A simultaneous derivatization of 3-monochloropropanediol and 1,3-dichloropropane with hexamethyldisilazane–trimethylsilyl trifluoromethanesulfonate at room temperature for efficient analysis of food sample analysis

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ARTICLE INFO

Article history:

Received 30 July 2015

Received in revised form

15 December 2015

Accepted 30 December 2015

Available online 4 January 2016

Keywords:

3-Monochloropropanediol (3-MCPD)

1,3-Dichloropropanol (1,3-DCP)

Acid hydrolyzed vegetable protein

(acid-HVP)

Silylation

Room temperature derivatization

Hexamethyldisilazane–trimethylsilyl

trifluoromethanesulfonate

(HMDS–TMSOTf)

ABSTRACT

This paper reports the application of hexamethyldisilazane–trimethylsilyl trifluoromethanesulfonate (HMDS–TMSOTf) for the simultaneous silylation of 3-monochloro-1,2-propanediol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) in solid and liquid food samples. 3-MCPD and 1,3-DCP are chloropropanols that have been established as Group 2B carcinogens in clinical testing. They can be found in heat-processed food, especially when an extended high-temperature treatment is required. However, the current AOAC detection method is time-consuming and expensive. Thus, HMDS–TMSOTf was used in this study to provide a safer, and cost-effective alternative to the HFBI method. Three important steps are involved in the quantification of 3-MCPD and 1,3-DCP: extraction, derivatization and quantification. The optimization of the derivatization process, which involved focusing on the catalyst volume, derivatization temperature, and derivatization time was performed based on the findings obtained from both the Box–Behnken modeling and a real experimental set up. With the optimized conditions, the newly developed method was used for actual food sample quantification and the results were compared with those obtained via the standard AOAC method. The developed method required less samples and reagents but it could be used to achieve lower limits of quantification (0.0043 mg L^{-1} for 1,3-DCP and 0.0011 mg L^{-1} for 3-MCPD) and detection (0.0028 mg L^{-1} for 1,3-DCP and 0.0008 mg L^{-1} for 3-MCPD). All the detected concentrations are below the maximum tolerable limit of 0.02 mg L^{-1} . The percentage of recovery obtained from food sample analysis was between 83% and 96%. The new procedure was validated with the AOAC method and showed a comparable performance. The HMDS–TMSOTf derivatization strategy is capable of simultaneously derivatizing 1,3-DCP and 3-MCPD at room temperature, and it also serves as a rapid, sensitive, and accurate analytical method for food samples analysis.

1. Introduction

Chloropropanols are a group of chlorinated compounds bearing the hydroxyl functional group. 3-Monochloro-1,2-propanediol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) are chloropropanols that exist in heat-processed food and drinking water. 3-MCPD and 1,3-DCP were first reported in protein hydrolysates [1]. 3-MCPD can be generated the presence of chloride ions and a glycerol precursor, under a high temperature treatment for a prolonged period. The compounds was found as a major constituent in acid-hydrolyzed

soy sauce, acid-hydrolyzed vegetable protein (acid-HVP), bakery products [2], acid-HVP contaminated smoked food products [3], and food products that come in contact with paperboard packaging [4]. 1,3-DCP, on the other hand can be found in contaminated water source. 3-MCPD is registered as a rodenticide by the Environment Protection Agency (EPA) [5] while 1,3-DCP is classified as a fumigant and nematicide [6]. In food samples, the concentration of 1,3-DCP is normally lower than the concentration of 3-MCPD. 1,3-DCP is typically be formed through a hydrolysis process that occurs when the concentration of 3-MCPD is high. The contamination of 3-MCPD in food products is a major concern as soy sauce and its related products are highly consumed, especially in the Asian region.

3-MCPD can cause the systematic failure of kidneys and reproductive organs if consumed in low or medium-low concentration.

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Fig. 1. Silylation mechanism of HMDS-TMSOTf.

Table 1
Maximum tolerable concentration of 3-MCPD in food [7].

Country	Maximum limit (mg kg ⁻¹)
Australia/New Zealand	0.20
Canada	1.00
China	1.00
European Union	0.02
Malaysia	0.02
Singapore	0.02
United States	1.00

When consumed in high concentrations, laboratory toxicity testing has linked 3-MCPD to hyperplasia, kidney and reproductive organ cancer in laboratory toxicity testing. Due to the toxicity reports, 3-MCPD has been categorized as a Group 2B carcinogen and a Proposition 65 (Prop. 65). Meanwhile, an exposure to a medium or high dosage of 1,3-DCP was reported to be cause carcinogenic effects in liver, kidney, oral epithelium, and tongue as well as thyroid glands. Due to the carcinogenic and hepatotoxic nature of 1,3-DCP, it has been labeled as a Group 2B carcinogen and a Prop. 65. Based on the toxicity reports of laboratory animals, various countries have also established a maximum tolerable concentration of 3-MCPD and 1,3-DCP. The maximum tolerable concentration of 3-MCPD can be categorized into three concentrations: 0.02 mg L⁻¹, 0.20 mg L⁻¹ and 1.00 mg L⁻¹ (Table 1) [7]. The concentrations of 3-MCPD and 1,3-DCP in food samples have been surveyed and reported in various countries including Belgium [8], United Kingdom [9], Singapore [10], Spain [11], Brazil [12], Taiwan [13], United States [14], and Hong Kong [15]. In these reports, food products (especially soy sauce related products) are collected and their 3-MCPD content was quantified. All the surveys from the aforementioned countries reported products with 3-MCPD concentrations that are over the tolerable limits.

