector [14] and mass spectrometry (MS) [12,15-20]. The Association of Official Analytical Chemists (AOAC) has published a method for the extraction, separation and identification of 3-MCPD in foods and ingredients using GC-MS [21]. Prior to GC analysis, appropriate sample pretreatment steps are usually required to clean up and enrich the target species, as well as the derivatization reactions with powerful agents such as acetone [18], n-butaneboronic acid [22], phenylboronic acid [13], and heptafluorobutyrylimidazole[14], etc. Although these methods offer

efficient and precise results, they are relatively time-consuming and relative expensive with respect to the derivated agent. Moreover, GC-MS is an expensive instrument and not popular in the soy sauce industries. Recently Lam et al. [23] used molecular imprint-based (MIP) chemosensor as semi-quantitative analytical tool to screen 3-MCPD in food products. However, the complicate matrix of soy sauce still interfered the binding of 3-MCPD on the MIP sensor.

High performance liquid chromatography-ultraviolet (HPLC-UV) is a widely used instrument with advantages of simplicity, inexpensive, convenient, and has diverse applications. It has potential to be an alternative to GC-MS method for determining 3-MCPD in soy sauce for analytical quality control purpose due to its popularity in food industries. However, to the best of our knowledge, the application of HPLC-UV to determine 3-MCPD has not been reported so far. In general, the high polarity of 3-MCPD limits its retention on a reversed-phase HPLC column and the lack of strong characteristic absorption in UV spectrum limits its detection by an UV detector. Therefore, a derivatization reaction is required to decrease the polarity of 3-MCPD as well as to create a characteristic UV-absorption. For this purpose, in this study, a derivatization reaction with acetophenone to form 2-methyl-2-phenyl-4-(chloromethyl)-1,3-dioxolane was examined to decrease the polarity of 3-MCPD and create a characteristic UV-absorption for HPLC-UV measurement. Moreover, the derivatization reaction by conventional heating/reflux method was usually a time-consuming process, thus the application of microwave irradiation is worth to investigate to speed-up the derivatization reaction. In our previous research publications, we demonstrated novel and rapid sample preparation/derivatization methods based on microwave irradiation and which has been successfully applied for the analysis of several pollutants in different aqueous samples matrix [24-26]. In continuation of our research work, we report here for the first time, a HPLC-UV method coupled to microwave-assisted derivatization (MAD) was established for fast determination of 3-MCPD in soy sauce posterior to the liquid phase column cartridge extraction/clean-up.

II. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Deionized water was produced using a Barnstead Nanopure water system (Barnstead, New York, USA) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. 3-MCPD was purchased from Fluka (FlukaChemieGmbh, Switzerland), acetophenone was from Acros Organics (Belgium), p-toluenesulfonic acid monohydrate was from Showa Chemicals (Japan), dypnone was from Wako pure chemical industries, LTD (Japan), and Toluene was from Tedia Company (USA). Sodium hydroxide, sodium chloride, ammonium acetate, and diethyl ether were from Riedel-deHäen (Hannover, Germany). Acetonitrile was obtained from Mallinckrodt (Kentucky, USA). Standard stock solutions (1000 μg mL^{-1}) of acetophenone and dypnone were prepared by dissolving 0.100 g in 95 mL acetonitrile and then adding acetonitrile to adjust the volume to 100 mL. The solutions were stored in brown glass bottles, and kept at 4 ºC. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The HPLC eluents were prepared as 50% and 80% acetonitrile in 0.01 M ammonium acetate buffer (pH 6.9). The eluents were filtered through a 0.45 μm poly (vinylidene difluoride) (PVDF) membrane filter and degassed ultrasonically. An Extrelut NT3 column (EM Science, Gibbstown, NJ, USA) was used to clean the sample matrix.

2.2 Apparatus and Instrumentation

HPLC used in this work was an Agilent 1100 series system equipped with a vacuum degasser, quaternary pump, and variable wavelength UV detector with a 20-μL flow cell (Agilent, Palo Alto, CA). A Waters reversed-phase LC-18 column (15 cm x 4.6 mm id. 5-μm particle) was used for separation. A Rheodyne 7125 injector (Rohnert Park, CA, USA) was used for sample injection. The detection wavelength was set at 241 nm. The microwave oven used to achieve the derivatization of 2-MCPD was a modified home-made version of the domestic SHARP R-340D system (2450 MHz, SHARP, Taipei, Taiwan) with a maximum power of 650 Watts, equipped with a cooling condenser connecting to tap water [24-25]. After the modification, the powers of microwave were 105, 188, 362, and 502 Watts for weak, medium low, medium, and medium high irradiation, respectively. The MAD system was set-up as shown in **Fig. 1**. In order to keep from the leak of microwave irradiation, aluminum foils was tacked on the inner-wall and the outer-wall of microwave body in the interface part. A microwave leak detector (MD-2000, Less EMF Inc., NY, USA) was used to check the safety aspects before the running.

