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Optimization and comparison of GC-FID and HPLC-ELSD methods for determination of lauric acid, mono-, di-, and trilaurins in modified coconut oil

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ABSTRACT

Modified coconut oil (MCO) obtained from the glycerolysis of virgin coconut oil (VCO) and glycerol under various conditions should have different amounts of bioactive fatty acids (FAs) and acylglycerols (AGs). Methods were developed to analyze lauric acid (LA), monolaurin (ML), dilaurin (DL), and trilaurin (TL) in MCO samples using gas chromatography - flame ionization detector (GC-FID) and high performance liquid chromatography - evaporative light scattering detector (HPLC-ELSD). The purpose of this research is to optimize and compare GC-FID and HPLC-ELSD methods for determination of LA, ML, DL, and TL in MCO samples. All the standard curves exhibited good linearity ($R^2 \geq 0.9995$), except for that of LA analyzed by HPLC-ELSD ($R^2 = 0.9971$). The limits of detection (LODs) and quantification (LOQs) were found to be in the range of 0.033–0.260 mg/ml and 0.099–0.789 mg/ml for the GC-FID method and 0.040–0.421 mg/ml and 0.122–1.277 mg/ml for the HPLC-ELSD method, respectively. The GC-FID method (LOD ≤ 0.033 mg/ml) was more sensitive than HPLC-ELSD method (LOD ≤ 0.421 mg/ml) and showed satisfactory recoveries for LA analysis while HPLC-ELSD method (LOD ≤ 0.040 mg/ml) was more sensitive than GC-FID method (LOD ≤ 0.260 mg/ml) and exhibited acceptable recoveries for TL analysis. Both methods were applied to determine the MCO samples produced under varied conditions for glycerolysis. The results revealed that the developed GC-FID method is suitable for the quantification of LA, ML, and DL while the developed HPLC-ELSD method is appropriate for the determination of ML, DL, and TL. Both developed GC–FID and HPLC–ELSD methods produced reproducible results for the determination of LA, ML, DL, and TL in MCO samples.

1. Introduction

Virgin coconut oil (VCO) is derived from cold pressing the oil out of the dried coconut flesh (copra). Modified coconut oil (MCO) is synthesized from the glycerolysis of VCO and glycerol under optimal conditions. This reaction can be described as shown in Fig. 1. The MCO resulting from the glycerolysis is composed of fatty acids (FAs) and acylglycerols (AGs) especially lauric acid (LA) and monolaurin (ML) exhibiting antimicrobial properties [1] against *Clostridium difficile* [2] and some foodborne pathogens (*Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enteritidis*) [3]. Consequently, MCO may be used to treat *C. difficile* colitis in humans and as a preservative to extend the shelf life of raw and processed food, respectively. Furthermore, LA may help suppress appetite in women with excess body fat [4]. Therefore, MCO obtained from the glycerolysis of VCO is eligible to apply for further research because of its potential capabilities. The development of analysis method for determination of LA, ML, dilaurin (DL), and trilaurin (TL) is important to follow the progress of the glycerolysis reaction and can also be used to evaluate the quality of MCO.

Gas chromatography equipped with a flame ionization detector (GC-FID) is an efficient separation and detection technique for the analysis of FAs and AGs in oils and fats. Silylation is the most common derivatization reaction used for providing fatty acids and acylglycerols to volatile and thermally stable silyl derivatives [5]. *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) is generally used, in the existence of the transition metal catalyst, trimethylchlorosilane (TMCS), to add the trimethylsilyl (TMS) group to the active hydrogen of the target analyte to create the trimethylsilyl derivative [6]. The resulting

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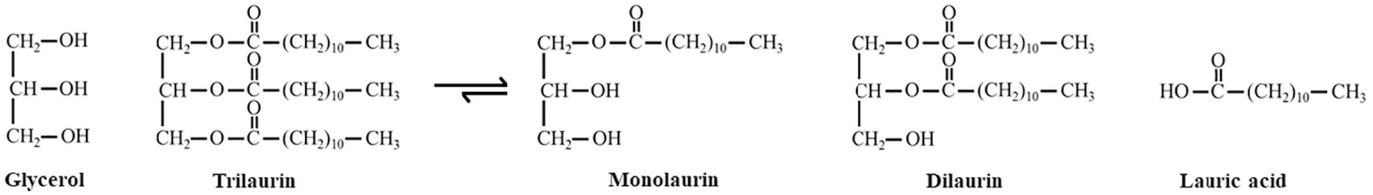

