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Original Article

Validation of an Extractive Spectrophotometric Method for Determining Lauric Acid in Virgin Coconut Oil

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ABSTRACT

Background: An easy, quick, sensitive and precise extractive spectroscopic approach has been devised for the determination of lauric acid in pure form as well as present in virgin coconut oil. The approach is based on the formation of a brightly coloured ion-pair complex between the anionic drug (lauric acid) and cationic dye (methylene blue) in an alkaline solution. **Materials and Methods:** This approach is stable and extracted into a chloroform layer (ion-pair complex). This complex is stable and can be extracted into a chloroform phase. All experimental conditions were optimized, and the maximum absorbance of the complex was observed at 653 nm in the visible spectrum, using chloroform as the reagent blank. To get the highest possible colour intensity, reaction conditions were modified. **Results:** The study demonstrated that Beer's law is applicable within a conc. range of 1-8 µg/mL, with a correlation coefficient (r^2), was found to be 0.9956. The approach was extensively validated following the guidelines set by the International Conference on Harmonization (ICH) and adhered to the criteria established by the U.S. Pharmacopoeia. It was successfully employed to analyze the target compound in pharmaceutical formulations, yielding satisfactory recovery rates. From this research, the limit of detection was 0.7002 µg/mL and the limit of quantification was 2.334 µg/mL. The method demonstrated reliable precision, with a Relative Standard Deviation (%RSD) below 2%, and an accuracy rate reflected in a recovery of 104.13%. **Conclusion:** Based on the results, the proposed method is suitable for quality control and routine analysis of ionic drugs such as lauric acid (anionic drug) in combination with methylene blue (cationic dye).

Keywords: Lauric acid, Methylene blue, Extractive spectroscopy, Ion pair complex, Quantification, Validation.

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INTRODUCTION

Lauric acid, commonly known as dodecanoic acid, is a saturated medium-chain fatty acid whose chemical formula is $C_{12}H_{24}O_2$ (Figure 1). They are commonly found in coconut oil, palm kernel oil, and other tropical oils, as well as in human and animal milk (Dayrit *et al*., 2014). Lauric acid is known for its antimicrobial properties and is used in a variety of applications, from cosmetics to food and pharmaceuticals (Sandhya *et al*., 2016). Various previously published work finds that lauric acid is not UV-active because it lacks conjugated double bonds or aromatic rings that would allow it to absorb Ultraviolet (UV) light. UV-active compounds generally have molecular structures with alternating single and double bonds (conjugated systems) or ring structures that create delocalized π-electrons. These delocalized π-electrons can absorb energy in the Ultraviolet range (UV-400-200 nm) and become excited to higher energy levels, resulting in UV absorbance (Silverstein *et al*., 1991; Chung *et al*., 2019). Various analytical techniques have been documented for the quantitative analysis of lauric acid, a Medium-Chain Fatty Acid (MCFA), including HPLC coupled with Evaporative Light Scattering Detection (ELSD) (Ponphaiboon *et al*., 2018), Gas chromatography, High-Performance Liquid Chromatography (HPLC, 2024) GC-MS, GC-FID (Spandana *et al*., 2019) and esterification. The reported spectrophotometric methods have certain limitations, such as requiring higher concentrations of the drug, being time-consuming and costly (Saiyed *et al*., 2022; Gavazov *et al*. 2023). The proposed aim is to develop an extractive spectrophotometric technique that relies on the formation of ion-pair complexes soluble in chloroform, which are produced between lauric acid and methylene blue in an alkaline buffer solution. This approach offers greater sensitivity and simplicity compared to existing spectrophotometric techniques. However,

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Methylene blue is often chosen as a reagent in extractive spectrophotometric analysis because of its excellent properties as a cationic dye with a strong absorbance in the visible range, typically around 660-650 nm. However, lauric acid reacts with sodium hydroxide, it undergoes a neutralization reaction. The carboxylic acid group of lauric acid reacts with the hydroxide ion from sodium hydroxide, forming sodium laurate (a fatty acid soap, sodium salt of lauric acid) and water as a product. The reaction between sodium laurate and methylene blue (a cationic dye) typically involves ionic interactions rather than a chemical reaction that forms a new covalent compound. It is also known as the ion pair complex (Omara *et al.*, 2012). This type of interaction is often exploited in dye-binding studies or to form complexes for analytical or material science applications. Its specific chemical interactions and intense colour make it useful in forming detectable complexes with certain analytes, which improves the sensitivity and selectivity of the analysis (Jurado *et al.*, 2006).