The detection of 3-MCPD in protein hydrolysate was first reported by a direct comparison method using gas chromatography [1]. Due to the complexity of food samples, a sample pre-treatment step had to be introduced to efficiently extract chloropropanols from food samples, followed by a derivatization process. The high

polarity of diol in 3-MCPD entailed a derivatization stage to reduce the analytes polarity before it can be quantified by instruments with a non-polar column. A few derivatization agents have been reported to be effective in derivatizing 3-MCPD, including phenylboronic acids (PBA) [16–21], bis(trimethylsilyl) trifluoroacetamide (BSTFA) [22–24], *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) [25], heptafluorobutyrylimidazole (HFBI) [26–31], heptafluorobutyricanhydride (HFBA) [32–34]. Other detection of 3-MCPD included ketal/acetone formation [35–37], and periodate oxidation [38]. Among the reported derivatizing agents, only BSTFA, HFBI and HFBA can derivatize both 3-MCPD and 1,3-DCP simultaneously. This is because PBA, along with the ketal/acetone formation and periodate oxidation processed, only reacts with the diol group of the analytes; thus this approach is not applicable to the 1,3-DCP compound which contains only one hydroxyl group. HFBI and HFBA substitute the hydroxyl groups in the analytes, rendering them non-polar. HFBI is the derivatizing agent recommended by AOAC [39]. The advantage of HFB derivatization lies with the derivatized analytes, which produce a number of characteristic ions that are useful for mass spectrometry (MS) quantification. Unfortunately, HFBI is expensive and has to be stored in a moisture free environment. Therefore, the usage of HFBA together with a catalyst, has become the preferred method in derivatize 3-MCPD [40]. Unlike HFBI, HFBA will produce acidic by-products at the end of the derivatization process and this inevitably reduces the column life span of a gas chromatography system. A washing step (with water) has to be carried out after the derivatization process to remove the acidic by-products before the sample can be analyzed using gas-chromatography. Another commonly used derivatizing agent is BSTFA, which is a silylation agent that substitutes the hydroxyl group with the trimethylsilyl (TMS) group. TMS-derivatized analytes are more heat stable and they can be stored, as well as quantified in different batches. Although TMS-derivatized analytes typically produce sharp and narrow peaks in gas chromatography, they tend to produce low confirmation ions. Therefore in our method, the GC-MS program uses a single ion monitoring (SIM) mode to achieve a better ion confirmation with improved limit of detection (LOD) and limit of quantification (LOQ).

Table 2

Comparison between various derivatizing agents used for derivatizing 1,3-DCP and 3-MCPD simultaneously.

	HMDS	BSTFA	MSTFA	HFBI (AOAC Standard Method)	HFBA
Derivatization mechanism	Silylation	Silylation	Silylation	Acylation	Acylation
Derivatized end products stability (storage at room temperature)	Thermally stable	Thermally stable	Thermally stable	Thermally labile (end-products dissociate with time)	Thermally labile (end-products dissociate with time)
Derivatizing agent storage condition	Dry, room temperature	Dry, room temperature	Dry, 2–8 °C	Dry, –20 °C	Dry, 2–8 °C
Derivatization period	10 min	10 min–2 h	40 s	20 min	20 min
Derivatization temperature	Room temperature	40–80 °C	230 °C	60 °C	60 °C
LOD	1,3-DCP: 0.0028 mg L ⁻¹ 3-MCPD: 0.0008 mg L ⁻¹	1,3-DCP: 1.4 ng mL ⁻¹ 3-MCPD: 11.2–0.04 mg kg ⁻¹	1,3-DCP: 0.35 ng g ⁻¹ 3-MCPD: 3.91 ng g ⁻¹	1,3-DCP: 10 µg kg ⁻¹ 3-MCPD: 5–100 µg kg ⁻¹	1,3-DCP: 0.6–5 µg kg ⁻¹ 3-MCPD: 0.7–5 µg kg ⁻¹
LOQ	1,3-DCP: 0.0043 mg L ⁻¹ 3-MCPD: 0.0011 mg L ⁻¹	1,3-DCP: 4.8 ng mL ⁻¹ 3-MCPD: 34.5 ng mL ⁻¹	1,3-DCP: NA 3-MCPD: NA	1,3-DCP: NA 3-MCPD: 10 µg kg ⁻¹	1,3-DCP: 3–5 µg kg ⁻¹ 3-MCPD: 3–5 µg kg ⁻¹
Instruments used for detection	GC–MS	GC–FID, GC–MS	GC–MS	GC–ECD, GC–MS, GC–MS/MS	GC–ECD, DC–MS
By-products	Ammonia	Hydrogen fluoride	<i>N</i> -Methyl trifluoroacetamide	No acid by-products	Acidic by-products
Cost of derivatizing agent (price as quoted by Sigma–Aldrich)	Very low (0.62 USD/mL)	Low (13.20 USD/mL)	High (14.62 USD/mL)	Very high (61.10 USD/mL)	High (17.30 USD/mL)
Catalyst	TMSOTf	TMCS	TMCS	–	Triethylamine
References	This study	[22–24]	[25]	[26–31]	[32–34]

NA means data not available.

Fig. 2. (A) Full EI scan of 1,3-DCP-TMS and 3-MCPD-TMS; (B) characteristic ions of 1,3-DCP-TMS; (C) characteristic ions of 3-MCPD-TMS.