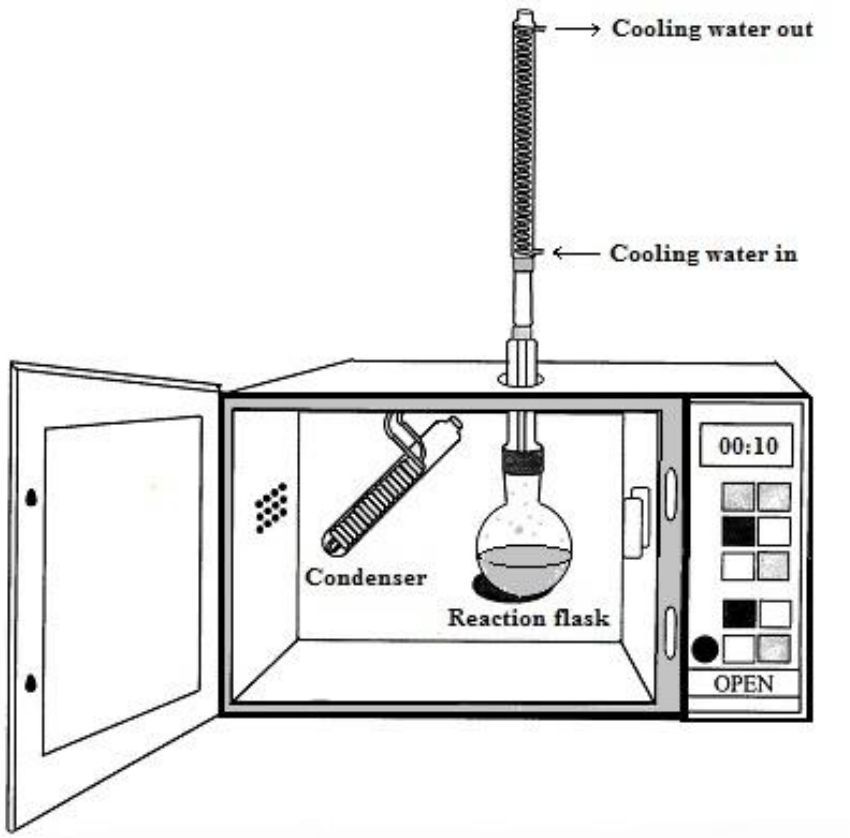

FIGURE 1. Apparatus of the microwave assisted derivatization (MAD) system

2.3 Preparation of calibration solutions of derivateddioxolane

Since the derivatization product 2-methyl-2-phenyl-4-(chloromethyl)-1,3-dioxolane, is not commercial available, varied concentrations of the derivateddioxolane were synthesized by mixing varied volumes of 3-MCPD (12, 24, 47, 95, 190, and 380 μL) and acetophenone in 1:3 weight ratio, respectively, and adding 20 mg of p-toluenesulfonic acid monohydrate in 25 mL toluene under microwave irradiation (362 watts) for 10 min. After cooling to ambient temperature, 10 mL of saturated sodium chloride solution was added and stirred thoroughly. After setting and removing the aqueous phase, the organic phase was made-up to 25 mL with toluene. The working solutions were prepared by diluting 0.1 mL of these solutions to 100 mL with acetonitrile and concentrations were ranged from 0.6 to 20.1 μg mL^{-1} (as 3-MCPD).

2.4 Sample preparation (LPE/clean-up) and MAD

Soy sauce (12 g) was added into a beaker containing 15 mL of saturated sodium chloride. After 10 min mixing and setting into two layers, the upper liquid layer was poured into an Extrelut NT3 cartridge column and allowed to equilibrate for 15 min. The column was eluted with 30 mL of diethyl ether and the eluent was collected in a vial. After removing water by adding anhydrous sodium sulfate, the resulting extract was then evaporated to dryness at room temperature (30 ºC) under nitrogen [27].

