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Verification of AOCS Cd 29a-13: 2013 Method for 3-Chloropropane-1.2-Diol Esters and Glycidol Esters Analysis in Palm Oil

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ABSTRACT

Fatty acid esters of 3-Chloropropane-1,2-diol(3-MCPD) and Glycidol were contaminants in fat-rich foods such as palm oil. These contaminants have received serious concern since they may cause cancer in humans. Several methods have been developed to analyze 3-MCPDE and GE especially in refined palm oil, including AOCS Cd 29a-13 2013 with aid of GC-MS. Principally, it involves transesterification promoted by H_2SO_4 in MeOH, then derivatized using PBA (Phenylboronic Acid). The verification was required before applying this method in laboratory. The instrument performance analysis showed that linearity response (R^2) reaching up to 0.997 for 3-MCPD and 0.998 for Glycidol, determined from a linear regression using internal standards and external standards at the range of 0.3-9.3 mg kg^{-1} (3-MCPD) and 0.6-21.3 mg kg^{-1} (Glycidol). The precision of retention time in 3-MCPD and Glycidol demonstrated satisfying results, RSD=0.03% (3-MCPD-d_5), RSD=0.02% (3-MCPD), RSD=0.03% (Gly-d_5) and RSD=0.03% (Glycidol). The LoD was observed at 0.037 mg kg^{-1} (3-MCPD) and 0.072 mg kg^{-1} (Glycidol), while the LoQ was found at 0.123 mg kg^{-1} (3-MCPD) and 0.241 mg kg^{-1} (Glycidol). The verification method showed that the precision of retention time results, RSD=0.05% (3-MCPD) and RSD=0.04% (Glycidol) and the precision of concentration results showing RSD Analysis value<2/3 RSD Horwitz. Recovery percentage for 3-MCPD and Glycidol was 92.19% and 88.38%. Within the RSD analysis of lab reproducibility was obtained at 0.4% (3-MCPD) and 0.58% (Gly) less than the value of RSDh. This method also have a good selectivity. Based on the verification results, this method meets all requirements and therefore can be applied for analysis in the laboratory.

Keywords: 3-MCPD, acid transesterification, GC-MS, Glycidol, verification method

INTRODUCTION

Food safety is very importantly considered both by food producers and consumers. According to indonesian food policy No. 18 in 2012, food safety is the necessary conditions and efforts to prevent food from biological, chemical and other objects contamination possibilitiesthat can interfere and be harmful,

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besides endangering human health and incontradict with religion, beliefs and society culture, thus safe to consume. Food contaminants can be biological, chemical or physical, originated from the food due tounsuitable food chain or derived from food processing and contaminated by the environment, then causing the food unsafe to beconsumed (Hussain 2016). One of contaminant types that currently has special concern is the processing contaminant or contaminants formed due to certain chemical reactions during the food processing, such as heating, roasting, canning, hydrolyzing or fermenting (Nerin *et al.* 2016). Some types of processing contaminants are 3-chloropropane-1.2-diol (3-MCPD) along with its ester and Glycidyl ester (GE).

3-MCPD compound is firstly discovered by Velisek *et al.* (1978) on hydrolised vegetable protein (HVP) used to produce soy sauce. These contaminants are found in food containing fats suspected to be derived from the chlorinated components and asilglycerol obtained from oil refining process (Ermacora & Hrnricirik 2013). A study conducted showed that 3-MCPD content in food was not only in its free form, but also as mono or di-estercompound with fatty acids at higher concentration (Svejkovská *et al.* 2004). Another study indicated 3-MCPD ester was discovered in various vegetable oils as the lowest content was discovered in soybean, corn, and olive oil with <0.25-0.35 mg kg^{-1}, while the highest content was in palm and peanut oil with 1.65-2.45 mg kg^{-1} (Raznim *et al.* 2012). The formation mechanism of 3-MCPD along with its esters in refined condition of vegetable oil states that the presence of fatty acids (or perhaps free hydrogen chloride) is indispensable for enhanced formation of 3-MCPD esters as the number of free fatty acids increases. 3-MCPD ester is formed 2-5 times faster from the derivation of diacylglycerol than from monoacylglycerol (or possibly from triacylglycerol) (Smidrkal *et al*. 2016). The existence of 3-MCPD compound and its esters in food causes kidney and testes damage in animal assay (Liu *et al.* 2012; Abraham *et al.* 2013). International agency for research on cancer (IARC) classifies that 3-MCPD is the 2B group that can possibly cause human cancer (IARC 2012). The commision regulation (EC) No. 1881/2006 establishes tolerable daily intake (TDI) of free 3-MCPD is 2 μg kg^{-1} per body weight.