Apart from *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and BSTFA, hexamethyldisilazane (HMDS) is another commercially available silylation agent which is a stable and easy to handle. However, the main disadvantage of using HMDS is the low silylation activity of the derivatizing agent and this often necessitates the use of a catalyst and high temperature during the derivatization process. The cost per mL for HMDS is lower compared to both MSTFA and BSTFA (Table 2) [41]. The catalysts used to promote the derivatization ability of HMDS are sulfonic acid-functionalized nanoporous silica [42], trichloroisocyanuric acid (TCCA) [43], InBr_3 [44], zirconyl triflate [45], K-10 montmorillonite [46], H- β zeolite [47], silica-supported perchloric acid [48], barbituric acid [49], iodine [50], MgBr_2 [51], *N*-bromosuccinimide (NBS) [52] and iron(III) trifluoroacetate [53]. The use of the aforementioned catalysts will only produces the harmful acidic by-products, but also involves long derivatization time, high temperature, a large amount of chemicals, and toxic metal catalysts.

The aim of this research is to use the hexamethyldisilazane-trimethylsilyl trifluoromethanesulfonate (HMDS-TMSOTf) derivatizing agent to derivatize 3-MCPD and 1,3-DCP in food samples. The use of HMDS as a derivatizing agent combined with TMSOTf as the catalyst was first reported in the derivatization of monosaccharide and trehalose building blocks [54]. The procedure was completed at room temperature and required only a short period of reaction time (10 min).

2. Experimental

2.1. Chemicals and reagents

3-MCPD (98%), HMDS and TMSOTf were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aluminium oxide for column chromatography (neutral, 70–230 mesh size), sodium carbonate (AR grade), sodium bicarbonate (AR grade), and sodium sulfate (AR grade) were purchased from R&M Chemicals (Selangor, Malaysia). 1,3-Dichloropropanol (98%) was supplied from Merck (Selangor, Malaysia). Dichloromethane (HPLC grade) and HFBI (GC derivatization grade) were purchased from Fisher Scientific (Selangor, Malaysia). Purified water (18.2 M Ω cm) was obtained from a Milli-pore Milli-Q Plus water purification system (Bedford, MA, USA).

2.2. Preparation of standards solutions

The standard solutions of 3-MCPD and 1,3-DCP were prepared by diluting 98% (w/v) of 3-MCPD (8.9 mol L⁻¹) and 1,3-DCP (7.6 mol L⁻¹) stock solutions with ethyl acetate to give a final concentration of 200 mg L⁻¹ each. Mixed calibration solutions containing 3-MCPD and 1,3-DCP were prepared by adding together an appropriate volume of the prepared stock solutions. There were seven calibration points covering the concentration range from 0.001 to 10 mg L⁻¹ and these solution concentrations were prepared from a dilution of the stock solutions and stored at 4 °C. The prepared mixed solutions of 3-MCPD and 1,3-DCP can be stored up to 3 months.

1,4-Butanediol (1,4-BD) was selected as the internal standard. The use of an internal standard is a quality control measure, which enables a comparison of the instrumental response between the target analytes and the internal standard. The concentration of the internal standard was set at 1.00 mg L⁻¹. Meanwhile, 1,5-pentadiol (1,5-PD) was selected as the surrogate standard. Surrogate standard is used as part of a quality control tool to calculate the recoveries of target analytes. The concentration of the surrogate standard was set at 1.00 mg L⁻¹. The standard solutions of 1,4-BD and 1,5-PD were prepared by diluting 98% (w/v) of 1,4-BD (10.9 mol L⁻¹) and 1,5-

PD (9.4 mol L⁻¹) stock solutions with ethyl acetate to give a final concentration of 200 mg L⁻¹ each.

2.3. Derivatization

To obtain the TMS derivatives, HMDS was used as the derivatization agent and TMSOTf was used as the catalyst. HMDS (50 μ L) was added to the ethyl acetate extract (prepared according to the description written in Section 2.7), and TMSOTf (10 μ L) was then added into the mixture. The vial was subsequently sealed and shaken by a vortex shaker. The derivatization process was carried out at room temperature for 10 min and 1.0 mL water was added to the vial to stop the derivatization process. The vial was then vortexed for 0.5 min. The organic layer was transferred to a 2 mL gas chromatography vial and a small quantity of Na_2SO_4 was added into the vial to remove any moisture prior to GC analysis.

For HFB derivatization, 50 μ L of HFBI was added to the extract, sealed and heated at 70 °C for 20 min. The extract was then cooled to 40 °C and 1 mL of water was added to the extract to quench the derivatization process. The extract was then vortexed for 0.5 min and transferred into chromatography vials.

2.4. Instrumentation and operating conditions

The quantification of 3-MCPD-TMS and 1,3-DCP-TMS was performed by a single quadrupole gas chromatography-mass spectrometer (Shimadzu GC-MS-QP 2010 Plus, Kyoto, Japan). The capillary column used was 30 m in length and 0.23 mm in diameter, with a 0.25 μ m film thickness (DB-5MS, Agilent Technologies, Selangor, Malaysia). The quantification of 3-MCPD-HFB and 1,3-DCP-HFB was carried out with the Agilent 7890A GC system (USA), equipped with an inert mass spectrometry detector. The column used was 30 m in length and 0.23 mm diameter with a 0.25 μ m film thickness (HP-5MS, Agilent Technologies, Selangor, Malaysia).

