Vol.4, No.8, Page 2324- 2333, 2026 https://doi.org/10.59628/jast.v4i8.3112 ISSN 2958-9568 JAST

SUN PUBLISHER

مجلة جامعة صنعاء للعلوم التطبيقية والتكنولوجيا

Sana'a University Journal of Applied Sciences and Technology

https://journals.su.edu.ye/index.php/jast/

Method Development for the Detection of Lard in Lotion Cosmetics Using Gas Chromatography-Flame Ionization Detection and Chemometrics

Raja'a A. H. Saif[1,2], Ranya A. Al-Alie[1*], Mahfoudh M. AL-Hamadi[1], Anass A. Al-Nedhary[3], Najwa S. AL-Darwish[1] and Fatima A. Murshed[1]

[1]**Department of Chemistry, Faculty of Science, Sana'a University, Sana'a, Yemen,**
[2]**Yemen Standardization Metrology and Quality Control Organization, Sana'a, Yemen,**
[3]**Department of Chemistry, Faculty of Education, Khawlan Branch, Sana'a University, Sana'a, Yemen**

*Corresponding author: r.alali@su.edu.ye

Abstract

A Gas Chromatography-Flame Ionization Detector method was optimized for the rapid detection of lard in cosmetic lotion samples. Lard was extracted using the Soxhlet method, and lotion oils were isolated via liquid-liquid extraction at 60 °C. The method demonstrated high linearity (R^2) ranging from 0.9983 to 0.9998 across a concentration range from 1 to 100 μg/mL for four fatty acids: C20:2, C20:3n6, C20:3n3, and C20:4, which are biomarkers for lard. The precision was high, with a Relative Standard Deviation not exceeding 5.82%. The recovery rates from the spiked samples ranged between 93.00% and 103.58%. The Limit of Detection and Limit of Quantitation were 0.16–0.25 μg/mL and 0.54–0.84 μg/mL, respectively. The validated method was applied to 20 commercial lotion brands collected from Yemeni markets. None of the analyzed samples showed the characteristic fatty acid markers of Lard. The developed GC-FID method, combined with principal component analysis provides a rapid, reliable, and cost-effective approach for halal authentication of cosmetic products.

Article Info

Keywords:
Lard, GC-FID, Lotion Cosmetics, Chemometrics, Yemen.

Article History:
Received: 29-April-2026,
Revised: 25-May-2026,
Accepted: 12-July-2026,
Published: 28 August 2026.

1. INTRODUCTION

The global consumption of personal care products, particularly lotions and creams, has increased tremendously [1, 2]. Given their constant and direct contact with the skin, the safety and suitability of the ingredients in these preparations are critical [2–4].

In some formulations, lard (pig fat) is used as a viscosity-increasing agent [5, 6]. While the Food and Drug Administration (FDA) recognizes lard as a safe substance, its inclusion in cosmetics is strictly prohibited in Islamic practice [7]. Consequently, there is a rising demand from both Muslim and non-Muslim consumers for high-quality, pig-derived-free products [8–10]. Therefore, lotion cosmetic products without pig derivatives are not only becoming more popular among Muslims, but also non-Muslims are becoming more discerning about the assurance that the cosmetic products are of the highest quality [11, 12].

To analyze lard, the development of an analytical method that offers fast and reliable results is required. Fourier-transform infrared (FTIR) spectroscopy, biochemical techniques (including DNA-based polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), electronic nose technique (EN), and chromatographic techniques) are the four main analytical techniques used in lard analysis, which allow the identification of lard in complex matrices [5, 7, 8].

Chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) are widely used in the detection

of lard adulteration. One of the developed methods is gas chromatography/flame ionization detector (GC/FID), which is a highly effective choice. It is a fast, specific, reproducible, sensitive, low-cost, rapid, and reliable tool for the separation and quantitation analysis of lard in food and cosmetics matrices [10, 13].

Previous research has shown GC/FID to be a method for analyzing lard, mainly in food products or lard mixture with other fats or oils [14–17]. Most studies have focused on detecting lard in food or food products [5–8, 14, 16–19]. In Yemen, a published study indicates that lard was detected in food commodities [20], but the detection of lard in cosmetics, specifically in lotion, has not been studied. Although several GC-based methods have been reported for food matrices, direct application of these methods to cosmetic products remains challenging because lotions contain complex formulations including emulsifiers, surfactants, preservatives, fragrances, and various lipid components that may interfere with lipid extraction and chromatographic determination.

Therefore, the optimization of extraction and derivatization conditions specifically for cosmetic matrices is necessary to achieve accurate and sensitive lard detection. The objective of this study was to develop and validate a rapid and reliable G/FID method combined with chemometric analysis for the detection of lard in cosmetic lotion products. In addition, this study evaluated the suitability of selected fatty acid methyl ester (FAME) biomarkers for the halal authentication of lotion products available in the Yemeni market.

2. MATERIALS AND METHODS

2.1. Instruments

A Shimadzu QP2010 (GC-FID) system (Tokyo, Japan) equipped with a DB-Wax polyethylene glycol capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) was used.