Besides 3-MCPDE, there are also other contaminants in food, namely Glycidyl fatty acid esters (GE). Cheng *et al.* (2016) states GE are formed due to the intramolecular elimination process by diacylglycerol (DAGs) and monoacylglycerol (MAGs), but underived from triacylglycerol (TAGs). The existence of GE in food become a concern as some studies stated that Glycidol can be transformed in two directions with 3-MCPD as the transformation rate from Glycidol to 3-MCPD was higher compared to 3-MCPD transformed into Glycidol in acidic conditions (Kaze *et al.* 2011). The existence of GE on food was discovered in 2009 where DAG-based oil manufactured by Kao Corporation was withdrawn from the market because it contained a large number of GE (Cheng *et al.* 2016). Study conducted by Hrncirik & Gerrit (2011) stated that GE are formed in the refining process of palm oil, especially during the deodorization process as dedorisation temperature at 180 °C resulted GE content of 0.5 mg kg^{-1}, while at 230 °C the GE content increased to 2.1 mg kg^{-1}. Glycidol is GE hydrolysate designated as a carcinogenic compound and classified as 2A group by IARC (IARC 2012).

Based on the contaminant exposure in food, the analysis of 3-MCPDE and GE content is very important in relation to food safety, especially in palm oil with

the highest contamination. The analysis method of 3-MCPDE and GE in food consist of two types, i.e direct and indirect methods. The direct method consists two extraction processes with a target of 3-MCPD monoester using solid-phase extraction (SPE) and the detection is performed directly using LC-ToF-MS, while the indirect method do not target the quantification of the ester directly, but using the acid or base transesterification ester to release 3-MCPD instead. Free 3-MCPD is derivated using phenylboronic acid (PBA) or heptafluorobutyrl imidazole (HFBI) which is subsequently quantifiedusing GC-MS instruments (Dubois *et al.* 2012). One of the indirect methods validated in Indonesia is the Weißhaar method 2008 with LOD 0.06 μg g^{-1} and LOQ 0.2 μg g^{-1} (Lanovia *et al.* 2014). However, this method has a deficiency in the salting out process using NaCl that causes Glycidol transform into MCPD, therefore method with another reagent, namely AOCS Cd 29a-13 2013 using GC-MS instrument.

The method of AOCS Cd 29a-13 2013 needs to be verified based on ISO 9000, as the test method verification is conductedto reconfirm the test method by completing the objective evidences, whether the method can meet the requirements set and in accordance with the objectives. Test method verification is done to prove that the relevant laboratory is capable of testing with the method with valid results. When the laboratory implements the existing standard methods but on a different matrix, validation is required, whereas when the matrix used is the same as the standard method, then the verification should be done (Magnusson & Ornermark 2014). Verification test was applied at laboratory of IPB University. Parameters observed to verify the test method were linearity, selectivity, accuracy, precision, also detection limits and quantitation.

MATERIALS AND METHODS

The standarts, pentadeurated 1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD-d_5) and pentadeurated Glycidol palmitate (Gly-P-d_5), 1,2-Dipalmitoyl-3-chloropropanediol (PP-3-MCPD) and Glycidol palmitate (Gly-P) standard (Santa Cruz Biotechnology, Amerika). Toluene (≥98%) (Merck, Germany), Tetrahydrofuran (≥99.8%) (Merck, Germany), Acetone analytical grade (Merck, Germany), NaBr (Sodium bromide) (Merck, Germany), sodium hydrogen carbonate ($NaHCO_3$) (Merck, Germany), sulfuric acid (H_2SO_4) (95-97%) (Merck), methanol analytical grade (Merck, Germany), sodium sulfate (Na_2SO_4) (≥99%) (Merck, Germany), n-heptane (≥99%) (Merck, Germany), phenylboronic acid (PBA) (≥97%) (Sigma Alridch, Amerika), Ultrapure water (Merck, Germany), and technical N_2 gas. Samples used were olive oil and palm oil obtained from the local supermarket.