Fig. 1. Glycerolysis reaction of VCO.

derivatives are subsequently determined by GC-FID. High-performance liquid chromatography combined with an evaporative light scattering detector (HPLC-ELSD) is a widely used technique to compare with GC-FID for the determination of FAs. Although GC-FID has some limitations such as the loss of analytes during a derivatization reaction and the degradation of thermal sensitive FAs, it is more suitable than HPLC when the determination of FA composition is required [7]. HPLC-ELSD has become the accepted technique for the separation of triacylglycerols (TGs) in the isocratic or gradient elution mode [8]. However, the drawbacks of HPLC-ELSD are that its response does not follow Beer's Law and its sensitivity is sometimes low [9].

The aim of this work is to optimize GC-FID and HPLC-ELSD methods for determination of LA, ML, DL, and TL in MCO. In order to validate the analytical methods, the validation parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy were evaluated. After validation was completed, the two analytical methods were applied to determine the composition of LA, ML, DL, and TL in MCO samples. This study also focuses on the comparison of the contents of LA, ML, DL, and TL obtained from the developed GC-FID and HPLC-ELSD methods.

2. Materials and methods

2.1. Reagents and solvents

Solvents such as pyridine, acetone, and acetonitrile (ACN) used in this study were of HPLC grade bought from Merck KGaA, (Darmstadt, Germany). Reference standards including LA (Lot No. N-12A-S8-T), ML (Lot No. M-134-Jy6-W), DL (Lot No. D-131-MA16-U), and TL (Lot No. T-130-A7-T) were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). BSTFA (99%) with TMCS (1%) (Lot No. BCBJ4275V) and n-tetradecane (Lot No. BCBF0800V) were obtained from Sigma-Aldrich (St. Louis, MO, USA). MCO samples were prepared by Pharmaceutical Biopolymer Group (PBiG), Faculty of Pharmacy, Silpakorn University (Nakhon Pathom 73000, Thailand).

2.2. Standard solutions

For GC-FID analysis, the internal standard solutions (5 and 20 mg/ml) were individually prepared by dissolving 125 and 500 mg of n-tetradecane (internal standard) in 25 ml of pyridine. A standard mixture stock solution composed of LA, ML, DL, and TL at concentrations of 5, 5, 10, and 10 mg/ml, respectively, was prepared by dissolving 25, 25, 50, and 50 mg of standard LA, ML, DL, and TL in 1.25 ml of 20 mg/ml internal standard solution and then adjusting the volume to 5.0 ml with pyridine. The standard mixture stock solution was diluted with 5 mg/ml internal standard solution to create a dilution series, which is used as a standard curve.

In case of HPLC-ELSD analysis, a standard mixture stock solution was prepared by dissolving 25, 2.5, 2.5, and 2.5 mg of standard LA, ML, DL, and TL in 5 ml of acetone to achieve final concentrations of 5, 0.5, 0.5, and 0.5 mg/ml, respectively. This stock solution was diluted with acetone to make an appropriate concentration range for creating a standard curve.

2.3. Sample preparation

MCO samples were dissolved separately in the internal standard solution and then adjusted the volume by adding pyridine to reach the final concentrations of 10 and 100 mg/ml for GC-FID analysis. The samples were individually dissolved in acetone to achieve the final concentration of 5 mg/ml for HPLC-ELSD analysis.

2.4. Silylation reaction for GC-FID analysis

The derivatization procedure was done according to the Association of Analytical Communities (AOAC) 969.33 procedure [10]. Silylation of the diluted standard solutions and sample solutions were separately carried out with excess silylation reagent, BSTFA-TMCS (99:1), for 30 min at 70 °C.

2.5. Chromatographic analysis

The silyl derivatives were analyzed using a GC-FID (6890 N Network GC System) fitted with a DB-5HT capillary column (30 m × 0.25 mm ID, 0.10 µm film) purchased from Agilent Technologies, USA. The oven temperature was held at 100 °C for 1 min, increased from 100 to 223 °C at 30 °C /min, from 223 to 227 °C at 1 °C/min, from 227 to 360 °C at 5 °C/min, and held at 360 °C for 10 min. Inlet and detector temperatures were 350 °C. The injection volume was 1.0 µl, the flow rate of helium was 1.4 ml/min, and injection was performed in the split mode (1:80). All measurements were performed in triplicate.