MATERIALS AND METHODS

Apparatus

The spectrophotometric analyses were performed using the software LabSolutions UV-vis (Version: 1.11), Shimadzu, UV-1900 Series (Model (S/N) UV 1900i (A12535881041) with 1 cm quartz cells.

Reagents

Lauric acid was procured from Acme Synthetic Chemicals Mumbai. Coconut oil was obtained from Veda Oils Pvt. Ltd., Haryana. Methylene Blue was procured from Qualigens Fine Chemicals Mumbai. Analytical grade solvents like methanol and chloroform were used.

Extractive Spectroscopy

Extractive spectrophotometric methods are widely favoured due to their high sensitivity in drug analysis. Hence, ion-pair extractive spectrophotometry has garnered significant interest for the quantitative assessment of various pharmaceutical substances (ESM, 2024). However, an ion pair complex is formed between two oppositely charged ions in solution, typically enhancing the extraction of ionic species into organic solvents. In extractive spectroscopy, ion-pair complexes play a key role in extracting charged analytes (like metal ions or ionic drugs) by converting them into neutral ion-pair complexes that are soluble in organic solvents (Saiyed *et al.*, 2022).

Assay procedure for pure drug

A standard stock solution of lauric acid (1000 µg/mL) was prepared using methanol. This solution was then diluted to obtain concentrations of 1, 2, 4, 6, and 8 µg/mL in methanol. The absorbance was measured at a UV range (400-200 nm, Shimadzu) against methanol as blank. No significant absorbance was observed in the prepared solutions, due to the saturated hydrocarbon chain of lauric acid lacking π-electrons or conjugated systems, and the carboxyl group having only weak UV absorbance (Kumar *et al.*, 2013).

Thus, for performing the extractive spectroscopy, an accurately measured 5 mL sample solution (For each serial dilution prepared above) of lauric acid was transferred into a 125 mL separating funnel, 1 mL of a 1% sodium hydroxide solution and 0.5 mL of a 0.1% Methylene Blue (MB) solution were added for each of the serial dilutions prepared earlier, and the mixture was gently shaken. After a period of 10 min, the ion-association complex was extracted three times with chloroform by shaking for approximately 2 min. Once the two layers had distinctly separated, the absorbance of the chloroform layer, which appeared light blue, was measured in the visible range at 653 nm using a Shimadzu spectrophotometer, with chloroform as the reagent blank. The calibration curve was constructed for lauric acid concentrations ranging from 1 to 8 µg/mL in chloroform (Kepekci Tekkeli *et al.*, 2013; El-Kommos *et al.*, 2013). The absorbance data from the measurement results of the standard lauric acid solution are presented in Table 1, while the standard solution calibration curve is presented in Figure 2.

Assay procedure for sample virgin coconut oil

A volume equivalent to 5 mL of virgin coconut oil was transferred into a 100 mL volumetric flask, mixed with 70 mL of methanol, and thoroughly shaken. The mixture was sonicated for approximately 30 min, brought up to the mark with methanol, and filtered, discarding the initial portion of the filtrate. The filtrate was then further diluted to prepare the required working sample solution (Thangabalan *et al.*, 2009). The diluted filtrate was taken and the volume was made up of methanol and appropriate aliquots were analyzed using the methods given above for performing extractive spectroscopy.

Method Validation

Detection and quantification limits

The limit of detection or LOD represents the smallest concentration of an analyte in a solution that can be detected but cannot be measured accurately within the testing parameters. The minimal amount of an analyte in a sample that can be accurately quantified within the test's parameters with a substantial level of precision and accuracy is referred to as the limit of quantification, or LOQ. The following formula was utilized to determine the LOD and LOQ (Kepekci Tekkeli *et al.*, 2013).