Instruments used were GC-MS instrument with QP2010 Plus model equipped with single quadrupole analyzer (Shimadzu Corp.,Japan), AOC-20i autosampler (Shimadzu Corp., Japan), and poly capilary column (dimethylsiloxane) (e.g., Supelco Equity-1, 30 m lengthx0.25 mm i.d.x1.0 μm film thickness).

3-MCPDE and GE Determination (Indirect Method)

The determination of 3-MCPDE and GE was carried out as AOCS Cd 29a-13 method (AOCS 2013), which consisted of three stage, extraction, derivation and GC-MS analysis. The initial stage began with measuring the sample as much as 100-110 mg and was inserted into the test tube. Samples must be homogenized before preparation step. The internal standard solution PP-3-MCPD-d_5 (40 μg mL^{-1}) 50 μL, internal standard solution Gly-P-d_5

(50 µg mL^{-1}) 50 µL and 2 mL tetrahydrofuran were added into the test tube.

All mixtures were vortexed for 15 seconds. Conversion GE to MBPDE was done by applying 30 µL of NaBr in the acid solution (NaBr 3 mg mL^{-1} in 5% acid solution), then vortexed back for 15 seconds and incubated at 50 °C for 15 minutes. The reaction was stoped by adding 3 mL $NaHCO_3$ 0.6% and 2 mL N-heptane to separate the oil and water phases. Mixtures were vortexed once again for 15 seconds and waited until the separation occured. The extraction process was done twice.

The oil phase was transferred to another reaction tube with N_2 gas was flown for 15-20 minutes until the entire solvent evaporated at 35-40 °C. The residue was dissolved by adding 1 mL of tetrahydrofuran. Next stage was performed by adding 1.8 mL H_2SO_4 in MeOH (1.8%) and vortexed for 10 seconds, then incubated for 16 hours at 40 °C in the waterbath. After incubation process, the reaction was stoped by adding 0.5 mL saturated $NaHCO_3$. After that, N_2 gas was flown until 1 mL solution left. Fatty acid methyl esters were separated from the samples by added with 2 mL of Na_2SO_4 (20%) and 2 mL of N-heptane, then vortexed for 10 seconds and waited until the separation occurred. The upper part that containing methyl ester fatty acids was eliminated at this process.

After that, the remaining solution was added 250 µL saturated PBA and vortexed for 10 seconds, then incubated for 5 minutes at the room temperature ultrasonic bath. After that, 3-MCPD and Glycidol derivative were extracted by adding 1 mL N-heptane and vortexed for 10 seconds, then taken the upper part and transferred into the other test tube. The derivate result was evaporated by N_2 gas until dry and added 400 µL n-heptane, then vortexed for 10 seconds. Afterwards, the upper part was taken from the solution and moved into 250 µL insert vial for getting injected into the GC-MS.

GC-MS Analysis

For GC-MS analysis, 1 µL of extracted sample was injected using splitless mode at 250 °C. Helium was used as carrier gas with 0.8 µL min^{-1} flow rate. The temperature program used was 80 °C (1 min), 80 °C until 170 °C (at 10 °C min^{-1}), 170 °C until 200 °C (at 3 °C min^{-1}), 200 °C until 300 °C (at 15 °C min^{-1}), then held for 15 minutes at 300 °C. For MS condition used, ion source, quadropole and line transfer temperature were 230 °C, 150 °C, and 300 °C respectively. SIM parameter mode was derivative PBA-3 MCPD (m z^{-1}) 147 (quantifier ion); 196, 198 (qualifier ion), derivative PBA-3 MCPD d_5 (m z^{-1}) 150 (quantifier ion for 3-MCPD), derivative PBA-3-MBPD (m z^{-1}) 147 (quantifier ion); 240 (qualifier ion), and derivative 3-MBPD-d_5 (m z^{-1}) 150 (quantifier ion); 245 (qualifier ion).