The derivatized sample (1 μ L) was injected into the oven at a split-less mode. The interference temperature was programed at 270 °C and the GC oven was programed as follows: 60 °C kept for 2 min, increased at a rate of 5–120 °C min⁻¹, followed by a further increase to 300 °C at a rate of 30 °C min⁻¹ and eventually maintained for 8 min. Purified nitrogen was used as the carrier gas. The whole temperature program took about 12 min. The confirmation of peaks was carried out by running the mass spectrometer in a scanning mode from m/z 50 to 500 whereas the quantitative analysis of the derivatized analyte was carried out in SIM mode. In SIM mode m/z 116, 119, and 147 were selected as the signature ions for the detection of 3-MCPD-TMS, while m/z 239 was selected as the quantifying ion. On the other hand, m/z 93, 154, and 151 were selected as the signature ions for 1,3-DCP-TMS whereas m/z 185 was chosen as the quantifying ion.

On the other hand, the HFBI derivatized 3-MCPD and 1,3-DCP were prepared using a method was adopted from Association of Analytical Communities (AOAC) with a slight modification [26]. The sample (1.5 μ L) was injected in a split-less mode with the interference temperature set at 270 °C. The oven temperature was programed as follows: the initial temperature was set at 50 °C for 1 min, followed by a 2 °C min⁻¹ increment until the temperature reached 90 °C, this was changed to 25 °C min⁻¹ until the temperature reached 270 °C, and held for 10 min. The entire temperature program took about 27 min. Helium gas was selected as the mobile phase, with a flow rate of 1 mL min⁻¹. For full scanning, the range was set between 100 and 800 amu. The characteristic ions selected for the quantification of 3-MCPD-HFB and 1,3-DCP-HFB were m/z 253 and 275, respectively. Meanwhile, the characteristic ions selected for the qualification of 3-MCPD-HFB were m/z 453, 291, and 289, and m/z 113, 111, and 75 for 1,3-DCP-HFB.

Table 3

Comparison of the inter-day and intra-day precision made between the HMDS–TMSOTf and HFBI derivatization.

% RSD	Concentration (mg L ⁻¹)	Number of replicates (N)	HMDS–TMSOTf		HFBI	
			1,3-DCP	3-MCPD	1,3-DCP	3-MCPD
Intra-day precision	0.005	3	4	5	ND	ND
	0.02	3	4	4	2	5
	1	3	3	3	7	1
Inter-day precision	0.005	3	4	5	ND	ND
	0.02	3	5	5	2	7
	1	3	2	2	4	4

ND means target compound not detectable.

2.5. Method optimization

There are three important parameters that must be optimized in generating a derivatization process that has the lowest cost and shortest reaction time. These parameters include temperature, incubation time and catalyzer volume. The Box–Behnken statistical experimental design was implemented to investigate the optimal conditions for the derivatization process (Supplementary material: Table a and b). Theoretical studies have been conducted to confirm and complement the experimental findings. Firstly, for the optimization of the incubation period (5–50 min), the volume of TMSOTf was fixed at 10 μ L while the incubation temperature was fixed at 35 °C. In contrast, for the optimization of the TMSOTf volume (5–30 μ L), the incubation time was set at 20 min while the incubation temperature was fixed at 35 °C. Finally, for the optimization of the incubation temperature (25–95 °C), the TMSOTf volume was set at 10 μ L while the incubation time was set at 20 min.

2.6. Method validation

Guidelines from EURACHEM were used to estimate the LOD and LOQ of the developed method [55]. A concentration of the target analyte that was close to the GC quantification limit was prepared and derivatized. The same concentration was then repeated 10 times. The mean and standard deviation values were then calculated and fitted into the following equation:

$$\text{LOD} \cong X_{M1} + 3S_{M1} \quad (1)$$

$$\text{LOQ} \cong X_{M1} + 10S_{M1} \quad (2)$$

where X_{M1} is the mean of the peak signal for a concentration close to the blank solution, and S_{M1} is the standard deviation of the peak signal for a concentration close to the blank solution.

The inter-day precision (intermediate) of the developed method was conducted by repeating the measurement of three replicates ($N=3$) of both 1,3-DCP and 3-MCPD at three different concentrations (low: 0.005 mg L⁻¹; medium: 0.02 mg L⁻¹; and high: 1 mg L⁻¹) standard solutions for 6 days. While for intra-day precision (repeatability), both the standard solutions of 1,3-DCP and 3-MCPD at three different concentrations (low: 0.005 mg L⁻¹; medium: 0.02 mg L⁻¹; and high: 1 mg L⁻¹) were repeatedly measured every hourly for 6 times over a day (Note: number of replicates, $N=3$ for every measurement performed).

2.7. Food sample preparation

About 2 g of the food sample (soy sauce, sweet sauce, chicken flavored seasoning and oyster sauce) was added to 8 g of aluminum oxide. The sample was homogenized and mixed gently and the surrogate standard was added to the mixture. 1 mg kg⁻¹ of 3-MCPD and 1,3-DCP were spiked into repeated food samples to check for recovery. After mixing again the sample was loaded into a chromatography column (2 cm internal diameter, 40 cm length, with

0 porosity sintered disc). Prior to sample addition, 1 g of cotton wool (pre-soaked with dichloromethane) and 1 g of anhydrous Na₂SO₄ were added to the column. During the addition of the mixed aluminum oxide, the chromatography column was gently tapped to minimize the formation of porous voids. After the addition of aluminum oxide another layer of Na₂SO₄ (1 g) was added on top. The chromatography column was then eluted with 250 mL of dichloromethane at a flow rate of about 8 mL min⁻¹. The eluate was collected and concentrated to near dryness under a gentle flow of purified nitrogen gas, followed by the immediate addition of ethyl acetate (1 mL) to the extract.