Limit of Detection (LOD)=3* standard deviation/slope

Limit of Quantification (LOQ)=10*standard deviation/slope

Optimization of reaction conditions

To identify the ideal reaction conditions for quantitatively characterizing ion-pair complexes, several exploratory experiments were carried out. It was found that the kind of buffer employed and its pH had an impact on how well the complex was extracted. It was observed that this method performed effectively with an alkaline buffer. By measuring the absorbance of solutions with varied amounts of the reagent and a constant amount of lauric acid separately, the effects of the reagents were examined. One milliliter of 0.1% methylene blue solution was used to get the complex maximal colour intensity. The coloured species were successfully extracted from the aqueous phase using many organic solvents (Nair *et al.*, 2015; Shoaibi *et al.*, 2012). Since quantifiable recovery of the complex could be obtained with only one extraction, chloroform was shown to be the optimal extractant. There was no appreciable change in the product's absorbance or colour, even when the reactants were added in a different order.

Quantification

A strong correlation was found when Beer's law graphs at λ_{max} values were subjected to regression analysis. The regression equation Y=ax+b (where Y represents absorbance, a denotes the slope, b indicates the intercept, and x is the concentration of the selected drug in µg/mL) outlines the relationships shown in absorbance versus concentration graphs, which have intercepts at zero (Barache *et al.*, 2019).

Accuracy, precision and recovery

The ICH guidelines number 46 describe the accuracy of an analytical approach as the degree of closeness between the values recognized as predictable true values or accepted reference values and the measured value (El-Kommos *et al.*, 2013). The proposed methods' accuracy was assessed by examining the recovery of each drug studied at three concentration levels within the specified range (El-Kommos *et al.*, 2013; Amanlou *et al.*, 2010).

Dilute 1 mL of secondary stock solution with 10 mL of methanol to create six solutions with the same concentration (2 µg/mL). These were ascertained through the application of Beer's law to six duplicates of the drug. It is implied that the techniques are accurate by the low Relative Standard Deviation (RSD) values. Measure the absorbance of this solution and repeat it intra-day at the visible range (653 nm) against the equivalent reagent blank. The percentage RSD was calculated using the formula:

$$\% \text{ RSD} = \text{SD}/\text{Mean} * 100$$

Recovery studies using typical addition procedures were performed to investigate the approach's accuracy. For each drug, varying volumes of pure sample solutions were combined with two different concentrations of standard drug solution and tested. Table 4 shows the findings of the analysis of virgin coconut oil. The average per cent recoveries were determined using the provided formula:

$$\% \text{ recovery} = \text{amount recover}/\text{amount added} * 100$$

Table 1: Absorbance of Lauric Acid.

Sl. No.	Concentration (µg/mL)	Absorbance (nm)
i.	1	0.148
ii.	2	0.204
iii.	4	0.449
iv.	6	0.697
v.	8	0.918

RESULTS

It was found that cationic dyes like methylene blue form ion-association complexes with positively charged drugs, such as lauric acid in this case. These drug-dye complexes consist of two oppositely charged ions held together by weak electrostatic interactions. Lauric acid interacts with methylene blue in an alkaline buffer to produce a chloroform-soluble ion-association complex, which shows an absorption maximum at 653 nm for methylene blue. The absorption spectra of the ion-association complex are presented in Figure 3.

Method Validation

The proposed approach was validated according to the guidelines given by the International Conference on Harmonization and adhered to the validation requirements of the United States Pharmacopeia (El-Kommos *et al.*, 2013).

Table 2: Optical characteristics of the proposed methods.

Parameters	Lauric acid- Methylene blue complex
λ_{max} (nm)	653
Limit of Detection (LOD) (µg /mL)	0.7002
Limit of Quantification (LOQ) (µg /mL)	2.334
Regression equation	Y=ax+b
a (Slope)	0.1137
b (intercept)	0.0055
Correlation coefficient (r^2)	0.9956
Intra-day (precision) (Abs)	0.205
Inter-day (precision) (Abs)	0.206

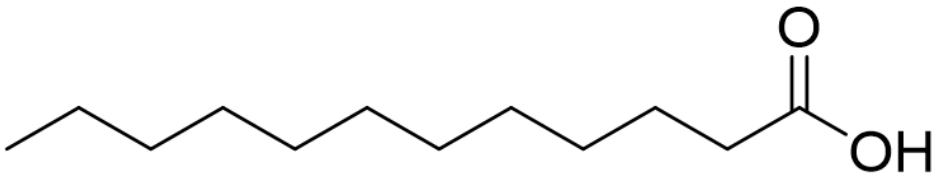


Figure 1: Chemical Structure of Lauric Acid.