Verification Method of 3-MCPDE and GE in Palm Oil GC-MS Performance Test

Before verifying the method, the instrument performance test was firstly performed to ensure GC-MS instrument worked properly. Instrument performance test included linearity, precision retention time, as well as LoD and LoQ. Linearity instrument was perfomed by making eight concentrations of standard solution added into olive oil sample for 3-MCPD (0.3 mg kg^{-1}-9.3 mg kg^{-1}) and Glycidol (0.6 mg kg^{-1}-21.3 mg kg^{-1}). Internal standard was added with the same concentration on all calibration standard solution, i.e 50 µL PP-3-MCPD-d_5 (40 µg mL^{-1}) and 50 µL Gly-P-d_5 (50 µg mL^{-1}) internal standard. Chromatogram results on each calibration solution were used for the standard curve design of area ratio between 3-MCPD

and 3-MCPD-d_5 (y-axis) and the standard solution concentration of 3-MCPD (µg) (x-axis), as well as the area ratio relationshipbetween Glycidol and Gly-P-d_5 (y-axis) and the standard solution concentration of Gly-P (µg) (x-axis). Through the curve, a linear equation and correlation value (R^2) were obtained.

The instrument precision can be seen through the relative standard deviation (RSD) value of analytic retention time obtained at one standard solution concentration of MCPD and Glycidol with seven replications, where the standard solution concentration of 3-MCPD was 2.1 mg kg^{-1} (20 µL) with the number of PP-3-MCPD-d_5 (40 µg mL^{-1}) 50 µL and Glycidol standard solution concentration of 4.7 mg kg^{-1} (20 µL) with the number of Gly-P-d_5 (50 µg mL^{-1}) 50 µL. The retention time obtained was averaged and calculated RSD value, therebyaccepting the RSD value of<2% in this test (AOAC 2002). RSD value of the appropriate retention time indicates the instrument is capable of providing good repeatable detection on the analysis.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) tests were perfomed by making one lowest concentration of 3-MCPD and Glycidol standard as much as three times, i.e 25 µL of 3-MCPD (0.3 mg kg^{-1}) and Glycidol (0.6 mg kg^{-1}), then added with the internal standard of PP-3-MCPD-d_5 (40 µg mL^{-1})as much as 50 µL and Gly-P-d_5 (50 µg mL^{-1}) as much as 50 µL. LoD and LoQ values are determined with standard deviation, LoD=(3x standard deviation) and LoQ=(10x standard deviation)

Parameters Test for Verification Method

Verification parameter tested were selectivity, accuration, precision and reproducibility. Selectivity was applied to find out that the analytical method can determine and measure the concentration of the analyte with other component in the sample. This stage was used a standard that containing of 0.3 mg kg^{-1} (3-MCPD) and 0.6 mg kg^{-1} (Glycidol). The preparation step was very important in this stage because the effective isolation can make the analytes separate from the matrix. Accuration was presented as recovery percentage.The test was performed using spiking with the medium concentration tested containing 20 µL of 3-MCPD (5.2mg kg^{-1}) and Glycidol (11.9mg kg^{-1}). Spiking was performed with six time replications.Accuration result was presented as follows in equation 1:

$$\frac{spiked\ sample - unspiked\ sample}{concentration\ of\ tested\ sample}\ x100 \quad (1)$$

Concentration and retention time precision test were performed with six time replications using unspike sample. The result of retention time and concentration of 3- MCPD and Glycidol was presented as the relative standard deviation (RSD). Standard deviation (SD) was presented as follows in equation 2 and for RSD value in equation 3 :

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n-1}} \quad (2)$$

$$RSD\ (\%) = \frac{SD}{\bar{x}}\ x100 \quad (3)$$

After obtaining RSD value, coefficient variance was calculated. Precision method was presented to meet all requirements whether the coefficient variance was smaller than 2/3 Horwitz coefficient as follows in equation 4.

$$KV/RSD\ Horwitz\ = \pm 2^{(1-0,5\ logC\ )} \quad (4)$$

C is the concentration presented as decimal fraction

Reproducibility was carried out in the same laboratory, equipment and operator on different days. This stage was used one concentration of the sample which performed by three times on the same day, then the average of the repetition was compared with the average of the

test performed on another day. Acceptance of reproducibility was determined based on RSD calculations, if the RSD analysis value lower than RSD Horwitz (RSDh) value, the reproducibility is good.