HPLC-ELSD (Agilent 1200 series, Agilent Technologies, USA) was used for analysis of FAs and AGs. HPLC separation was carried out on a ZORBAX Eclipse Plus C_{18} column (4.6 × 250 mm, 5 µm) bought from Agilent Technologies, USA, operating column temperature at 25 °C. The mobile phase used was a gradient of ACN with 0.01% acetic acid (A) and acetone (B). Elution was carried out at a flow rate of 1.0 ml/min with a linear gradient of A: B (v/v) as follows: 0 min, 90: 10; 5 min, 70: 30; 10 min, 50: 50; 15 min, 30: 70; 20 min, 20: 80, holding at 20: 80 for 10 min and returning to the initial conditions within 5 min. The injection volume was 10 µl. The effluent was monitored by an ELSD detector, with the following settings: ELSD temperature 40 °C, nitrogen pressure 3.5 bar, ELSD gain 5, and ELSD filter 2 s. All the assays were done in triplicate.

2.6. Method validation

The developed methods were validated with regard to linearity, LOD, LOQ, accuracy and precision. For GC-FID method, six concentration levels of the standard mixture solutions dissolved in 5 mg/ml internal standard solution were used for evaluation of linearity. The standard curves were constructed at six concentration levels in the concentration ranges of 0.51–2.55 (LA), 0.51–2.55 (ML), 1.02–5.10 (DL), and 1.00–5.02 (TL) mg/ml. The linearity was subsequently estimated by the linear regression equation. After creating standard curves in the MS Excel, the values of the residual standard deviation (σ) and slope can be obtained from the LINEST function. LOD and LOQ can be calculated according to the following formulas: LOD = (3.3 × σ)/slope and LOQ = (10 × σ)/slope, respectively [11].

Validation of the precision of a GC-FID method was evaluated at three steps. The first step was system precision based on multiple injections (n = 10) of a single preparation of each standard on the same day. The second step was method precision (multiple preparations, n = 5) obtained from the same MCO sample using the same measurement procedure and one set of chemical reagents on one particular day. The third step was intermediate precision (n = 5) performed on different days on the same MCO sample. Precision data was calculated as the percent relative standard deviation (% RSD) according to the formula: % RSD = (S.D./mean) × 100%.

The spike recovery method was used to evaluate the accuracy of the GC-FID method. The spiked samples containing LA, ML, DL, and TL in the concentration ranges of 0.51–2.56, 0.51–2.56, 1.02–5.09, and 1.00–5.04 mg/ml, respectively, were prepared by adding three different concentrations of standard mixture solutions to the MCO sample solutions using 5 mg/ml internal standard solution as the diluent. The average recoveries were calculated using the formula: recovery (%) = [(observed amount − original amount)/spiked amount] × 100%.

In case of HPLC-ELSD method, six concentration levels of the standard mixture solutions were prepared in the ranges of 0.80–5.03 (LA), 0.03–0.44 (ML), 0.02–0.35 (DL), and 0.02–0.34 (TL) mg/ml by diluting in acetone. Standard curves were plotted as the peak area versus the concentration of each standard. The linearity was evaluated by linear regression analysis and then LODs and LOQs were determined as mentioned above. The precision of the HPLC-ELSD method was assessed by the evaluation of the system, method, and intermediate precision in relation to the aforementioned procedures. The recovery test was used to evaluate the accuracy of the HPLC-ELSD method. Accurate amounts of the standard mixture solution were added to 500 µl of each stock MCO solution using acetone as the diluent to achieve the spiked samples in the concentration ranges of 0.04–2.01 (LA), 0.05–0.27 (ML), 0.04–0.22 (DL), and 0.04–0.21 (TL) and subsequently analyzed by HPLC-ELSD. Finally, the achieved average recoveries from spiked samples were calculated.

2.7. Statistical analysis

The LODs and LOQs were calculated from the regression data [12]. The comparison between the developed GC-FID and HPLC-ELSD methods was performed using the Student's t-test with 0.05 as the significant level of the mean experimental values [12].

3. Results and discussion

3.1. Optimization of GC-FID and HPLC-ELSD conditions

For GC-FID method, detector temperatures at 350 °C had no effect on the peak heights and areas of analytes including LA, ML, DL, and TL. Therefore, the FID detector temperature was set at 350 °C. Furthermore, there was no evidence to show thermal decomposition of analytes and IS with the inlet temperature up to 350 °C. The results of this study indicate that the DB-5HT capillary column can be used for analysis of silyl derivatives of LA, ML, and DL because three peaks of the silyl derivatives are separated within 16 min displayed sharp and symmetrical peaks with high resolution values (Rs = 1.52–9.96) except for TL (Rs ≈ 0.81) as shown in Fig. 2A and B. The retention time increases in the order: LA < ML < DL < TL.