Helium (purity 99.99%) was used as the carrier gas, with a flow rate of 0.89 mL/min and pressure-controlled mode at 141 kPa. 1 μL of the sample was injected in splitless mode. The injection and detection temperatures were 50 °C and 250 °C, respectively. The GC/FID temperature program used was as follows: initial 50 °C, then increased by 10 °C min.$^{-1}$ to 250 °C and held for 31 min.

Additional equipment included an Analytical balance GR-202 (Japan) and a heater (DLAB, MS7-H550-S). Drying oven (Memmert, Germany) was used. Centrifuge (Sigma, Germany). Soxhlet Apparatus (Gerhardt, Germany). 15 and 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes with ethylene-tetrafluoroethylene screw closures (Nalgene, Rochester, NY) were used.

2.2. Reagents and Materials

A 37-component Fatty Acid Methyl Esters (FAMEs) mix (purity 99%) from Supelco, Sigma-Aldrich, USA was used to identify the fatty acid composition of the pure and adulterated lotion samples. The lard samples were sourced from Tunisia.

Analytical grade solvents used for fat extraction and GC/FID system, n-hexane (99.9% w/w), diethyl ether (99.5% w/w), and (14% w/v) boron trifluoride in methanol were purchased from Sigma-Aldrich; methanol (99.9% w/w) and ethanol (96% w/w) were purchased from Scharlau. Also, the solid materials used–sodium hydroxide (96% w/w), sodium sulfate anhydrous (98.5% w/w), and potassium hydroxide (99.9% w/w)–were purchased from Scharlau.

2.3. Preparation of Standard Solutions

Intermediate standard solutions (100-300 μg/mL) were prepared from a stock solution containing a mixture of 37 fatty acid methyl ester (200-600 μg/mL). A Calibration mixture with six concentration levels (1–100 μg/mL) was prepared in n-hexane from an intermediate standard solution.

2.4. Extraction of Pig Fat (Lard) used as a Reference

The pig fat (lard) was extracted from a pig tissue-meat mixture once time using a Soxhlet apparatus to serve as a reference material. A 5.0 g sample was placed into a cellulose thimble and dried to remove residual moisture before being subjected to extraction with 150 mL n-hexane. The process was maintained for approximately 3 h at 70 °C. Following the extraction process, the solvent was evaporated at room temperature to obtain concentrated lard lipids [20, 21].

2.5. Optimization of Extraction Parameters

To achieve maximum recovery and sensitivity for the rapid detection of lard in lotion matrices, five extraction variables were optimized:

- **Extraction Solvent:** The efficiency of n-hexane and diethyl ether as perfect solvents was tested to determine the most effective solvent for fatty acid recovery.
- **Solvent Volume and Ratio:** Different ratios of 15 mL and 20 mL of n-hexane to ethanol (2:1 and 3:1) were tested to ensure a sufficient yield of approximately 100 mg of oil.
- **Shaking Time:** The effect of mechanical shaking was evaluated over a range of 1 to 10 min. to establish the minimum time required for effective lipid extraction.

- **Extraction Cycles:** The multiple extraction runs were performed to establish the required number of extraction runs for the complete recovery of lard from the pig sample.
- **Centrifugation Time:** Separation durations from 1 to 10 min. were investigated at 4,000 rpm to determine the optimal time for phase separation and oil layer recovery.

2.6. Optimization of Esterification Procedures

Two derivatization methods were compared to identify the most efficient method for converting lotion-extracted lipids into fatty acid methyl esters.

Method 1: Oil 100 mg was dissolved in n-hexane(10 mL) and treated with 100 μL of 2M potassium hydroxide in methanol. The mixture was shaken for 30 seconds and centrifuged for 5 min. at 4000 rpm, the supernatant was separated for analysis [12].

Method 2: 100 mg of oil was dissolved in 10 mL of 0.5M sodium hydroxide in methanol and boiled at 60 °C for 5 min. Subsequently, 10 mL of 14% w/v boron trifluoride in methanol was added and boiled for 2 min. Then, adding 10 mL of n-hexane and mixing well, the organic layer was separated and dried with anhydrous sodium sulfate for analysis [22, 23].

2.7. Sample Collection

Twenty lotions (imported and locally manufactured brands) were randomly collected from markets in Sana'a, Yemen. The samples were transported to the laboratory in their original containers.

2.8. Spiked Sample Preparation

5.0 g of lotion was homogenized before spiking, then spiked with lard at concentrations of 5–50% w/w, to evaluate the method performance. Lipids were esterified before GC/FID analysis.

2.9. Validation Study

The method was validated for specificity, linearity, accuracy, precision, limits of detection (LOD), and quantification (LOQ), according to the AOAC [24].

2.10. Chemometric Analysis

To differentiate between lard, vegetable oils, and lotion samples, the Unscrambler X10 software was used to employ the chemometric method of principal component analysis (PCA).

3. RESULTS AND DISCUSSION

3.1. Optimization Results of the Extraction Parameters

To enhance the sensitivity and efficiency of the rapid detection method, five parameters were optimized for the extraction of lipids from lotion matrices.

3.1.1. Effect of Extraction Solvent

The efficiency of two organic solvent systems, n-hexane/ethanol (2:1 v/v), and diethyl ether/ethanol (2:1 v/v), was evaluated for the extraction of fatty acids from lotion samples at room temperature (R.T). As shown in Figure 1, the extraction efficiencies differed significantly between the two solvent systems. Based on the results, a mixture of n-hexane/ethanol (2:1 v/v) was selected for all subsequent lipid extractions.