RESULT AND DISCUSSION

Verification Method of 3-MCPDE and GE in Palm Oil GC-MS Performance Test

Instrument performance test of GC-MS conducted before implementing the verification method of AOCS Cd 29a-13 (AOCS 2013) for analyzing 3-MCPDE and GEwasthe selectivity, linearity, retention time precision, also LoD and LoQ. Linearity is the ability of analytical method to provide proportional response against analyte concentration in the sample. Linearity is usually expressed in terms of variances around the regression line direction calculated based on the mathematical equations of data obtained from analyte test results in samples with analyte various concentrations (Magnusson & Ornermark 2014). The instrument linearity was conducted by making eight standard solution concentrations of 3-MCPD (0.3 mg kg^{-1}-9.3 mg kg^{-1}) and Glycidol (0.6 mg kg^{-1}- 21.3 mg kg^{-1}) with twicereplications (duplicate). This was in accordance with AOAC (2002), which stated that the minimum linearity curve should use six to eight concentrations with zero calibration standard excluded. The results showed that there was a linear relationship between 3- MCPD/3-MCPD-d_5 (x-axis) and area ratio of 3-MCPD/3-MCPD-d_5 (y-axis) with the equation y=0.0573x+0.013 and R^2 =0.9969 (Figure 1). Similary in Glycidol, where there was a linear relationship between Gly/Gly-d_5 concentration ratio (x-axis) and area ratio of 3-MBPD/3-MBPD-d_5 (y-axis) with y=0.0497x-0.0164 and R^2=0.9983 (Figure 2). The 3-MCPD linearity curve and Gly obtained have qualified the instrument linearity criteria as R^2 value obtained >0.99 (AOAC 2002).

Instrument precision test was performed by calculating the RSD value obtained through the retention time resulted from the concentration of 20 µL of 3-MCPD standard solution (2.1 mg kg^{-1}) and Gly standard solution (4.7 mg kg^{-1}) also 50 µL of 3-MCPD-d_5 internal standard (40 µg mL^{-1}) and Gly-d_5 internal standard Gly-P-d_5 (50 µg mL^{-1}) with 7 time replications.

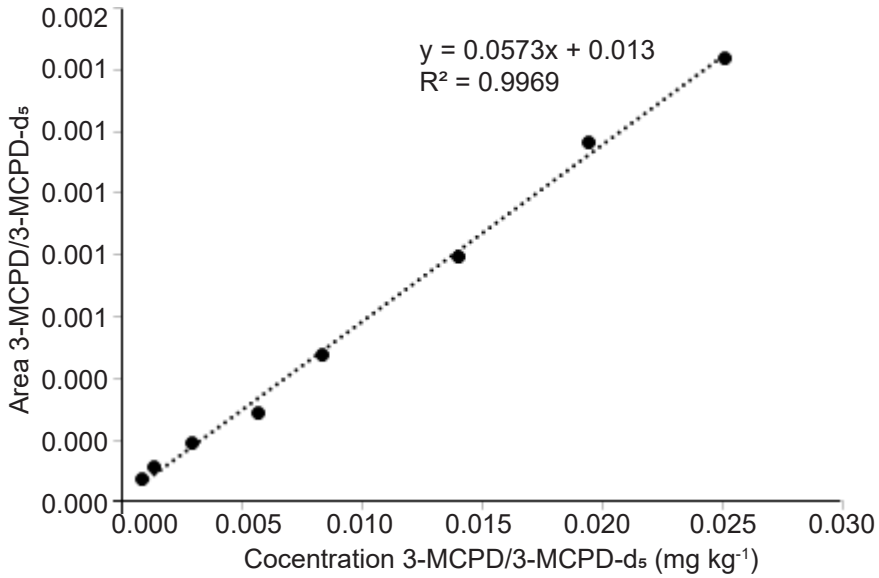

Figure 1 Linearity curve of 3-MCPD.