The residue in vial was re-dissolved by adding 365 μL of acetophenone and 20 mg of p-toluenesulfonic acid in 25 mL toluene. After mixing thoroughly, the solution was put into microwave oven for derivatization under 362 watts for 10 min. After cooling to ambient temperature, 0.1 M NaOH was used to titrate the solution to pH 7.0 and 10 mL of saturated sodium chloride solution was added and stirred thoroughly. After setting, the aqueous phase was removed and the organic phase was dried with 5 g of anhydrous sodium sulfate and purged with nitrogen to near dryness. The residue was re-dissolved by adding 20 mL of acetonitrile for HPLC analysis (injection volume: 20 μL).

III. RESULTS AND DISCUSSION

3.1 Optimization of HPLC conditions

In the derivatization, in spite of the derivated product, 2-methyl-2-phenyl-4- (chloro-methyl)-1,3-dioxolan, dypnone was also produced through the dimerization of acetophenone. Reactions are shown in **Fig. 2**. Therefore, the optimum chromatographic conditions for resolving acetophenone, dypnone, and 2-methyl-2- phenyl-4-(chloromethyl) -1,3-dioxolane were examined. Results were listed in **Table 1**. Under the conditions, the acetophenone, 2-methyl-2-phenyl-4-(chloromethyl)-1,3-dioxolane, and dypnone were with retention time of 3.81, 11.17, and 27.42, respectively. Obviously, the derivateddioxolane was separated from the derivatization agent and its dimer product. All chromatograms were virtually free of any interference.

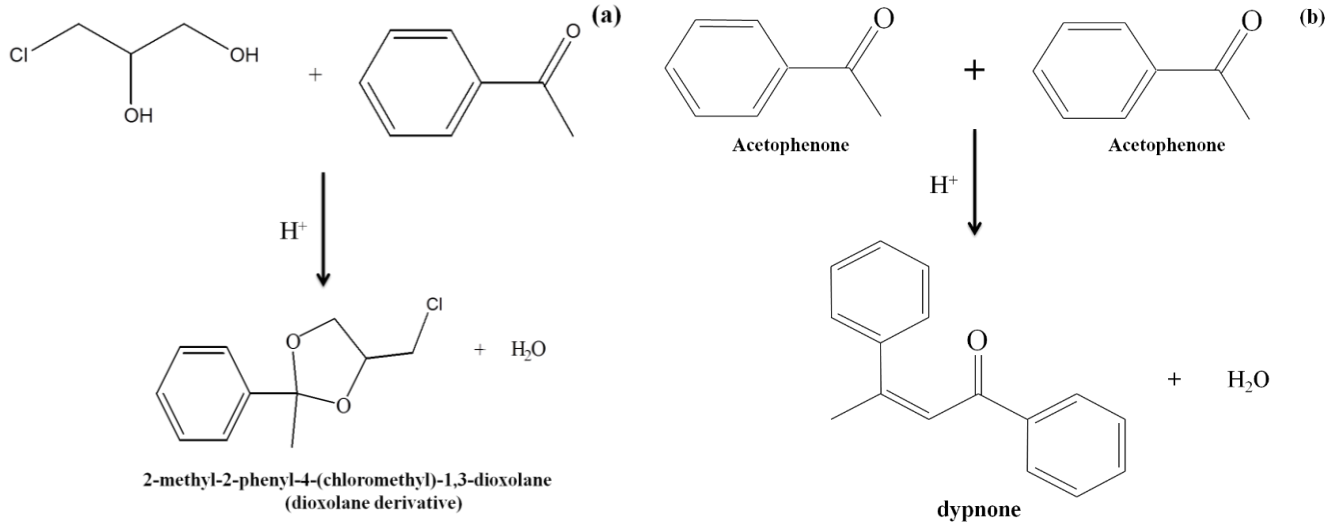

FIGURE 2. (a) Reactions in the derivatization of 3-MCPD with acetophenone, and (b) Dimerization of acetophenone

TABLE 1
THE OPTIMAL HPLC ELUTING CONDITION FOR SEPARATION

Time	Eluent composition	Flow rate
1-13 min	50% acetonitrile in pH 6.9 ammonium acetate buffer	1.0 mL min^{-1}
13-16 min	80% acetonitrile in pH 6.9 ammonium acetate buffer	1.0 mL min^{-1}
16-20 min	50% acetonitrile in pH 6.9 ammonium acetate buffer	1.0 mL min^{-1}

Quantification of 3-MCPD in samples was based on external standards using the calibration solutions described in Section 2.3. The derivateddioxolane (1.3 μg mL^{-1} as 3-MCPD) was with retention time of 11.17 min with 1.08% RSD and the peak area for quantitative determination was with 2.47% RSD for three determinations.