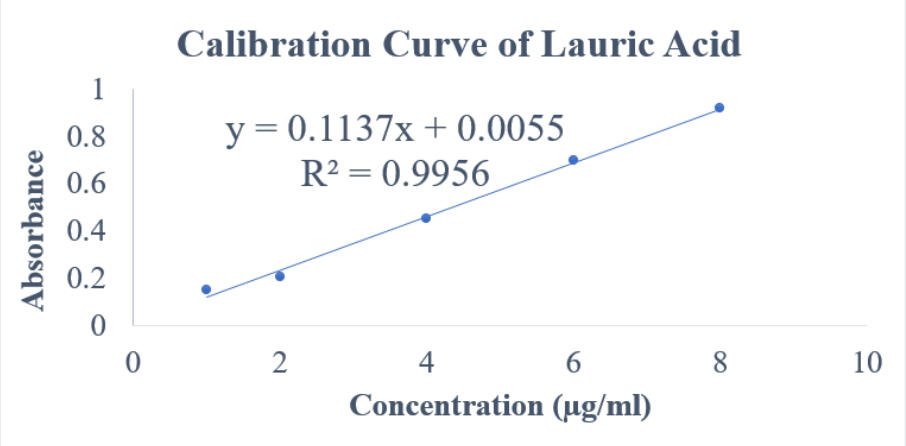


Figure 2: Standard calibration curve of lauric acid in chloroform.

Table 3: Assay results in precision studies.

Concentration (µg/mL)	Absorbance	Mean±SD	Repeatability	Inter-day	Intra-day	%RSD
2	0.207	0.203±0.0028	0.215	0.209	0.208	1.427
2	0.205		0.211	0.207	0.209	
2	0.204		0.212	0.208	0.204	
2	0.202		0.207	0.206	0.207	
2	0.201		0.209	0.205	0.203	
2	0.199		0.210	0.205	0.202	

Quantification and detection limits

The Limit of Detection (LOD) for the lauric acid-methylene blue complex represents the lowest concentration of analytes in the sample that can be detected, though not necessarily quantified, under the given experimental conditions. The limit of detection was found to be 0.70026 µg/mL. The Limit of Quantification (LOQ), which refers to the lowest concentration of analytes that can be accurately measured. The limit of quantification was found to be 2.334 µg/mL, respectively and presented in Table 2. This data indicates the high molar absorptivity of the ion pair complex and the high sensitivity of the proposed method.

Accuracy, precision and recovery

Precision is quantified through standard deviation or relative standard deviation (coefficient of variation), represented as repeatability or reproducibility. In the validation of the test method, repeatability was used, which is the precision of the method when performed by the same analyst under the same conditions in a short time interval. The data presented in Table 3 were obtained from measurements of the sample 6 times under the same conditions and a short period of time. The results show that the relative standard deviation expressed in % RSD (Relative Standard Deviation) of the lauric acid methylene blue complex determination using a UV-visible spectrophotometer was 1.427%, which is less than the 2% acceptance criterion for precision. This indicates that the determination of ion-pair complex using a UV-visible spectrophotometer has good precision.

Accuracy is a parameter that indicates the degree of closeness of the results of the analysis to the actual analyte concentration. It is expressed as the per cent recovery of the analyte added to the sample. The standard addition method is often used to determine the accuracy of an analytical approach. The results of determining the accuracy of the ion pair complex determination by spiking the sample with 50%, 100% and 150% of lauric acid standard solution are presented in Table 4. The average percentage of recoveries for virgin coconut oil was 104.13%. The average percentage

recoveries were quantifiable, indicating that the approaches were highly accurate.