To determine the accuracy of the results produced by using our newly developed method, 3-MCPD and 1,3-DCP concentrations in solid, paste and liquid food samples were derivatized with both HMDS–TMSOTf and HFBI derivatizing agents. Both derivatization methods were repeated three times and the results obtained were compared using a Student's *t*-Test for determining the significant difference between these two derivatization methods.

2.8. Calculation of sample concentration

The calibration curves were plotted using the responds ratio of 3-MCPD/1,4-BD or 1,3-DCP/1,4-BD against the concentration of 3-MCPD/1,3-DCP in mg L⁻¹.

To determine the unknown concentrations of 3-MCPD and 1,3-DCP in food samples, the following formula was used:

$$\begin{aligned} \text{Concentration, mg L}^{-1} \\ = (\text{Ratio of 3-MCPD or 1, 3-DCP to 1, 4-BD})(1/C)/ \\ \text{sample weight, g.} \end{aligned}$$

where C is the slope of the calibration curve.

3. Results and discussion

3.1. Derivatization mechanism

As compared to other silylating agents (e.g., BSTFA and MSTFA), the HMDS–TMSOTf shows additional and very important advantages in terms of short derivatization period and the derivatization process could be performed at room temperature. A careful comparison is made between silylation and acylation where the advantages, disadvantages and limitations encountered for each derivatization mechanism using different types of derivatizing agents are as detailed in Table 2. Therefore, our method, which does not require reaction to be performed at high temperature and long derivatization time, is a feasible alternative to existing protocols.

The proposed derivatization mechanism for the silylation of 3-MCPD and 1,3-DCP is illustrated in Fig. 1. The addition of TMSOTf to HMDS will form (bis(trimethylsilyl)ammonio)trimethyl(((trifluoromethyl)sulfonyl)oxy)silicate(IV), which will then react with the hydroxyl functional group in 3-MCPD, to give 1-chloro-3-

Fig. 3. (A) Calibration curves of TMSOTf derivatized 1,3-DCP (blue line) and 3-MCPD (red line); (B) Calibration curves of HFBI derivatized 1,3-DCP (blue line) and 3-MCPD (red line). Error bars are standard deviations obtained from triplicate experiments.

(trimethylsilyl)propan-2-ol, a partially silylated 3-MCPD. After the silylation step, (bis(trimethylsilyl)ammonio)trimethyl(((trifluoromethyl)sulfonyl)oxy)silicate(IV) will turn into trimethyl(methyl(trimethylsilyl)ammonio)(((trifluoromethyl)sulfonyl)oxy)silicate(IV), which further derivatize 1-chloro-3-(trimethylsilyl)propan-2-ol to (3-chloropropane-1,2-diyl)bis(trimethylsilane) (a fully derivatized 3-MCPD-TMS). After the derivatization process, (bis(trimethylsilyl)ammonio)trimethyl(((trifluoromethyl)sulfonyl)oxy)silicate(IV) will turn into (dimethylammonio)trimethyl(((trifluoromethyl)sulfonyl)oxy)silicate(IV). It is worth mentioning that the regenerated TMSOTf could be reused for the next derivatization cycle. (bis(trimethylsilyl)ammonio)trimethyl(((trifluoromethyl)sulfonyl)oxy)silicate(IV) was found to effectively derivatize two hydroxyl groups simultaneously in 3-MCPD. The emission of gaseous ammonia is not expected to deteriorate the performance of the detector used in the GC-MS system. The by-products generated from BSTFA and MSTFA derivatization mechanism are hydrogen fluoride and *N*-methyl trifluoroacetamide. Hydrogen fluoride when in contact with water, it will form hydrofluoric acid which will be detrimental to mass spectrometry detector if got injected.

3.2. 3-MCPD-TMS and 1,3-DCP-TMS chromatogram

Fig. 2A shows the full election ionization (EI) scan chromatogram over the mass range of m/z 100–500 at 1 s scan^{-1} , for 1,3-DCP-TMS and 3-MCPD-TMS. From the chromatogram, the retention time for 1,3-DCP was 10.402 min while the retention time for 3-MCPD was 13.991 min. Fig. 2B and C shows the characteristic ions in the EI mass spectra for 1,3-DCP-TMS and 3-MCPD-TMS, respectively. The TMS derivatized 1,3-DCP and 3-MCPD shared a few characteristics ions, such as m/z 55, 73, and 93. This is due to the similarity of the molecular structure between these two molecules. To increase the LOD for the targeted analytes, SIM mode was used for GC-MS quantification. The characteristic ions m/z 151, 93, 154 were chosen to differentiate and confirm the TMS-derivatized whereas a m/z 185 was selected as the quantification ion for 1,3-DCP-TMS (in the SIM mode). For the TMS derivatized 3-MCPD m/z 116, 119 and 147 were selected as the qualitative ion and m/z 239 as the quantitative ion. A calibration curve consisting of seven data points was used to quantify the concentrations of 1,3-DCP and 3-MCPD. The standardized calibration concentrations were 0.01 mg L^{-1} , 0.02 mg L^{-1} ,