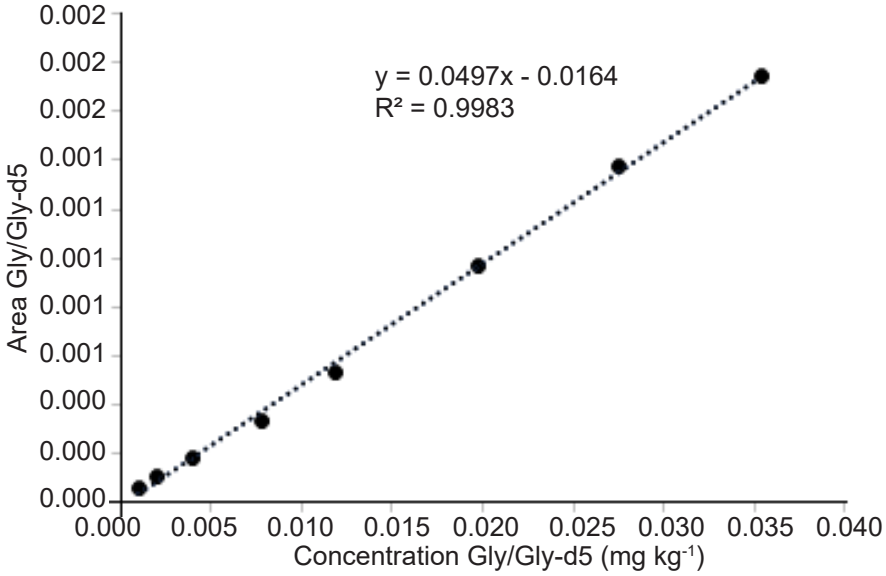

Figure 2 Linearity curve of glycidol.

Table 1 Retention time of 3-MCPD, 3-MCPD-d_5, Gly-d_5 and Gly using AOCS Cd 29a-13 method

No	Retention time (minute)			
	3-MCPD-d5	3-MCPD	Gly-d5	Gly
1	20.611	20.744	23.925	24.067
2	20.613	20.744	23.934	24.072
3	20.62	20.747	23.937	24.078
4	20.62	20.751	23.943	24.083
5	20.624	20.751	23.944	24.083
6	20.625	20.755	23.946	24.085
7	20.625	20.753	23.946	24.085
Average	20.62	20.749	23.939	24.079
SD	0.006	0.004	0.008	0.007
RSD (%)	0.028	0.021	0.033	0.029

Precision is the size that indicates how closed one test result to another. Precision is usually presented as statistical parameter which describes the obtained result as the standard deviation where this parameter is calculated from repeated measurement on certain condition (Magnusson & Ornermark 2014). The result (Table 1) indicated that RSD value on the retention time of derivative 3-MCPD-d_5 was 0.03% and 3-MCPD was 0.02%. RSD value on the retention time of derivative Gly-d_5 was 0.03% dan Gly 0.03%. Based on the result obtained, it can be stated that the retention time of both derivatives has met the requirements based on AOAC (2002), whether RSD analysis value with <2% indicates the GC-MS instrument is capable of presenting good repeatable detection analysis.

Limit of detection (LoD) and limit of quantification (LoQ) were obtained from testing the lowest concentrations of 3-MCPD and Glycidol standard as much as three times, i.e 0.3 mg kg^{-1} (3-MCPD) and 0.6 mg kg^{-1} (Glycidol) with PP-3-MCPD-d_5 (40 µg mL^{-1}) and Gly-P-d_5 (50 µg mL^{-1}) internal standard. Detection limit is the smallest amount of analyte in the detectable sample that still provides significant response compared to blank, while the quantitation limit is a parameter on the renic analysis interpreted as the smallest quantity of analyte in the sample that are still able to meet careful and thorough criteria (ISO/IEC 17025). LoD value obtained for 3-MCPD and Glycidol analysis respectively was 0.037 mg kg^{-1} and 0.072 mg kg^{-1}, while LoQ of 3-MCPD and Glycidol analysis respectively was 0.123 mg kg^{-1} and 0.241 mg kg^{-1}. The results of LoD and LoQ obtained are presented on the following (Table 2). LoD and LoQ

value from another research are presented on (Table 3).

Verification Method

Verification method test is to reconfirm a method by testifying the method based on the objective evidence completion, therefore the method meets the requirements set and in accordance with the objectives. Method verification test is performed to prove that the relevant laboratory is capable of testing using the method with valid result (ISO 9000). According to ISO 17025:2005, each laboratory must ensure or confirm that all components in the laboratory can apply the standard method properly before testing the material matrix. If the laboratory implements existing standard methods but on a different matrix, required validation should be done, whereas the matrix used is the same as the standard method, then the verification has to be done. Parameters confirmed for the verification method include selectivity, accuration, precision and reproducibility.