DISCUSSION

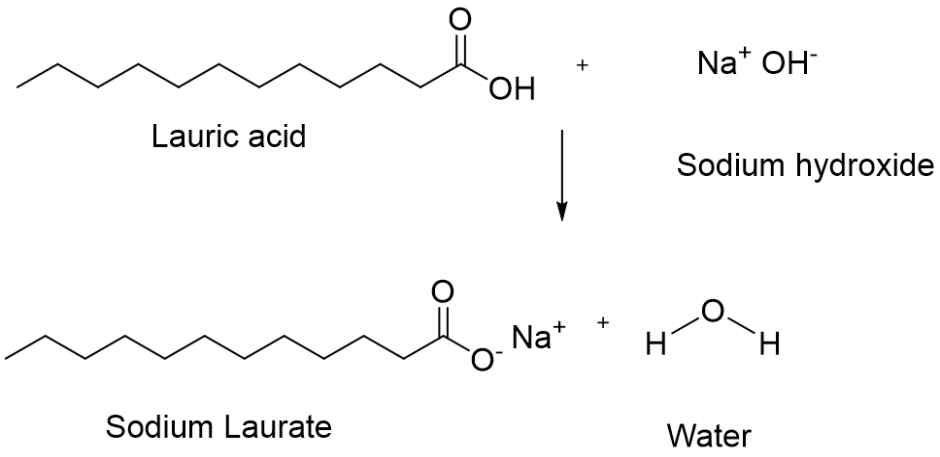
Under the experimental setup, the blank reagent exhibited minimal absorbance, allowing for optimal analytical conditions for the quantitative analysis of lauric acid. In the established UV-Vis method, the calibration curve was determined to be linear within the range of 1-8 µg/mL of lauric acid. The correlation coefficient (r^2) value was in the range of 0.9956, indicating good linearity. Zhao *et al.*, employed the same technique to determine thiosulfate in desulfurization solutions through the decolorization of methylene blue. The method demonstrated excellent linearity, with a correlation coefficient (r^2) value of

Table 4: Assay results in % recovery study.

Drug	Standard Solution of Pure Lauric acid (µg/mL)	Estimated amount of Lauric acid (µg/mL)	Spike Level (%)	Amount of drug added (µg/mL)	Amount of drug recovered (µg/mL)	% Recovery	Mean Recovery
Virgin Coconut Oil	3	3.089	50	1.5	1.401	93.4	104.13
			100	3.0	3.310	111.03	
			150	4.5	4.858	107.96	

The chemical reaction of lauric acid with methylene blue

Step 1:



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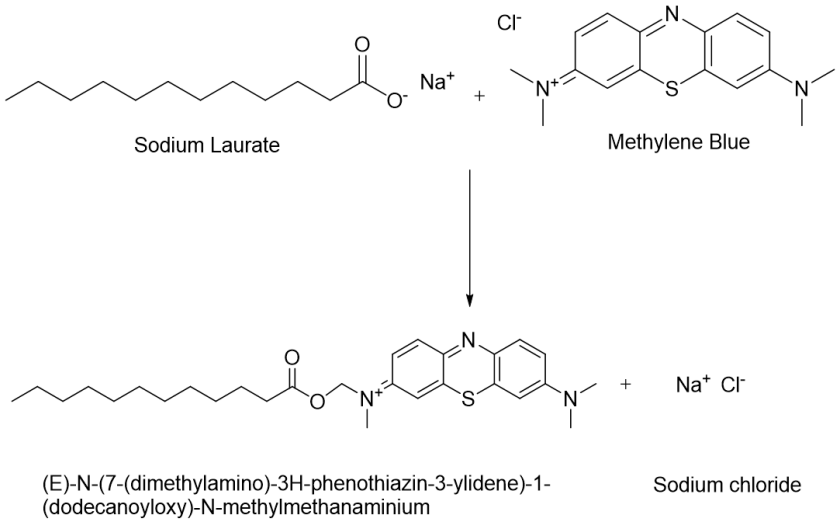