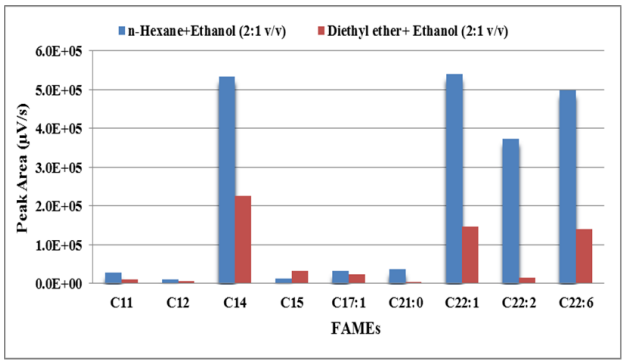

Figure 1. Comparative efficiency results of diethyl ether/ethanol and n-hexane/ethanol mixture for lipid extraction from cosmetic lotions, at RT, sample weight 5.0 g.

3.1.2. Effect of Shaking Time

The influence of shaking time on the extraction efficiency was evaluated over a range of 1 to 10 min. using the n-hexane/ethanol mixture (2:1 v/v) at room temperature. The experimental results indicated that a shaking time of 1 min. was sufficient to achieve effective lipid extraction from lotion samples (Figure 2). Consequently, 1 min. was determined to be the optimal shaking time.

3.1.3. Effect of Solvent Ratios

Two different ratios of the extraction solvent mixture n-hexane to ethanol (2:1 and 3:1 (v/v)) were optimized to ensure maximum recovery. The results demonstrated that a 2:1 (v/v) ratio was sufficient to provide a yield (Figure 3).

3.1.4. Effect of Extraction Cycles

The influence of the number of extraction cycles on recovery was investigated. Chromatographic analysis (Figure 4) demonstrated no substantial difference in the fatty acid methyl esters (FAMEs) profiles between single and multiple extraction cycles. Therefore, a single-step ex-

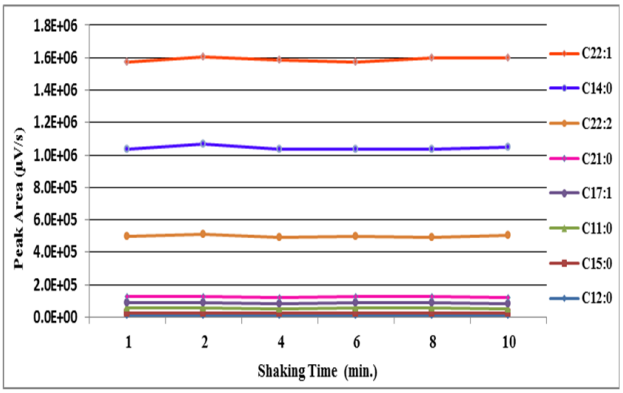

Figure 2. Effect of shaking time on the extraction efficiency of oil from the lotion sample using n-hexane/ethanol (2:1 v/v), at (R.T), sample weight 5.0 g.

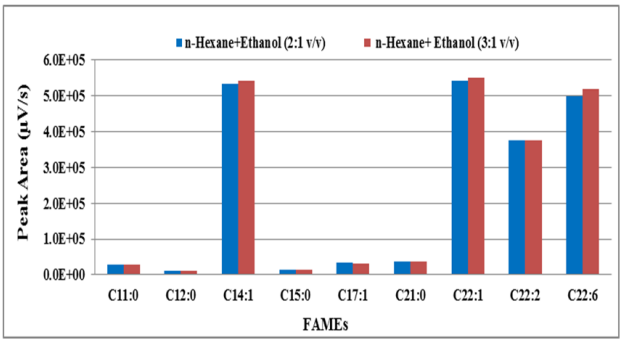

Figure 3. Effect of solvent mixture ratios on recovery yield of fatty acids from lotion samples at (R.T), sample weight 5.0 g.

traction was adopted to ensure that the method remained rapid and cost-effective.

3.1.5. Effect of Centrifuge Time

Centrifugation duration was evaluated from 1 to 10 min. The time was between 5 and 10 min. yielded no significant increase in fat quantity. Thus, a centrifugation time of 5 min. was established as the optimal duration for the experiment.

3.1.6. Final Extraction Protocol

Under the optimized conditions, 5.0 g of the homogenized lotion sample was mixed with a 2:1 v/v n-hexane/ethanol solvent system. The mixture was then centrifuged at 4,000 rpm for 5 min. at room temperature. The resulting supernatant was separated and dried at 60 °C to obtain the fat residue for further analysis.

3.2. Optimization of Esterification Procedures

Two esterification methods were investigated to determine the most effective derivatization procedures. The efficiency was assessed based on the ability to completely convert the extracted lipids into FAMEs. As shown in Figure 5, no significant differences were observed in the FAMEs profiles of the two procedures. Therefore, method 1 was selected for subsequent analysis due to its simplicity, speed, and lower reagent consumption.