0.05 mg L^{-1} , 0.5 mg L^{-1} , 1 mg L^{-1} , 5 mg L^{-1} , and 10 mg L^{-1} . Both the calibration curves for 1,3-DCP and 3-MCPD have an R^2 value of 0.999 (Fig. 2A). Fig. 3B shows the calibration curves of the HFBI-derivatized 1,3-DCP and 3-MCPD, both having an R^2 value of 0.999. Fig. 3A shows that the detection sensitivity of the HMDS-TMSOTf-derivatized 1,3-DCP and 3-MCPD analytes was nearly identical using the new approach as both calibration curves are close to each other. On the contrary analytical method using the HFBI derivatization process showed a significant difference in terms of the detection sensitivity of the analytes as there was a big gap between the two calibration curves (Fig. 3B). There is a significant difference in the detection sensitivity of the HFBI-derivatized 1,3-DCP and 3-MCPD because their respective derivatization process occurs through a different reaction mechanism (S_N1 versus S_N2). In contrast, HMDS-TMSOTf can react with 1,3-DCP and 3-MCPD simultaneously via the same S_N2 thus resulted in similar sensitivity. It is important to include 0.02 mg L^{-1} as one of the tested values for 3-MCPD during the preparation of the calibration curve as this is the maximum tolerance amount of 3-MCPD permissible in food under the regulation in European countries [56].

3.3. Optimization of HMDS-TMSOTf derivatization

3.3.1. Box-Behnken experimental design optimization

From the Box-Behnken optimization prediction, the polynomial equation generated for the HMDS-TMSOTf derivatization process is as follows:

$$\begin{aligned} \text{Peak Area} = & -1.66058E + 0.05 + 294.04823 \times \text{Time} \\ & + 27507.60568 \times \text{Temperature} - 5022.48561 \\ & \times \text{Catalyst Volume} - 20.21674 \times \text{Time} \times \text{Temperature} \\ & + 11.22133 \times \text{Time} \times \text{Catalyst Volume} - 21.39070 \times \text{Temperature} \\ & \times \text{Catalyst Volume} + 40.98592 \times \text{Time}^2 - 343.64132 \\ & \times \text{Temperature}^2 + 233.84978 \times \text{Catalyst Volume}^2. \end{aligned}$$

The ANOVA regression model showed that the established quadratic model was suitable for the prediction of the 3-MCPD-TMS peak area, using Fisher's *F*-test (F modal = 64.08) with a very low probability value (P -modal < 0.0001) (Supplementary material: Table c). There was only a 0.01% chance that a "Model *F*-Value" of 64.08 could occur due to noise. The predicted R^2 value

for the optimization of 3-MCPD derivatization was 0.9569 and the adequate R^2 value was predicted at 0.9726. The adequate precision analysis was used to measure the signal to noise ratio. A ratio greater than 4 was desirable. And ratio in the established model was 20.114, indicating that the model could be implemented to predict the optimum conditions that would provide the highest peak response for the derivatization. Substituting of different values of concentration, incubation time and catalyst volume, the prediction model could provide predicted peak areas, which will be very helpful in reducing the cost of detection depending on the facilities available in the testing laboratory.

3.3.2. Derivatization time optimization

The optimization of derivatization time was carried out within window of 5–50 min (Fig. 4A). There was no significant change in peak area from the 5th to 25th min. However, from the 25th to 30th minute, there was an increase in the recorded area. In between the 30th and 50th minute, no significant changes in peak area were observed, suggesting that the optimum derivatization time was at 30 min. Similarly based on the findings of the Box–Behnken optimization prediction (Supplementary material: Fig. a and b), the optimized peak area was also predicted at the 30th min mark.

3.3.3. TMSOTf volume optimization

The optimization for the volume of the TMSOTf catalyst used was carried out using 5–30 μ L of the reagent (Fig. 4B). It was worth mentioning that there was no significant difference found between all the catalyst volumes used in both the experimental optimization and Box–Behnken modeling (Fig. 4B Supplementary material: Fig. b and c). The catalyst volume used did not affect the obtained peak area recorded as the TMSOTf catalyst will be recovered at the end of the reaction and it could be reused in the next derivatization cycle. As such, a minimum of 5 μ L of TMSOTf is sufficient for to facilitate the HMDS derivatization of 3-MCPD and 1,3-DCP.

3.3.4. Temperature optimization

The optimal derivatization temperature was investigated in between 25 and 90 °C (Fig. 4C, Supplementary material: Fig. a and c). The responding peak areas of 3-MCPD and 1,3-DCP increased from 25 to 55 °C. However, the responding area decreased from 55 °C onward. A similar trend was predicted by the Box–Behnken modeling, where the responding area was found to increase from 27 to 56 °C (the highest peak) followed by a decrease thereafter. The decrease in the recovery of responding area at higher temperatures might be due to the evaporation of the derivatized 3-MCPD and 1,3-DCP. A temperature higher than 77 °C is likely to decrease the responding area as the boiling point of TMSOTf is 77 °C [57]. Using the optimal TMSOTf volume of 5 μ L, the evaporation of the catalyst will reduce the efficiency of HMDS in derivatizing 3-MCPD and 1,3-DCP. A Student's *t*-Test was performed to determine whether the derivatization temperature (25 and 55 °C) had any impact on the responding area and the results showed that there was no significant difference between these two temperature (Fig. 4C) ($p = 0.06$). Since there was no significant difference between 25 and 55 °C, the new HMDS–TMSOTf derivatization approach could be performed at room temperature (25 °C) and this is most convenient compared to the AOAC standard method (70 °C) [26].