3.2 Optimization of MAD conditions

The isolated (eluent) solution obtained from (LPE)/clean-up was subjected to derivatization with acetophenone to form 2-methyl-2-phenyl-4-(chloromethyl) -1,3-dioxolane for the sensitive HPLC-UV analysis. When using the conventional heating/reflux protocol for the derivatization of 3-MCPD with acetophenone with 110 ^{o}C oil bath, an 18-hour reaction was required. Therefore, the microwave irradiation was investigated to accelerate the derivatization. In the studies, factors that affect the derivatization such as quantity of acetophenone and catalyst (p-toluenesulfonic acid), power and time of microwave irradiation were investigated.

3.2.1 Effect of microwave irradiation power and time

Microwave irradiation power and time is an important parameter affecting the efficiency of the derivatization of 3-MCPD posterior to the LPE/clean-up of soy sauce sample [24-26]. In order to optimize the microwave irradiation, various effective

powers of 105, 188, 362, and 502 watts were examined with 190 µL of 3-MCPD, 735 µL of acetophenone, and 20 mg of p-toluene sulfonic acid dissolved in 25 mL toluene, at microwave irradiation times ranging from 5 to 25 min. After a series of tests, results (in **Fig. 3**) indicated that the derivatization efficiency of 3-MCPD increases when the microwave irradiation power was increased from 105 to 362 W. The effective microwave irradiation power of 362 W showed maximum derivatization efficiency for the target analyte (3-MCPD). However, when microwave was irradiated at 105 and 188 W, a minimum quantity of 3-MCPD derivative was detected due to the insufficient microwave energy for the derivatization of 3-MCPD. Conversely, increasing the microwave irradiation to 502 W showed less extraction efficiency due to the sequential degradation of derivatization reagents from the derivatization vessel under high microwave irradiation. Similarly, experimental results indicated that the peak areas increased with increasing microwave irradiation time in the range of 5–10 min, and maximum derivatization quantity was obtained at 10 min (**Fig. 4**). However, with longer microwave exposure times (>10 min), the peak areas began to decrease, possibly due to the degradation of the derivatization product resulting from higher temperature. Therefore, microwave irradiation with effective irradiation power of 362 W for 10 min was selected for the subsequent analysis.

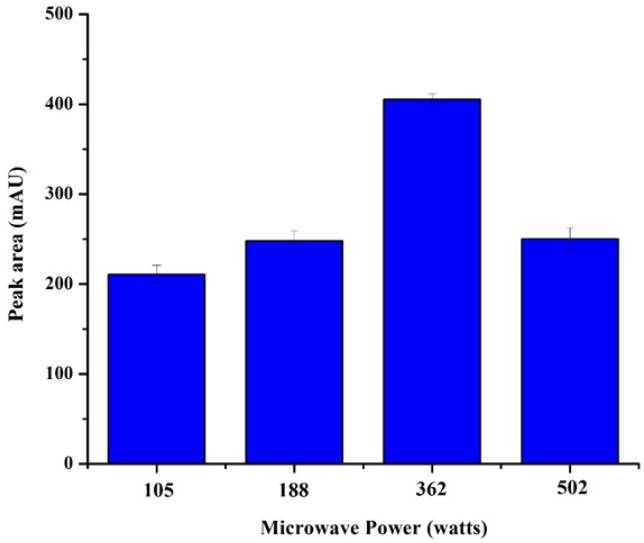

FIGURE 3. EFFECT OF THE MICROWAVE IRRADIATION POWER ON DERIVATIZATION

Conditions: 190 µL of 3-MCPD, 735 µL of Acetophenone, and 20 mg of p-toluene sulfonic acid dissolved in 25 mL toluene, under varied microwave power irradiation for 10 min.