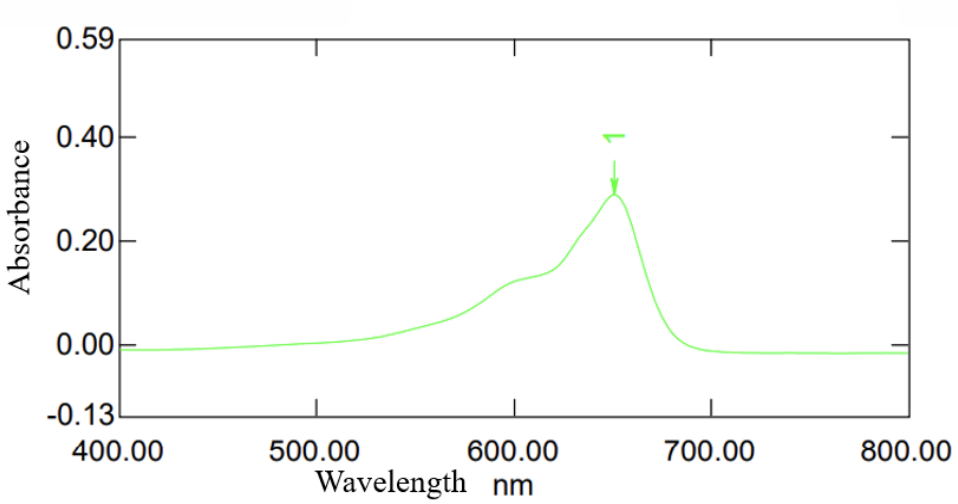

Figure 3: Absorption spectra of ion association complex (lauric acid-methylene blue) extracted into 10 mL of chloroform.

0.9977 (Zhao *et al.*, 2023). This comparison indicates that the results of the studied methods are not significantly different from those of the reference method. Another study by, Michael *et al.* applied a similar method to analyze seven Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in both their pure (anionic drug) forms and pharmaceutical formulations, such as tablets, capsules, and syrups, using methylene blue (cationic dye). Their study reported strong linearity, with correlation coefficient (r^2) values ranging from 0.9993 to 0.9997. The Limits of Detection (LOD) were between 0.013 and 0.24 µg/mL, while the Limits of Quantification (LOQ) ranged from 0.04 to 0.75 µg/mL. The percentage Relative Standard Deviation (%RSD) varied from 0.20 to 1.60, and recoveries were found to be between 96.8% and 102.5% (Kepekci Tekkeli *et al.*, 2013). In an another, study by Thangabalan *et al.*, they employed a similar technique to quantify cinitapride (cationic) in its pure form and pharmaceutical formulations using Bromocresol Green (BCG) and Bromothymol Blue (BTB) (anionic dye). However, an ion pair complex is formed between two oppositely charged ions in solution, typically enhancing the extraction of ionic species into organic solvents. Their findings demonstrated excellent linearity, with correlation coefficient (r^2) values ranging from 0.9998 to 0.9999. The Limits of Detection (LOD) were reported to be between 0.220 and 0.780 µg/mL, while the Limits of Quantification (LOQ) ranged from 0.66 to 2.36 µg/mL. Recovery rates were found to be between 100.06% and 100.17% (Thangabalan *et al.*, 2009). The comparison revealed no significant differences between the results of our studied methods and the reference method.

CONCLUSION

The use of the extractive spectrophotometric technique for the determination of chemicals in a multicomponent mixture is a unique advantage. The equipment is cheap and easy to use, in contrast to Gas chromatography and HPLC. The approaches' significance lies not in the sophisticated equipment but rather in the chemical interactions that support them. Because it offers a unique possibility for the evaluation of components in complex dosage forms, this aspect of spectrophotometric analysis is crucial to analytical pharmacy. The recommended methods use less expensive and more widely available chemicals, and they don't require any time-consuming basic preparations or essential reaction conditions. Little variations in the experimental variables, such as pH and reagent concentration, have little effect on the process. Moreover, common non-ionic excipients and additives do not interfere with the processes. However, the ionic excipients or polymers have the potential to interact with dyes, leading to challenges in maintaining the stability and accuracy of the colourant, which could be considered as a limitation. Such interactions may compromise the reproducibility of results, especially in systems relying on precise colorimetric responses. However, the developed extractive spectroscopy procedures effectively quantify lauric acid in Virgin Coconut Oil (VCO) using a cationic dye such as methylene blue. These methods may also support the quantification of VCO-based drug formulations with non-ionic surfactants, additives or polymers, and highlight the method's broader applicability in pharmaceutical quality assurance and innovative formulation development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LA: Lauric acid; **VCO:** Virgin coconut oil; **MB:** Methylene Blue; **ICH:** International Conference on Harmonization; **Abs:** Absorbance; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **CONC:** Concentration; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation.

AUTHOR CONTRIBUTIONS

VR and AEP conceived and designed the study. AR and AEP were involved in the experiments and contributed to the preparation of the manuscript. VR performed experimental analysis of the data and contributed to the preparation of the manuscript. VR critically reviewed the entire manuscript. VR and AEP supervised the study, involved in the design and execution of the study. All authors discussed in the manuscript and approved its final version. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

DATA AVAILABILITY

This study includes all generated and analyzed data.

PUBLISHER'S NOTE

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