The HPLC condition was developed by modifying the elution gradient. Analytes including LA, ML, DL, and TL were quantitatively determined using gradient elution with acetone: ACN containing with 0.01% acetic acid. It was found that all analytes were splendidly eluted at a flow rate of 1.0 ml/min and column temperature of 25 °C. The high resolution chromatograms (Rs = 1.32–6.79) for ML, DL, and TL and the low resolution chromatograms for LA (Rs ≈ 1.04) are shown in Fig. 2C and D. The elution time increases in the order: ML < LA < DL < TL. Under the optimal HPLC condition, ML, DL, and TL are clearly resolved within 20 min (Fig. 2C and D), indicating that this method can be used for determination of ML, DL, and TL in the MCO sample.

3.2. Method validation

The mixed standards including LA, ML, DL, and TL were all used to validate both HPLC–ELSD and GC–FID. Fig. 3 shows standard curves of ML obtained from GC-FID and HPLC-ELSD. Table 1 shows the equations of the standard curves, the LODs, and the LOQs for mixed standards. All the standard curves exhibited good linearity ($R^2 \geq 0.9998$), except for those of TL ($R^2 = 0.9995$) and LA ($R^2 = 0.9971$) analyzed by GC-FID and HPLC-ELSD, respectively. The LODs and LOQs for mixed standards were found to be in the range of 0.033–0.260 mg/ml and 0.099–0.789 mg/ml for the GC-FID standard curves and 0.040–0.421 mg/ml and 0.122–1.277 mg/ml for the HPLC-ELSD standard curves, respectively (Table 1).

The precision and accuracy of the GC-FID and HPLC-ELSD methods were performed by spiking the MCO sample with accurate quantity of mixed standards of LA, ML, DL, and TL. The mean recovery was calculated on three assays (n = 3) for each standard. Table 2 shows the accuracy of the GC-FID method. The overall recoveries ranged from 103.95 ± 2.01 to 108.05 ± 3.40% for LA, 104.82 ± 1.82 to 114.77 ± 3.58% for ML, 107.42 ± 2.34 to 118.06 ± 2.14% for DL, and 104.82 ± 2.25 to 114.66 ± 1.09% for TL. As shown in Tables 2 and 3, the GC-FID method showed high reproducibility for the quantification of the LA, ML, and DL, with good system, method, and intermediate precision of 0.36, 1.13, and 2.37% for LA, 0.69, 0.32, and 1.92% for ML, and 0.73, 0.50, and 0.44% for DL, respectively, except for poor system (1.32%) and intermediate (8.36%) precision of TL. The % RSD was taken as a measure of precision. These results demonstrated that the GC-FID method was accurate and precise for the quantitative determination of LA, ML, and DL in the MCO sample.

The developed HPLC-ELSD analytical method exhibited good accuracy with the satisfactory recoveries from 98.30 ± 2.95 to 105.27 ± 7.95% for ML, 92.56 ± 0.65 to 109.78 ± 2.99% for DL, and 96.06 ± 1.98 to 98.65 ± 7.23% for TL but it showed poor accuracy with the low recovery from 58.56 ± 2.05 to 65.60 ± 2.65% for LA (Table 2). The measurement of system, method, and intermediate variabilities was used to determine the precision of the developed HPLC-ELSD method. As presented in Table 3, the appropriate precision showed the low values of system, method, and intermediate % RSD of 1.20, 2.45, and 1.97% for ML, 1.29, 1.51, and 1.73% for DL, and 1.20, 1.52, and 3.11% for TL, respectively, except for poor system precision (3.50%) of LA. Therefore, the developed HPLC–ELSD method was accurate and precise for simultaneous quantitative evaluation of ML, DL, and TL in the MCO sample apart from LA which not only had high LOD (0.421 mg/ml) and LOQ (1.277 mg/ml) but also showed low % recovery (from 58.56 ± 2.05 to 65.60 ± 2.65%) as shown in Tables 1 and 2, respectively. The obtained results from method validation were consistent with the acceptance criteria [13].