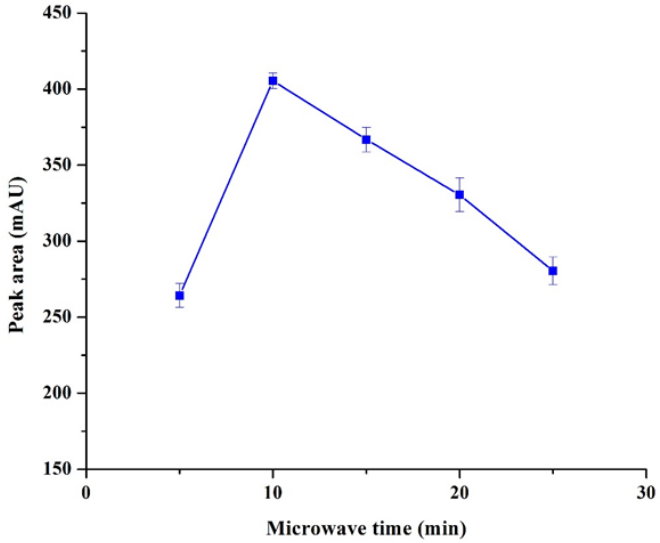

FIGURE 4. EFFECT OF THE MICROWAVE IRRADIATION TIME ON DERIVATIZATION

Conditions: 190 µL of 3-MCPD, 735 µL of acetophenone, and 20 mg of p-toluene sulfonic acid dissolved in 25 mL toluene, under 362 watts microwave irradiation

3.2.2 Optimization of derivatization agent and catalyst

The optimization of derivatization agentwas examined. Aftera series of experiment runs, the maximum derivatization efficiency was achieved at the ratio of derivatization agent (acetophenone) to 3-MCPD and was optimum at 3:1 under MAD. Regarding the quantity of catalyst required in the derivatization, there was no significant difference in detection peak area of the derivateddioxolane from 10 to 60 mg of p-toluenesulfonic acid in the reaction of 190 µL 3-MCPD with 735 µLof acetophenone. Therefore, 20 mg of p-toluenesulfonic acid was added as catalyst in the derivatization.

3.3 Method validation and real sample analysis

In order to examine the applicability of the proposed LPE/clean-up coupled with MAD followed by HPLC-UV for quantitative determination of 3-MCPD in soy sauce, standard spiked soy sauce samples were used for calibration after they were subjected to overall procedure as described in Material and Methods Section. An HPLC-UV chromatogram of derivateddioxolane from 3-MCPD standards spiked in soy sauce under chromatographic condition described in the Material and Methods section is showed in **Fig. 5(a)**. Calibration plot was built-up over the concentration range of 0.6 – 20.1 µg mL^{-1} (as 3-MCPD). Linear relationship between the peak area and the injected quantity was in good agreement with the correlation coefficients (r^2) being 0.9991. Detection limits of the proposed method was calculated based on three times the standard deviation (n=7) of the lower concentration in the method calibration plot (0.6 µg mL^{-1}divided by the detection sensitivity

(slope of calibration plot), which was 80 ng mL^{-1}. The precision of this method was estimated by performing 3 derivatizations and HPLC-UV determinations of sample spiked 3-MCPD at 5 µg mL^{-1}. The precision was 5.6 % RSD, which should be satisfactory for determining the 3-MCPD in soy sauce.In order to examine the applicability of the method to determine 3-MCPD in real sample,soy sauce sample was obtained from local traditional store in Silo town (Taiwan) and analyzed by the proposed method. The chromatogram was shown in **Fig. 5(b)**. Peak related to 3-MCPD was not found. When spiked a 5 µg mL^{-1} of 3-MCPD in real sample prior to the pretreatment and derivatization, the chromatogram was shown in **Fig. 5(c)**. The peak with retention time of 11.7 min was related to the derivateddioxolane from 3-MCPD.The recovery was ranged between 78.5 - 82.3% and the precision was 5.4 % RSD (n=5).