3.4. Method validation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the signal to noise ratio (S/N) of the peak signal for a concentration close to the blank solution. (please refer to Section 2.6 on the details of how calculations made). The peak to peak signal to noise ratio of 3 was established to determine the LOD and a peak to peak signal to noise ratio of 10 for the

determination of LOQ. Based on our newly developed method, low LOD values of 0.0028 mg L^{-1} for 1,3-DCP and 0.0008 mg L^{-1} for 3-MCPD were achieved while the LOQ values achieved for 1,3-DCP and 3-MCPD were 0.0043 mg L^{-1} and 0.0011 mg L^{-1} , respectively (Table 2). The obtained LOD by using HMDS–TMSOTf derivatization method is found lower compared to the HFBI derivatization method (10 $\mu\text{g kg}^{-1}$ for 1,3-DCP and 5–100 $\mu\text{g kg}^{-1}$ for 3-MCPD) indicates that TMS silylation is more sensitive than HFB acylation method [7]. The LOQ reported for HMDS–TMSOTf derivatized 1,3-DCP and 3-MCPD is lower than BSTFA, HFBI and HFBA derivatization (Table 2). Although the end products for HMDS–TMSOTf and BSTFA is the same, but the HMDS–TMSOTf has a lower LOQ as compared to BSTFA. In other words, this finding has proven that the new method has higher sensitivity as compared with the BSTFA, HFBA and AOAC derivatization method. The established HMDS–TMSOTf method is suitable to detect both 1,3-DCP and 3-MCPD within the maximum tolerable limit set by the European Union (please refer to Table 1).

Apart from the LOD and LOQ determinations, the Intra-day and inter-day precision were performed in this study as well to determine the reproducibility and repeatability of the newly developed method as compared to the AOAC method (Table 3). The % RSD obtained for the intra-day precision study using HMDS–TMSOTf derivatization method was 3% for both standard solution of 1,3-DCP and 3-MCPD tested at high concentration (1 mg L^{-1}) each. For intermediate concentration (0.02 mg L^{-1}), the % of RSD obtained for both 1,3-DCP–TMS and 3-MCPD–TMS were 4%. At low concentration (0.005 mg L^{-1}), % of RSD obtained for 1,3-DCP–TMS was 4% and 5% for 3-MCPD–TMS. Conversely, by using HFBI derivatization method (standard AOAC method), the intra-day precision obtained was 7% for 1,3-DCP and 1% for 3-MCPD at high concentration (1 mg L^{-1}); for intermediate concentration (0.02 mg L^{-1}), the % of RSD obtained for 1,3-DCP–HFB was 2% and 5% for 3-MCPD–HFB. However, for low concentration (0.005 mg L^{-1}), both 1,3-DCP–HFB and 3-MCPD–HFB were undetectable (Table 3).

For inter-day precision, the % RSD obtained using HMDS–TMSOTf derivatization method was 2% for both 1,3-DCP and 3-MCPD tested at high concentration (1 mg L^{-1}). For intermediate concentration (0.02 mg L^{-1}), the % of RSD obtained for both 1,3-DCP–TMS and 3-MCPD–TMS were 5%. For low concentration (0.005 mg L^{-1}), % of RSD obtained for 1,3-DCP–TMS was 4% and 5% for 3-MCPD–TMS. On the other hand, the % RSD obtained for the inter-day precision using HFBI derivatization method was 4% for both 1,3-DCP and 3-MCPD tested at high concentration (1 mg L^{-1}). For intermediate concentration (0.02 mg L^{-1}), the % of RSD obtained for 1,3-DCP–HFB was 2% and 7% for 3-MCPD–HFB. Similar to the intra-day precision, both 1,3-DCP–HFB and 3-MCPD–HFB were undetectable when tested at low concentration (0.005 mg L^{-1}). The % RSD obtained for both the intra-day precision and inter-day precision by using the HMDS–TMSOTf derivatization method was found lower ($\leq 5\%$) and better than the expected precision as required by AOAC which is (11%) [58], thus these important findings have further validated the precision and reliability of results obtained through the newly developed method.

3.5. Analysis on food samples

In order to validate the newly developed method it was applied to the analysis of various soy sauces related products. Food samples were chosen randomly from a Malaysian market and they included liquid (soy sauce, thick sweet sauce and oyster sauce) as well as solid (chicken flavored cube). The detection of 3-MCPD and 1,3-DCP in the aforementioned samples proved that the derivatization method works in actual samples. In actual sample analysis, a sample clean up procedure is required to remove any hydrophilic compounds from the samples. The pre-cleaning protocol used in this study involves a self-packed alumina chromatography col-

Fig. 4. Experimental optimization of (A) derivatization period; (B) TMSOTf volume used; (C) derivatization temperature. Error bars are standard deviations obtained from triplicate experiments.

Table 4

Derivatization step by using HMDS–TMSOTf versus HFBI technique for 3-MCPD and 1,3-DCP detection in solid, paste, and liquid food samples.