Selectivity test results stated that there was a clear separation of the peak chromatogram between the 3-MCPD sample with 3-MCPD d_5 and Gly with Gly-d_5. It can be concluded that the method has a good selectivity (Figure 3). Accuracion and precision concentration test were

Table 2 The results of instrument performance test using AOCS Cd 29a-13 method

Instrument performance test	3-MCPD	Gly	Requirements
Linearity (R^2)	0.997	0.998	>0.99 (AOAC 2002)
Precision of retention time (% RSD)	0.021	0.029	<2% (AOAC 2002)
Limit of detection (LoD) (mg kg^{-1})	0.037	0.072	
Limit of quantification (LoQ) (mg kg^{-1})	0.123	0.241	

Table 3 The results of LoD and LoQ from several types of method

Samples	LoD (mg kg^{-1})		LoQ (mg kg^{-1})		References
	3-MCPD	Gly	3-MCPD	Gly	
Palm oil	0.037	0.072	0.123	0.24	Verification method result
Palm oil	0.05	0.1	0.15	0.2	Sim *et al.* 2018
Palm oil	0.1	0.24	0.3	0.6	Goh *et al.* 2018
Palm oil	0.06	-	0.2	-	Lanovia *et al.* 2014
Palm oil	0.17	-	0.59	-	Lioe *et al.* 2015
Infant formula	0.08	0.16	0.10	0.20	Arisseto *et al.* 2017
Human breast milk	0.1	0.3	-	-	Zelinkova *et al.* 2008

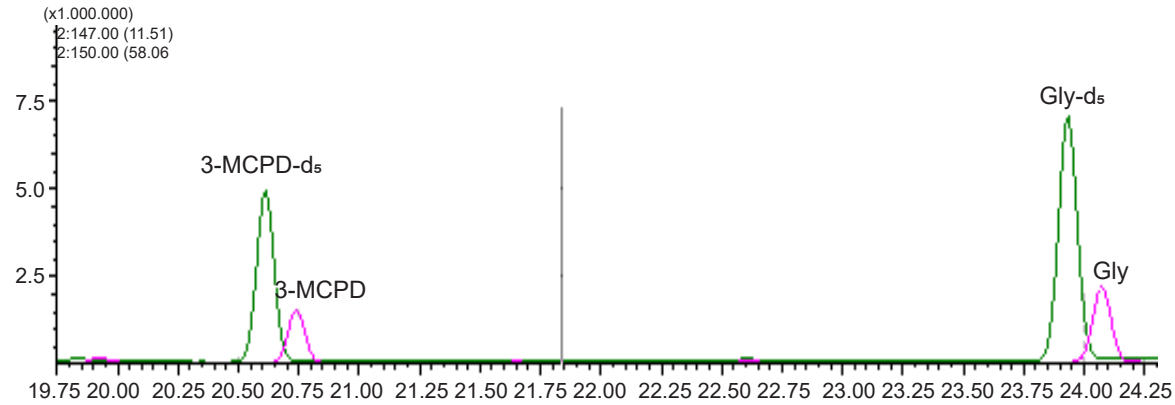

Figure 3 Chromatogram profile of 3-MCPD-d_5, 3-MCPD, Gly-d_5, Gly.

performed with the unspiked also spiked sample. The palm oil sample was spiked by medium concentrations 3-MCPD (5.2 mg kg^{-1}) and Glycidol (11.9 mg kg^{-1}) repeated six times. Accuracy shows the degree of closeness of the analysyst resulst to a reference value (Magnusson & Ornermark 2014).

Retention time precision result indicated all compounds had RSD<2% value, i.e RSD=0.05% (3-MCPD) and RSD=0.04% (Glycidol) (Table 4), as complying with AOAC requirements (2002). In addition, concentration precision (Table 5) indicates the relative standard deviation value on 3-MCPD and Glycidol respectively was 3.42% and 3.64%, lower than 2/3 horwitz relative standard deviation with 9.28% and 8.99%. This has been met the requirements of method verification result based on AOAC (2002) as the method precision is declared to meet the requirements whether the coefficient variance value is smaller than 2/3 horwitz coefficient.

The accuracy test result (Table 6) showed that the recovery obtained at 3-MCPD was 92.19% and Glycidol was 88.38%. This was in accordance with AOAC (2002), where the acceptable recovery should range between 80-110%.