3.3. Validation Study

3.3.1. Linearity

Linearity was evaluated at six concentrations of four characteristic fatty acids (C20:2, C20:3n3, C20:3n6, and C20:4), ranging from 1–100 μg / mL. The calibration curves of four biomarkers, FAMEs, are illustrated in Figure 6. The correlation coefficient (R^2) ranges from 0.9983 to 0.9998 (Table 1), which reflects high linearity.

The selection of C20:2, C20:3n6, C20: 3n3 and C20:4 as target biomarkers was based on their ability to discriminate differentiate lard from commonly used vegetable oils. Major fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1), are abundant in both animal fats and vegetable oils, therefore lack specificity. In contrast, the selected minor fatty acids occur in characteristic proportions in lard and have been reported in previous studies as useful markers for halal authentication [5, 8, 20]. Therefore, these compounds were selected as the principal indicators for qualitative identification, quantitative evaluation, and chemometric classification of the samples.

3.3.2. Precision (Repeatability)

Method repeatability was assessed via intraday variation of FAME standards in triplicate (Table 1). All the relative standard deviation (%RSD) values were within the acceptable limit (<20%), which is justified as fit-for-purpose in accordance with AOAC [24]. The highest observed %RSD was 5.8%, which remained well within the acceptable limit. These results confirmed the high repeatability and reliability of this analytical method. Additional inter-day precision and robustness studies were conducted, yielding %RSD values below 10%, indicating satisfactory method robustness.

3.3.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Sensitivity was determined using signal-to-noise ratios of 3:1 for LOD and 10:1 for LOQ, respectively. The equations used were as follows:

$$LOD = 3 \times C_{std} \times H_{noise} / H_{std} \quad (1)$$

$$LOQ = 10 \times C_{std} \times H_{noise} / H_{std} \quad (2)$$

Where C_{std}, H_{noise}, and H_{std} are the concentration of the standard, height of the noise peak, and height of the standard peak, respectively.

The LOD ranged from 0.16 to 0.25 μg/mL, and the LOQ ranged from 0.54 to 0.84 μg/mL (Table 2), demonstrating the high sensitivity of the method for identifying fatty acid components.

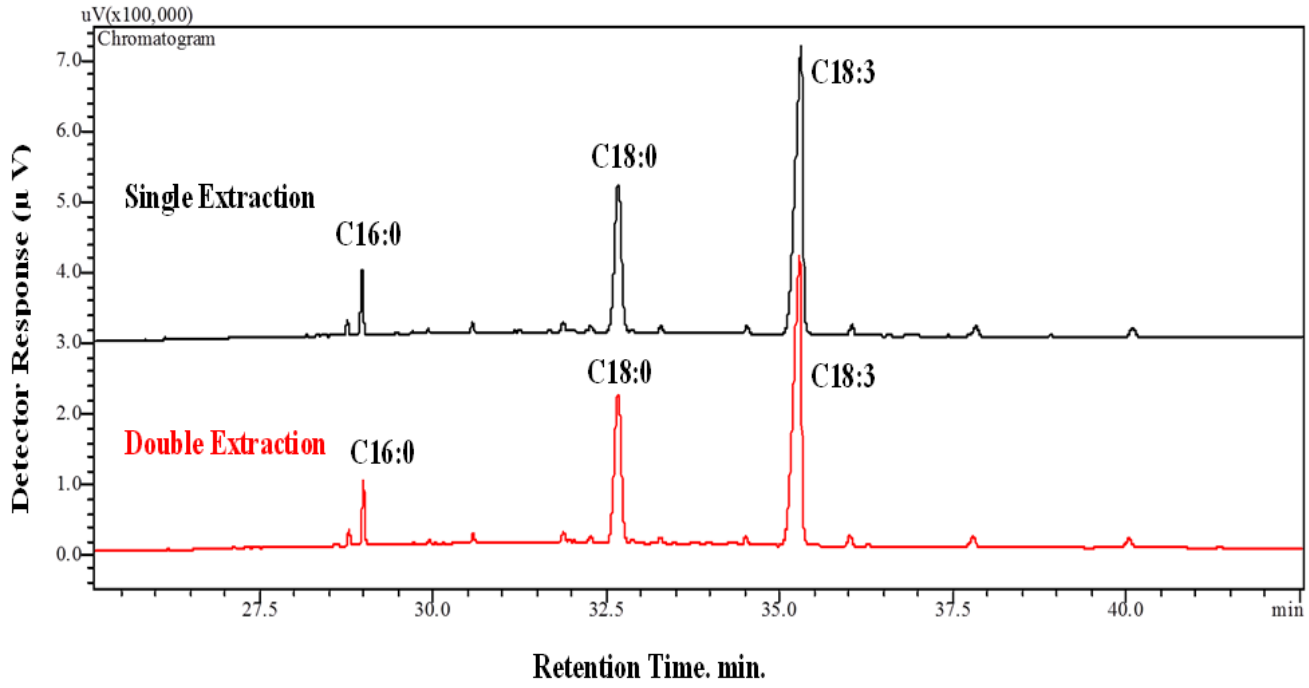

Figure 4. Chromatograms of FAMEs extracted from cosmetic lotions using single and double extraction cycles.

Table 1. Precision data for the target biomarkers C20:2, C20:3n6, C20:3n3, C20:4.