3.3. Application and comparison of the developed methods

GC-FID determination of LA, ML, and DL was performed by converting the target analytes to their silyl derivatives while HPLC-ELSD determination of ML, DL, and TL was carried out by direct analysis. The GC and HPLC chromatograms of the MCO sample obtained from developed GC-FID and HPLC-ELSD methods are shown in Fig. 2B and D, respectively. For the analysis of MCO samples, the developed GC-FID method was profitably applied to simultaneous determination of LA, ML, and DL while the developed HPLC-ELSD method was appeared to be specific for ML, DL, and TL. In terms of analyte's retention time, HPLC-ELSD method was slightly faster than GC-FID method. The total run time of HPLC-ELSD method was 20 min while that of GC-FID method was 26 min. For GC-FID method, the retention times of LA, ML, and DL were 3.5, 5.8, and 16.2 min, respectively, while for HPLC-ELSD

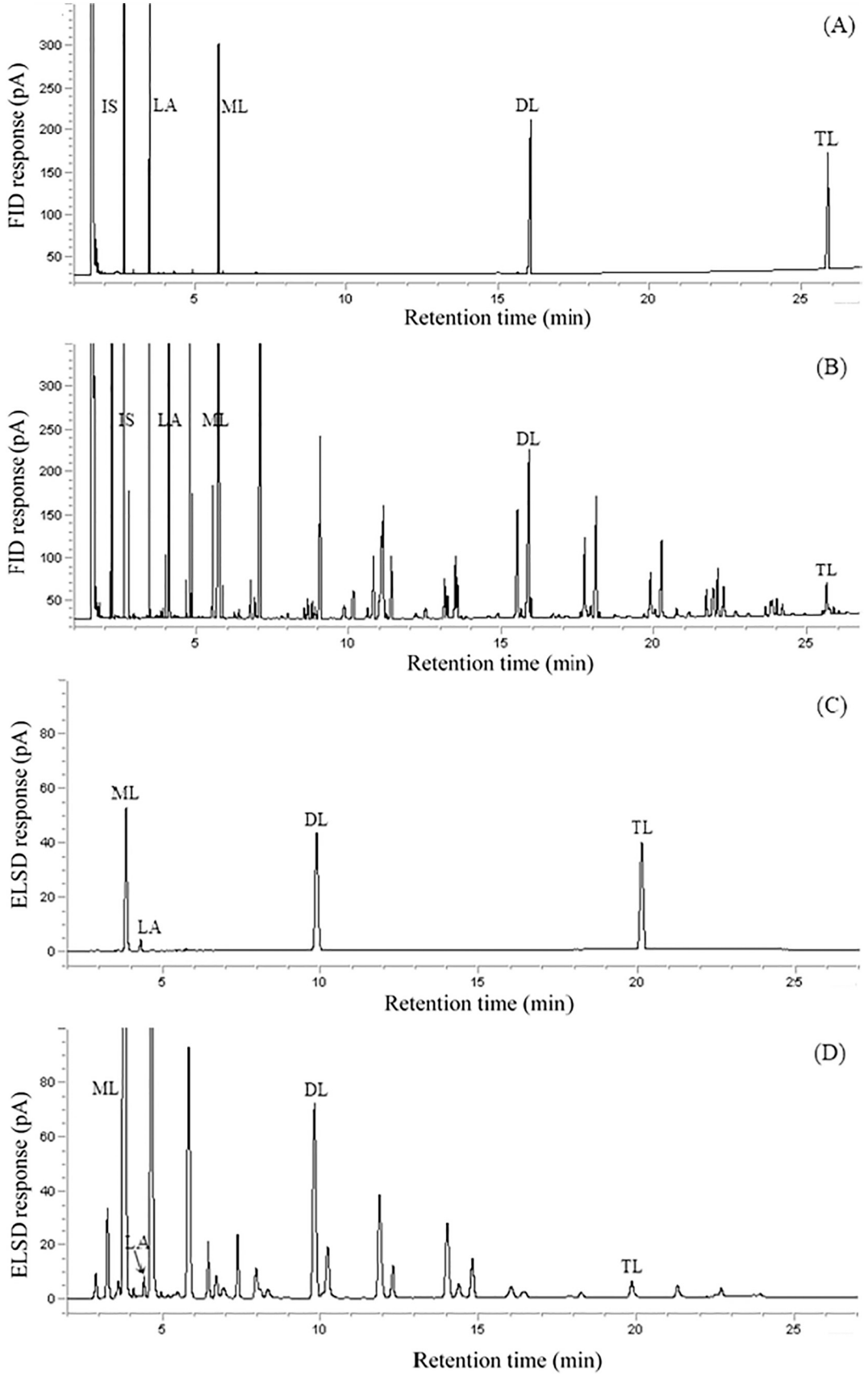

Fig. 2. The representative GC chromatograms of the mixed standards (A) and MCO sample (B). Column: DB-5HT capillary column (30 m × 0.25 mm ID, 0.10 μm film), detector: FID. The representative HPLC chromatograms of mixed standards (C) and MCO sample (D). Column: ZORBAX Eclipse Plus C_{18} column (4.6 × 250 mm, 5 μm), detector: ELSD. Lauric acid (LA), monolaurin (ML), dilaurin (DL), trilaurin (TL), n-tetradecane (IS).