Sample	Soy sauce		Sweet sauce		Chicken flavored seasoning		Oyster sauce	
Derivatizing agent	HMDS	HFBI	HMDS	HFBI	HMDS	HFBI	HMDS	HFBI
Recovery (%)	92 ± 6	94 ± 3	92 ± 2	98 ± 7	96 ± 1	93 ± 1	94 ± 3	83 ± 4
1,3-DCP (mg kg ⁻¹)	0.03 ± 3E-3	0.04 ± 3E-3	ND	ND	0.06 ± 5E-3	0.08 ± 3E-3	0.05 ± 8E-4	0.06 ± 6E-3
3-MCPD (mg kg ⁻¹)	0.01 ± 2E-4	0.01 ± 4E-4	ND	ND	0.01 ± 3E-4	ND	ND	ND

ND means target compound not detectable.

umn. Self-packing allows the modification of alumina content (the weight required), depending on sample matrices and weight. A self-packed chromatography column also allows a solid sample to be packed together in the column. The derivatization agents are sensitive to moisture and therefore, the sample clean up procedure can reduce the required amount of derivatization agent. Besides, samples that have been subjected to a chromatography column cleaning will generate better chromatograms due to the removal of interfering analytes.

Table 4 shows the analysis results of 3-MCPD and 1,3-DCP in actual food samples derivatized with HMDS–TMSOTf (our method) and HFBI (AOAC's standard method). The concentrations of 3-MCPD and 1,3-DCP were determined based on the calibration curve based on the ratio of chloropropanols to the internal standard with known concentrations. 1,4-Butanediol was selected as the internal standard as the derivatized deuterated 3-MCPD (with TMS) could not be effectively separated in the chromatogram in a single run. In contrast, 1,4-butanediol, which is similar to 3-MCPD (containing two hydroxyl functional groups) gives a peak that could be differentiated from those of 3-MCPD and 1,3-DCP. Hence, 1,4-butanediol can be used as a suitable substitution of an expensive deuterated internal standard. In order to validate the quantified 3-MCPD and 1,3-DCP, the samples were fortified with 1.00 mgL⁻¹ of 1,5-pentadiol as a surrogate standard. The introduction of a surrogate standard eliminates the requirement for a repetition of the quantification procedure in order to obtain

a percentage recovery. For the data to be valid, the percentage recovery of 1,5-pentadiol should fall from 80% to 120%. Table 4 shows the recoveries obtained for all the samples derivatized using HMDS–TMSOTf and HFBI ranging between 83% and 98% of recovery. For better data interpretation purpose, a Student's *t*-test was performed to investigate whether there is any significant difference found between the percent of recoveries obtained by the two different derivatizing agents. From the Student's *t*-test, we found that there is no significant difference for the percentage of recoveries obtained between our newly developed method as compared to AOAC method ($p = 0.69$) and this finding shows that the analytical concentration of 3-MCPD and 1,3-DCP in actual food samples (soy sauce, sweet sauce, chicken-flavored seasoning and oyster sauce) can be accurately and precisely determined using the newly developed method. Apparently Malaysia does not regulate the maximum permissible concentration of 1,3-DCP in food sources but the maximum allowable concentration of 3-MCPD is regulated at 0.02 mgL⁻¹. Based on our findings, the 3-MCPD concentrations quantified in all the selected food samples are below the maximum allowed concentration.

4. Conclusion

The simultaneous derivatization of 3-MCPD and 1,3-DCP with HMDS at room temperature was successfully developed. To our best knowledge this study is the first known silylation procedure of

chloropropanols using HMDS at room temperature. The analytical method was optimized experimentally, aided by a theoretical study utilizing Box–Behnken modeling software. Based on the calculated polynomial equation, the optimum conditions of the derivatization time, TMSOTf volume and derivatization temperature were accurately predicted depending on different laboratory settings. The experimental results were in line with the predicted values for all the parameters tested in these optimization studies. Meanwhile, the LOD and LOQ (0.0008–0.0043 mg L⁻¹) obtained using the HMDS–TMSOTf derivatization method was found lower as compared to the standard AOAC method (5–100 µg kg⁻¹). On top of this, a relatively low % RSD obtained for the inter-day (0.03%) and intra-day precision (0.01–0.02%) indicate that the newly developed method is highly reproducible. Recovery studies were conducted on both liquid and solid food samples and the results were found to be accurate (ranging between 80% and 100%). Results from with the newly developed method were also accurate as they closely agree with the results obtained using the AOAC method. For the analysis of actual food samples, three out of four selected food samples were found to contain 3-MCPD below the maximum tolerable limit (0.02 mg L⁻¹) with another sample containing no detectable level of 3-MCPD. On the other hand, high concentrations of 1,3-DCP were found in all the food samples due to the lack of an official maximum tolerable limit. Compared to the AOAC method, the consumption of chemicals using this approach is reduced. As the derivatization process can be performed at room temperature, this is also an added advantage of the HMDS–TMSOTf derivatization protocol. Furthermore, the reaction by-products (ammonia) produced by this method is relatively harmless to human health in small quantities. In short, the newly developed HMDS–TMSOTf derivatization method for quantifying 1,3-DCP and 3-MCPD simultaneously at room temperature is precise, accurate, reproducible and highly sensitive.

Acknowledgments

This research was supported by RP012C-14SUS (University of Malaya Research Grant), FP014-2013A (Fundamental Research Grant Scheme from Ministry of Higher Education Malaysia), and PG030-2014A (University of Malaya Postgraduate Research Grant). Bai Qin Lee would like to express his sincere gratitude to University of Malaya Fellowship Scheme for supporting his Ph.D. study. The authors would like to thank Miss Moo Eng Lim and Dr. Guan Serm Lee for the permission to use the gas chromatography-mass spectrometry in University of Malaya High Impact Research center.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2015.12.087>.

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