Cn	Conc. (μg/mL)	Regression equation	R^2	%RSD
C20:2	1–100	$y = 4001.3x + 3011.9$	0.9983	1.02–2.34
C20:3n6	1–100	$y = 3949.6x - 3543.1$	0.9994	0.53–2.71
C20:3n3	1–100	$y = 4370.5x + 1940.8$	0.9993	1.25–4.06
C20:4	1–100	$y = 4304.6x - 3787.8$	0.9998	0.70–5.82

%RSD: Relative Standard Deviation as Single Mean Value, R^2: Correlation Coefficient, the Regression mode is linear least squares.

Table 2. Limit of Detection and Limit of Quantification data for the C20:2, C20:3n6, C20:3n3, C20:4.

Cn	Conc. Std (μg/mL)	H_{noise}	H_{std}	H_{noise}/H_{std}	LOD (μg/mL)	LOQ (μg/mL)
C20:2	50.0	50.0	35524.0	0.00141	0.21	0.70
C20:3n6	50.0	48.0	34049.0	0.00141	0.21	0.70
C20:3n3	50.0	45.0	26673.0	0.00169	0.25	0.84
C20:4	50.0	30.0	27622.0	0.00109	0.16	0.54

Std: standard, H: height of peak, LOD: Limit of Detection, LOQ: Limit of Quantification.

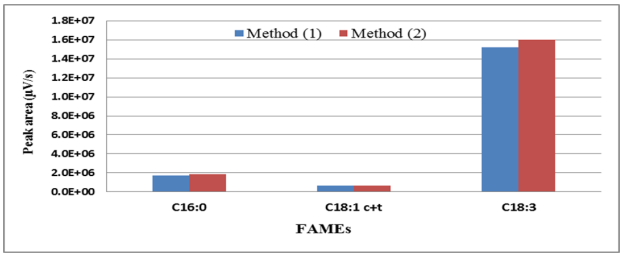

Figure 5. Effect of esterification method on the percentage of high FAMEs yield.

3.3.4. Spiked Lotion Analysis

To validate the method in a complex matrix, lotion samples were spiked with lard at concentrations ranging from 5%–100% [12]. As shown in Table 3, the method achieved high recovery (%R) rates (93.00% to 103.58%), while the corresponding chromatograms in Figure 7 confirm the method's sensitivity for detecting lard adulteration. The recovery was calculated using the following equation:

$$\%R = \frac{\text{Spiked concentration}}{\text{Measured concentration}} \times 100 \qquad (3)$$

3.4. Real Lotion Samples Analysis

The validated method was used to screen 20 local and imported lotion brands from Yemeni markets for lard adulteration. Authentication depends on the simultaneous identification of four characteristic FAME markers: C20:2, C20:3n6, C20:3n3, and C20:4. Although these fatty acids may appear individually in some vegetable oils, their collective presence is a definitive indicator of lard.

As shown in Figure 8 and Table 4, a single fatty acid peak at 100% indicates that only one compound exists in the lotion sample, which confirms the high purity of the

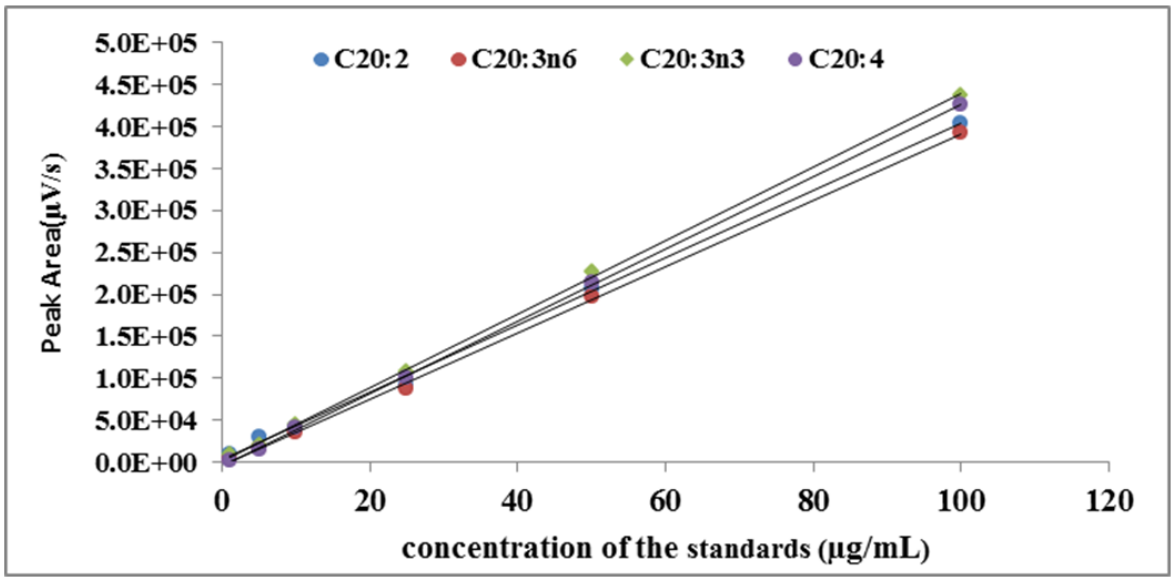

Figure 6. Calibration curves of the biomarker of FAMEs of Cn standards.