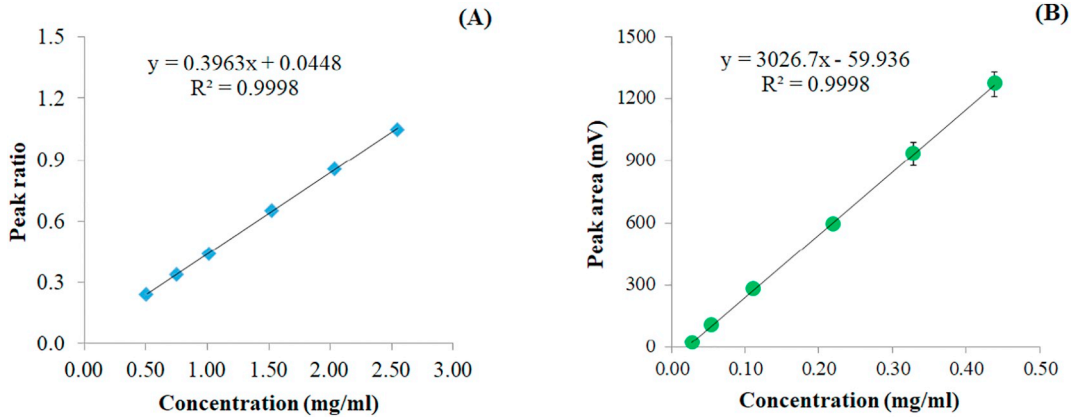

Fig. 3. Calibration curves of ML obtained from GC-FID (A) and HPLC-ELSD (B).

Table 1
Linearity of standard curves, LODs, and LOQs for lauric acid (LA), monolaurin (ML), dilaurin (DL), and trilaurin (TL) analyzed using GC-FID and HPLC-ELSD.

Compounds	Calibration curve	R^2	LOD (mg/ml)	LOQ (mg/ml)
GC-FID method[a]				
Lauric acid (LA)	y = 0.3810x + 0.0067	0.9998	0.033	0.099
Monolaurin (ML)	y = 0.3963x + 0.0448	0.9998	0.072	0.217
Dilaurin (DL)	y = 0.3780x + 0.0172	0.9999	0.093	0.282
Trilaurin (TL)	y = 0.3358x − 0.1117	0.9995	0.260	0.789
HPLC-ELSD method[b]				
Lauric acid (LA)	y = 33.042x − 16.219	0.9971	0.421	1.277
Monolaurin (ML)	y = 3026.7x − 59.936	0.9998	0.054	0.162
Dilaurin (DL)	y = 3793.2x − 51.645	0.9998	0.046	0.138
Trilaurin (TL)	y = 3819.0x − 56.028	0.9998	0.040	0.122

[a] y, peak area ratio (analyte/internal standard); x, concentration of analyte (mg/ml).
[b] y, peak area (mV); x, concentration of analyte (mg/ml).

Table 2
Accuracy of GC-FID and HPLC-ELSD methods for the determination of lauric acid (LA), monolaurin (ML), dilaurin (DL), and trilaurin (TL).