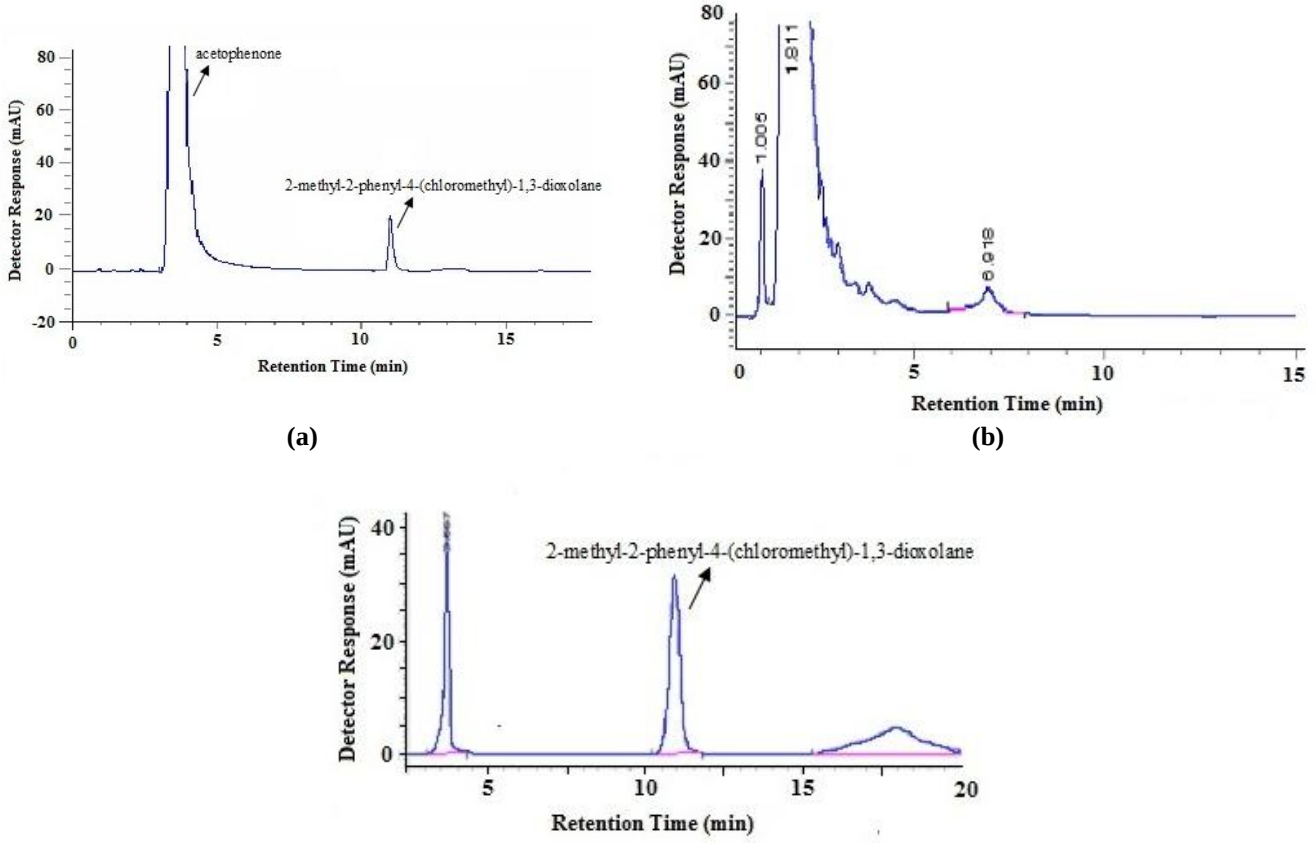

FIGURE 5. HPLC-UV chromatogram of derivateddioxolane
(a) from 3-MCPD standard solution;
(b) from soy sauce sample;
(c) from spiked soy sauce sample (5 µg mL^{-1}).

3.4 Characteristics of the proposed method

In the proposed method, the conventional HPLC-UV coupled to LPE/cleanup with MAD was developed to determine 3-MCPD in soy sauce. The detection limit (80 ng mL^{-1}) was comparable to GC-ECD and FID [13-14], although it was higher than GC-MS (15-17). Moreover, it meets the local requirement (1.0 µg/mL) and also with china and Japan's regulations [8-12]. The sensitivity of the presented method can also be improved further by decreasing the volume of re-dissolving solvent (acetonitrile) or evaporation prior to HPLC analysis. The derivatization reaction time has also been accelerated under microwave irradiation when compared with previous reports [13-15]. It takes only 10 min to complete the derivatization of 3-MCPD with acetophenone compared to several hours in other methods.

IV. CONCLUSION

In this study, a fast and sensitive LPE/*cleanup*-MAD method combined with HPLC-UV has been developed for the analysis of trace level of 3-MCPD in soy sauce samples. From the results of the applicability test for the analysis of 3-MCPD in commercial soy sauce sample, the present approach showed good linearity, good precisions, and satisfactory relative

recoveries, thus proving to be an efficient, convenient, and cost-effective method. Thus, the present technique possesses great potential in the fast preconcentration and analysis of 3-MCPD in soy sauce samples and represents an attractive alternative to both traditional and recently developed methods.

ACKNOWLEDGMENT

The authors thank the Ministry of Science and Technology of Taiwan (MOST) under the grant number NSC-91-2113-M-005-024 and NSC-101-2811-M-005-014 for financial supports.

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