Table 3. Recovery results of spiked lotion samples at lard-specific markers.

Sample Code	C s_{pike}%	C20:2			C20:3n6			C20:3n3			C20:4		
		P.A. S.S	R.P.A. (%)	%R	P.A. S.S	R.P.A. (%)	%R	P.A. S.S	R.P.A. (%)	%R	P.A. S.S	R.P.A. (%)	%R
L-0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-S1	5	5005.0	4.70	94.04	890.0	5.17	103.40	2690.0	4.83	96.61	815.0	4.86	97.22
L-S2	10	10385.0	9.76	97.57	1601.0	9.30	93.00	5490.0	9.86	98.59	1690.0	10.08	100.80
L-S3	20	20377.0	19.14	95.72	3255.0	18.91	94.54	10944.0	19.65	98.26	3127.0	18.65	93.26
L-S4	30	29953.0	28.14	93.80	5022.0	29.17	97.24	16096.0	28.90	96.35	4921.0	29.35	97.84
L-S5	40	40647.0	38.19	95.47	7071.0	41.08	102.69	21560.0	38.72	96.79	6297.0	37.56	93.90
L-S6	50	49893.0	46.87	93.75	8915.0	51.79	103.58	26831.0	48.18	96.36	7997.0	47.70	95.40
L-100	0	106439.8	N/A	N/A	17214.5	N/A	N/A	55686.7	N/A	N/A	16765.3	N/A	N/A

L-0: Unspiked Lotion, L-S (1–6): Spiked samples (1–6), L-100: 100% Lard (Pure), C_{spike} (%): Spike Conc. (%), P.A. S.S: Peak Area of the Spiked Sample. The Relative Peak Area (R.P.A.) (%) formula calculates recovery or response efficiency in spiked vs. unspiked samples during GC method validation, particularly for accuracy or recovery studies. %R: Recovery Percentage. N/A: Not Applicable.

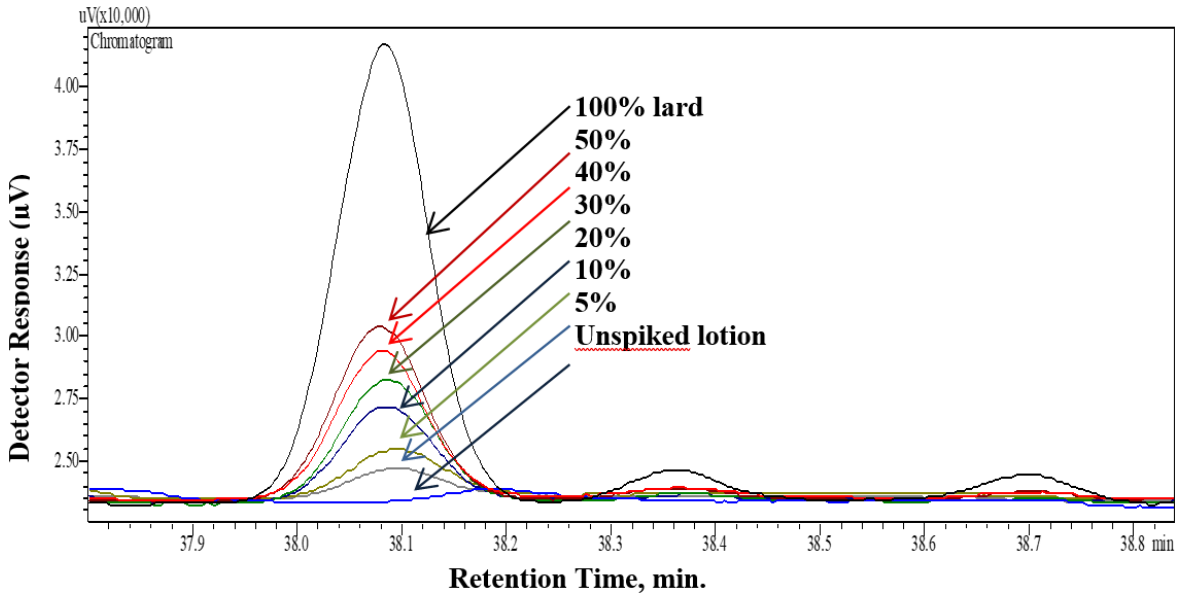

Figure 7. Chromatograms of unspiked and spiked lotion sample with 5–100% Lard.

tested lotion and that it is free from lard. The results of the lotion samples were categorized into six groups.