Standard	Concentration (mg/ml)	% Recovery			Average ± SD
		No. 1	No. 2	No. 3	
GC-FID method					
Lauric acid (LA)	0.51	105.42	111.88	106.85	108.05 ± 3.40
	1.53	102.75	105.72	106.50	104.99 ± 1.98
	2.56	101.63	105.25	104.97	103.95 ± 2.01
Monolaurin (ML)	0.51	115.04	111.06	118.21	114.77 ± 3.58
	1.54	104.13	110.08	107.75	107.32 ± 3.00
	2.56	102.75	105.57	106.14	104.82 ± 1.82
Dilaurin (DL)	1.02	119.54	115.60	119.03	118.06 ± 2.14
	3.05	112.18	112.29	111.80	112.09 ± 0.26
	5.09	109.43	107.97	104.86	107.42 ± 2.34
Trilaurin (TL)	1.00	113.41	115.44	115.14	114.66 ± 1.09
	3.02	108.47	107.93	109.03	108.48 ± 0.55
	5.04	106.97	104.99	102.49	104.82 ± 2.25
HPLC-ELSD method					
Lauric acid (LA)	0.04	67.92	62.72	66.16	65.60 ± 2.65
	1.21	60.25	59.16	56.28	58.56 ± 2.05
	2.01	66.46	63.37	63.67	64.50 ± 1.70
Monolaurin (ML)	0.05	111.53	96.32	107.96	105.27 ± 7.95
	0.16	100.86	103.29	94.28	99.48 ± 4.66
	0.27	98.27	101.27	95.36	98.30 ± 2.95
Dilaurin (DL)	0.04	107.96	113.23	108.14	109.78 ± 2.99
	0.13	94.68	96.42	97.37	96.16 ± 1.36
	0.22	92.86	91.81	92.99	92.56 ± 0.65
Trilaurin (TL)	0.04	98.18	106.10	91.66	98.65 ± 7.23
	0.13	98.71	94.15	98.58	97.15 ± 2.59
	0.21	97.88	93.94	96.35	96.06 ± 1.98

method, those of ML, DL, and TL were 3.8, 9.8, and 19.8 min, respectively (Fig. 2).

Ten MCO samples were prepared by Pharmaceutical Biopolymer Group (PBiG) under different conditions including concentrations of reactants, reaction temperatures, and times. The developed GC-FID and HPLC-ELSD methods were applied to compare the determination efficiency for LA, ML, DL, and TL in MCO samples (Table 4). The concentrations (mg/ml) of ML and DL in all MCO samples from the GC-FID method were in good agreement with the results obtained from the HPLC-ELSD method (Table 4). In the event of LA and TL, the Student's *t*-test was used for statistical data analysis. There is a statistically significant difference between GC-FID and HPLC-ELSD methods for determination of LA and TL in individual MCO samples (Table 4). Based on the results of method validation, the GC-FID method (LOD ≤ 0.033 mg/ml) was more sensitive than the HPLC-ELSD method (LOD ≤ 0.421 mg/ml) and showed satisfactory recoveries (matrix spiked recoveries range from 103.95 ± 2.01 to 108.05 ± 3.40%) for LA analysis while the HPLC-ELSD method (LOD ≤ 0.040 mg/ml) was more sensitive than the GC-FID method (LOD ≤ 0.260 mg/ml) and exhibited acceptable recoveries ranging from 96.06 ± 1.98 to 98.65 ± 7.23% for TL analysis as shown in Tables 1 and 2. The results of these experiments showed that the GC-FID method was suitable for analysis of LA while the HPLC-ELSD method was appropriate for determination of TL composed of medium-chain fatty acids. These findings were consistent with previous studies [7,8,14].

The higher contents of LA, ML, and DL and the lower contents of TL in each of the MCO samples compared to those in the VCO could be the useful markers to determine the optimal condition for the chemical glycerolysis of VCO and glycerol. Because VCO had higher content of TL than MCO samples, it is expected that TL was converted into hydrolyzed products such as DL, ML, and LA by the glycerolysis reaction as shown in Fig. 1. The MCO-04 sample should be synthesized under the optimal condition for the glycerolysis because it showed the highest contents of the bioactive ML (240.32 ± 5.63 and 247.25 ± 2.78 mg/ml) from GC-FID and HPLC-ELSD methods, respectively (Table 4). Consequently, the developed GC-FID and HPLC-ELSD methods are appropriate for determining the contents of LA, ML, DL, and TL in MCO samples, the products of glycerolysis, and can also be used to estimate the quality of the MCO related to the content of ML.

4. Conclusions

The GC-FID method is more sensitive toward the determination of LA than the HPLC-ELSD method while the HPLC-ELSD method is more sensitive to quantify TL than the GC-FID method. Although LOD

Table 3
System, method, and intermediate precision of GC-FID and HPLC-ELSD methods for the determination of lauric acid (LA), monolaurin (ML), dilaurin (DL), and trilaurin (TL).