Table 4 Retention time of 3-MCPD, 3-MCPD-d_5, Gly-d_5 and Gly using AOCS Cd 29a-13 method

No	Retention time[a] (minute)			
	3-MCPD-d_5	3-MCPD	Gly-d_5	Gly
1	20.565	20.693	23.882	24.021
2	20.571	20.697	23.89	24.025
3	20.576	20.700	23.896	24.031
4	20.580	20.707	23.900	24.033
5	20.583	20.712	23.901	24.041
6	20.585	20.718	23.900	24.046
Average	20.577	20.705	23.895	24.033
SD	0.008	0.010	0.007	0.009
RSD (%)	0.037	0.046	0.031	0.039

[a] The retention time of 3-MCPD and Gly on palm oil are not added with standard

This suggets that AOCS Cd 29a-13 analysis method has good accuracy for measuring both compounds.

Reproducibility was used one concentration of the sample which is unspiked sample of palm oil and performed by

Table 5 Concentration precision value of AOCS Cd 29a-13 method on GC-MS instrument

No	3-MCPD equiv.[a] (mg kg^{-1})	Gly equiv.[a] (mg kg^{-1})
1	2.515	2.903
2	2.654	3.075
3	2.581	3.231
4	2.525	3.183
5	2.393	3.096
6	2.510	3.107
Average	2.530	3.099
SD	0.086	0.113
RSD (%)	3.417	3.636
RSD horwitz (%)	13.914	13.495
2/3 RSD horwitz (%)	9.276	8.997

[a] The concentration of 3-MCPD and Gly on palm oil are not added with standard

Table 6 Accuration value of AOCS Cd 29a-13 method recovery test on GC-MS instrument

Sample	3-MCPD equiv. (mg kg^{-1})	Gly equiv. (mg kg^{-1})
Spike 1	7.093	12.701
Spike 2	7.305	13.159
Spike 3	7.271	13.885
Spike 4	7.575	14.348
Spike 5	7.097	12.681
Spike 6	7.599	14.925
Average spike[a]	7.323	13.617
Unspike 1	2.515	2.903
Unspike 2	2.654	3.075
Unspike 3	2.581	3.231
Unspike 4	2.525	3.183
Unspike 5	2.393	3.096
Unspike 6	2.510	3.107
Average unspike[b]	2.530	3.099
Recovery (%)	92.188	88.383

[a] The concentration of 3-MCPD and Gly on palm oil that had been added with standard

[b] The concentration of 3-MCPD and Gly on palm oil are not added with standard

three times on the same day, then the average of the repetition was compared with the average of the test performed on another day. This stage was carried out for three times every three days. Based on the results, it can be seen that the RSDa value of 3-MCPD (0.58%) and Gly (0.4%) was lower than RSD horwitz, 3 MCPD (13.87%) and Gly (13.52%) (Table 7). Acceptance of reproducibility was determined base on RSD calculations, if the RSD analysis value lower than RSD Horwitz (RSDh) value, the reproducibility is good.

Table 7 Reproducibility test results of AOCS Cd 29a-13 method on GC-MS instrument

Day	3-MCPD equiv (mg kg^{-1})	Average	SD	RSDa	RSDh
1	2.583	2.579	0.015	0.577	13.873
4	2.563				
7	2.592				

Day	Gly equiv (mg kg^{-1})	Average	SD	RSDa	RSDh
1	3.069	3.063	0.012	0.404	13.519
4	3.049				
7	3.071				

CONCLUSION

AOCS Cd 29a-13 2013 method for 3-MCPDE and GE analysis on the edible oil with the aid of GC-MS can be applied at Laboratory of IPB University. This method meets all requirements and therefore can be applied for analysis in the laboratory. Instrument performance test showed that linearity response (r) reached 0.997 for 3-MCPD and 0.998 for Glycidol. Retention time precision for 3-MCPD and Glycidol showed the RSD analysis was <2%, whereas RSD=0.02% for 3-MCPD and RSD=0.03% for Glycidol. LoD produced was 0.037 mg kg^{-1} (3-MCPD) and 0.072 (Glycidol), while LoQ was 0.123 mg kg^{-1} (3-MCPD) and 0.241 mg kg^{-1} (Glycidol). Verification method indicated that the precision result on the retention time indicated the RSD value of <2%, whereas RSD=0.05% (3-MCPD) and RSD=0.04% (Glycidol), while concentration precision result showed <2/3 Horwitz RSD. Accuration value as recovery percentage for 3-MCPD and Glycidol was 92.19% and 88.38%. Within lab reproducibility was obtained at 0.4% (3-MCPD) and 0.58% (Gly) less than the value of RSDh. This method also have a good selectivity.

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