- (Lo. 1): contains C20:3n6 at 100%, and the other biomarker FAMEs were not detectable.
- (Lo. 8, 11, 13): contained C20:2 in 100%, and the

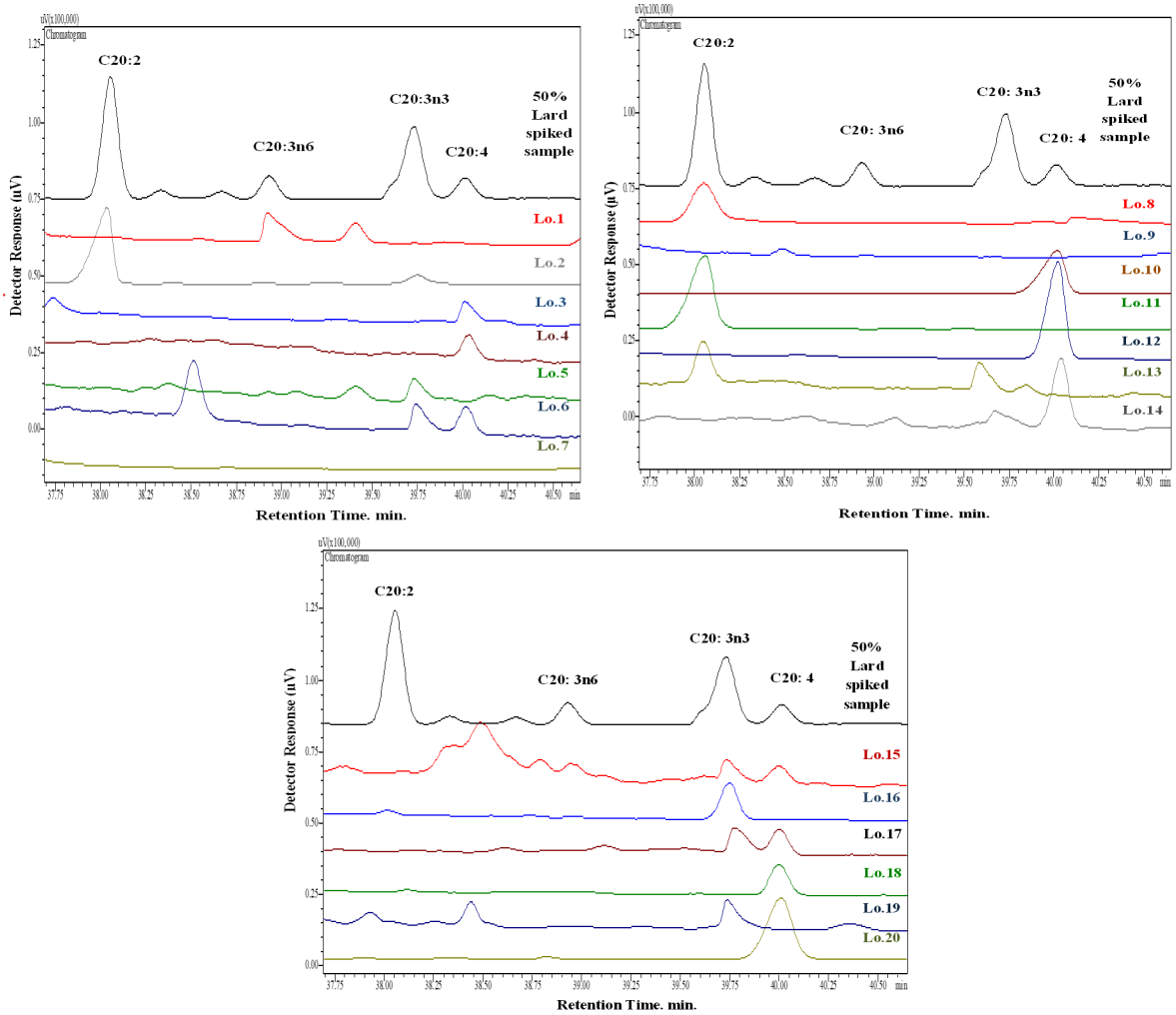

Figure 8. Chromatograms of some commercial lotion samples vs. a 50% Lard spiked sample.

other biomarker FAMEs were not detectable.

- (Lo. 3, 4, 10, 12, 18, 20): contained C20:4 in 100%, and the other biomarker FAMEs were not detected.
- (Lo. 5, 16, 19): contains C20:3n3 in 100%, and the other biomarker FAMEs were not detectable.
- (Lo. 2, 6, 14, 15, 17): have different ratios of the two FAMEs, and the other two biomarker FAMEs were not detectable.
- (Lo. 7, 9): none of the four biomarker FAMEs were detectable.

All results confirmed that none of the commercial samples contained the full suite of markers. These results confirm that the tested lotions are free of lard and meet halal authentication standards.

3.5. Statistical Analysis: Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was applied to the FAME profiles to evaluate the discrimination between lard and commercial lotion samples. The PCA score plot demonstrated a clear separation between the lard reference and analyzed lotion samples. PC1 and PC2 explained the majority of the total variance in the dataset (the exact percentages are reported on the PCA axes), indicating that the selected biomarkers contributed significantly to the sample classification. The absence of overlap between lotion samples and lard confirms the effectiveness of the developed method for halal authentication and cosmetic quality assessment.

Figure 9 shows that a PCA score can be differentiated between lard from the studied oils, which were separated from the lotion samples. PC1 variance explains 65% of the total variance in the data, and PC2 variance explains 19% of the total variance. The total variance could explain more than >84%, with a significant distance between the lard and lotions groups, confirming fundamental fatty acid composition differences. No overlap observed between the market lotion samples and the lard reference. PCA provides statistical confirmation of manual peak identification, establishing a robust "fingerprinting" for halal authentication in complex cosmetic lotion matrices.

Table 4. Relative contribution (%) of detected marker peaks in lotion samples.