Precision		% RSD			
		Lauric acid (LA)	Monolaurin (ML)	Dilaurin (DL)	Trilaurin (TL)
GC-FID method					
System precision (n = 10)		0.36	0.69	0.73	1.32
Method precision (n = 5)		1.13	0.32	0.50	0.51
Intermediate precision	Day 1	1.13	0.32	0.50	0.51
(n = 5)	Day 2	0.19	0.33	0.32	0.20
	Average	2.37	1.92	0.44	8.36
HPLC-ELSD method					
System precision (n = 10)		3.50	1.20	1.29	1.20
Method precision (n = 5)		0.29	2.45	1.51	1.52
Intermediate precision	Day 1	0.29	2.45	1.51	1.52
(n = 5)	Day 2	0.28	1.66	2.11	2.63
	Average	0.28	1.97	1.73	3.11

Table 4
Comparison of the concentrations (mg/ml) of lauric acid (LA), monolaurin (ML), dilaurin (DL), and trilaurin (TL) in virgin coconut oil (VCO) and modified coconut oil (MCO) samples using GC-FID and HPLC-ELSD methods.

Sample code	Concentration (mg/ml)			
	Lauric acid (LA)	Monolaurin (ML)	Dilaurin (DL)	Trilaurin (TL)
GC-FID method				
VCO	1.74 ± 0.23	1.01 ± 0.17	4.44 ± 2.10	163.68 ± 10.71
MCO-01	7.14 ± 0.10	64.21 ± 2.56	71.67 ± 0.14	57.81 ± 0.29
MCO-02	9.35 ± 0.08	132.34 ± 1.64	88.06 ± 0.45	43.46 ± 0.21
MCO-03	12.04 ± 0.09	107.02 ± 1.99	85.10 ± 0.41	45.14 ± 0.25
MCO-04	13.18 ± 0.06	240.32 ± 5.63	57.73 ± 0.21	17.05 ± 0.14
MCO-05	14.20 ± 0.12	188.04 ± 4.38	72.36 ± 0.24	24.64 ± 0.15
MCO-06	15.60 ± 0.06	184.46 ± 2.85	69.78 ± 0.95	24.21 ± 0.21
MCO-07	16.48 ± 0.03	236.56 ± 4.10	64.00 ± 0.71	20.48 ± 0.27
MCO-08	16.96 ± 0.15	167.46 ± 2.99	65.24 ± 0.28	19.53 ± 0.12
MCO-09	17.30 ± 0.22	158.50 ± 2.50	69.40 ± 0.69	26.65 ± 0.24
MCO-10	19.02 ± 0.04	126.43 ± 1.41	62.33 ± 0.44	25.95 ± 0.16
HPLC-ELSD method				
VCO	ND	ND	ND	159.50 ± 1.33
MCO-01	12.85 ± 0.09	64.18 ± 2.05	71.77 ± 0.75	54.54 ± 0.64
MCO-02	13.81 ± 0.21	129.76 ± 1.19	90.76 ± 0.41	36.03 ± 0.25
MCO-03	15.14 ± 0.05	102.70 ± 1.03	79.87 ± 1.35	39.60 ± 0.17
MCO-04	16.47 ± 0.03	247.25 ± 2.78	52.65 ± 0.75	12.27 ± 0.11
MCO-05	18.23 ± 0.19	189.53 ± 1.89	74.23 ± 1.11	20.28 ± 0.29
MCO-06	17.64 ± 0.13	195.14 ± 1.16	63.07 ± 0.76	19.76 ± 0.40
MCO-07	19.08 ± 0.31	238.98 ± 2.23	60.69 ± 0.42	17.61 ± 0.04
MCO-08	18.50 ± 0.11	161.31 ± 0.62	64.53 ± 0.81	18.28 ± 0.28
MCO-09	19.41 ± 0.11	151.22 ± 1.93	61.00 ± 0.87	21.01 ± 0.08
MCO-10	19.86 ± 0.06	127.72 ± 1.57	58.80 ± 0.93	21.20 ± 0.11

ND: not determined.

concentration of the GC-FID method for TL was not as sensitive as the HPLC-ELSD, it has been successfully applied for determination of LA, ML, and DL in MCO samples. Nevertheless, the HPLC-ELSD showed low % recovery of LA, it has been profitably applied for determination of ML, DL, and TL in MCO samples. Finally, we conclude that the developed GC-FID and HPLC-ELSD methods provide fast and reliable determination of LA, ML, DL, and TL in MCO samples. In this study, the developed GC-FID and HPLC-ELSD are new methods for the analysis of MCO samples.

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