Lotion sample	C20:2, %	C20:3n6, %	C20:3n3, %	C20:4, %
Lo. 1	ND	100.00	ND	ND
Lo. 2	57.52	ND	42.48	ND
Lo. 3	ND	ND	ND	100.00
Lo. 4	ND	ND	ND	100.00
Lo. 5	ND	ND	100.00	ND
Lo. 6	ND	ND	64.74	35.26
Lo. 7	ND	ND	ND	ND
Lo. 8	100.00	ND	ND	ND
Lo. 9	ND	ND	ND	ND
Lo. 10	ND	ND	ND	100.00
Lo. 11	100.00	ND	ND	ND
Lo. 12	ND	ND	ND	100.00
Lo. 13	100.00	ND	ND	ND
Lo. 14	ND	ND	25.08	74.92
Lo. 15	ND	ND	63.96	36.04
Lo. 16	ND	ND	100.00	ND
Lo. 17	ND	ND	51.67	48.33
Lo. 18	ND	ND	ND	100.00
Lo. 19	ND	ND	100.00	ND
Lo. 20	ND	ND	ND	100.00
Lard sample	54.28	8.78	28.40	8.55

Lo.: Lotion Samples, ND = not detectable. Percentages represent the relative distribution of the detected marker peaks only and do not represent the total fatty acid composition of the sample.

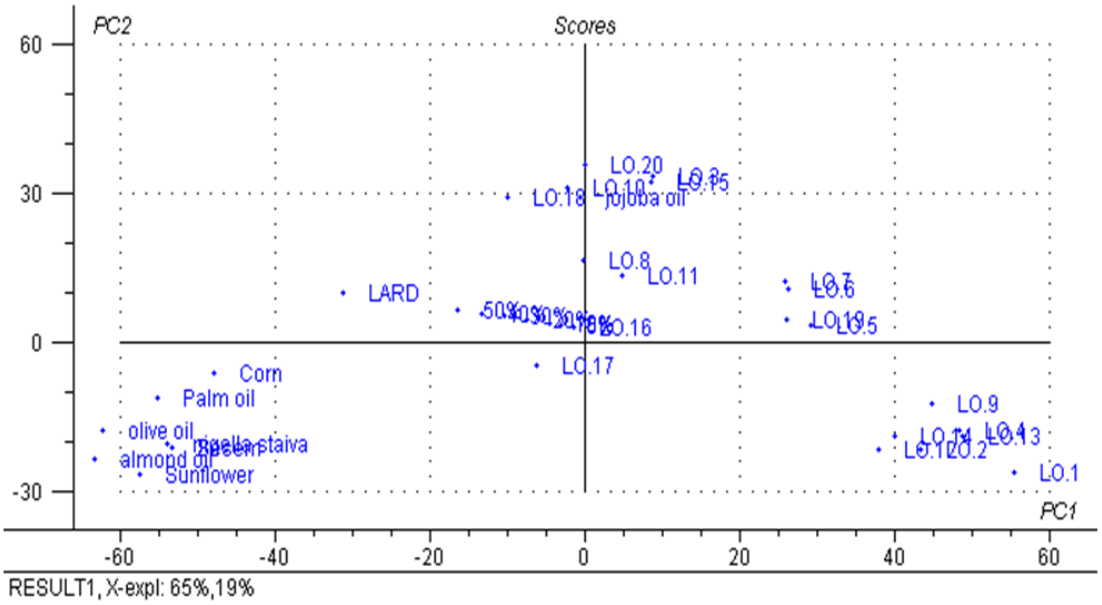

Figure 9. PCA Score plot of the PCS model based on fatty acid methyl esters (FAMEs) compositions.

4. CONCLUSION

This study successfully developed and validated a GC/FID method combined with chemometrics analysis for the detection of lard in cosmetic lotion products. The optimized extraction and derivatization procedures provided high recovery, excellent linearity, satisfactory precision, and adequate sensitivity of the method. The selected FAMEs biomarkers enabled reliable differentiation between the lard and lotion matrices. The application of the method to 20 commercial lotion samples collected from Yemeni markets showed no evidence of lard adulteration. The proposed approach offers a practical, cost-effective, and reliable tool for halal authentication and routine quality control of cosmetic products. Future studies may extend this approach to other cosmetic formulations and employ GC/MS for further confirmatory analysis.

ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to the Yemen Standardization Metrology and Quality Control Organization (YSMO) for allowing us to work in their laboratories and for providing instruments and materials.

DECLARATIONS

Ethical Approval

This article does not contain any studies involving animals or human participants performed by any of the authors. We declare that this manuscript is original, and is not currently being considered for publication elsewhere. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of interest

The authors declare no conflict of interest.

Competing interests

The authors have no financial or proprietary interest in any material discussed in this article.

Authors' contributions

All authors contributed to the study's conception and design. All authors performed the simulations, data collection and analysis and commented on the present version of the manuscript. All the authors have read and approved the final manuscript.

Funding

No funding was received from any organization for this work.

Availability of data and materials

No datasets were used in the present study.

Consent for publication

The authors confirm that informed consent was obtained for the publication of the data contained in this article.

Consent to participate

Informed consent was obtained from all the participants.

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