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Assessment of the European pharmacopoeia method for fatty acid composition of hydrogenated castor oil using gas chromatography

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Abstract

Background: Hydrogenated castor oil is widely used as a pharmaceutical excipient due to its emulsifying and solubilizing properties [1, 5]. The quality and consistency of this excipient are critically dependent on its fatty acid composition, which must comply with the specifications outline in the European Pharmacopoeia (Ph. Eur.) [1, 5]. This study presents an assessment of the European Pharmacopoeia gas chromatography (GC) method for the determination of fatty acid composition in hydrogenated castor oil intended to pharmaceutical manufacturing. Key performance activities including specificity, precision and system suitability, were evaluated in accordance with pharmacopoeia and ICH guidelines [4, 6]. The GC method demonstrated adequate resolution of major saturated fatty acids characteristic of hydrogenated castor oil, with reproducible retention times and consistent quantitative results.

Purpose: The aim of this study is to verify a gas chromatographic method for the identification and quantification of fatty acids presents in castor oil namely Methyl Myristate, Methyl Palmitate, Methyl Stearate, Methyl Oleate, Methyl Arachidate, Methyl Eicosenoate, Methyl Behenate, 12-Oxostearic acid, Methyl 12-Hydroxystearate, Methyl Lignocerate. The challenge of this EP method was to find the Exact GC column mention by monograph. This method will be able to identify and quantify the fatty acid composition within a single gas chromatographic procedure using a specific brand column.

The findings confirm that the European Pharmacopoeia GC method is suitable for routine quality control and regulatory compliance testing of hydrogenated castor oil in pharmaceutical products.

Method: A gas chromatography (GC) equipped with a liquid injector and a flame ionization detector [2, 7], and a column InertCap Wax, Material: Fused Silica, Size: l = 30m, Ø = 0.32 mm, Film thickness 0.25µm, Brand-GL Science was used to develop this method. The GC oven temperature was 215 °C over the run time 55 min. Nitrogen was used as a carrier gas at a flow rate of 0.9 mL per minute. 1, 1- dimethyl ethyl methyl ether was selected as sample solvent. N-heptane used as blank.

Fatty acid methyl ester analysis by GC-FID is a widely accepted approach for the determination of fatty acid composition [2, 3, 7].

Results: The developed method is precise and specific. The percent RSD for the areas of six replicate injections of this gas chromatographic method was within 5%.

Keywords: Method Development; Gas Chromatography; Compendia Method; Specificity; Precision

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1. Introduction

Hydrogenated castor oil (HCO) is an important pharmaceutical excipient widely used in tablets, capsules, ointments, and controlled-release formulations due to its lubricating, emulsifying, and stabilizing properties [1]. The quality and performance of HCO are highly dependent on its fatty acid composition, particularly the content of 12-hydroxystearic acid and related long-chain fatty acids [1, 2]. Therefore, accurate determination of fatty acid composition is essential to ensure product quality and compliance with pharmacopoeial specifications.

The European Pharmacopoeia (Ph. Eur.) recommends gas chromatography with flame ionization detection (GC-FID) for the analysis of fatty acid methyl esters (FAMEs) in hydrogenated castor oil [1]. GC-FID is a well-established analytical technique offering high sensitivity, selectivity, and reproducibility for fatty acid profiling [2, 3]. Although compendia methods are considered validated, method verification is necessary to confirm their suitability under laboratory-specific conditions and to ensure reliable analytical performance according to regulatory expectations [4].

The present study aimed to verify the Ph. Eur. GC-FID method for determining the fatty acid composition of hydrogenated castor oil by evaluating system suitability, specificity, and precision. The study demonstrates the applicability of the method for routine quality control analysis in a pharmaceutical laboratory.

2. Materials and methods

2.1. Materials

The source of chemicals used in this development study from the following suppliers:

n-heptane (Scharlau), Methyl 12-Hydroxy Stearate (Sigma Aldrich), Methyl Palmitate (Sigma Aldrich), Methyl Arachidate (Sigma Aldrich), Methyl stearate (Sigma Aldrich), Methyl Tetracosanoate (Methyl Lignocerate) (Sigma Aldrich), Methyl behenate (Sigma Aldrich), Methyl Oleate (Sigma Aldrich), Methyl Myristate (Sigma Aldrich), Methyl Cis-11-eicosenoate (Methyl Eicosenoate) (Sigma Aldrich), Methyl Stearate (Sigma Aldrich), Methyl 12- Hydroxy Stearate (Sigma Aldrich) was used to prove the specificity and recovery of the method.

2.2. Methods

2.2.1. Instrumentation

A gas chromatography instrument with a flame ionization detector and a liquid injector was utilized. Model & manufacturer: Thermo Scientific Trace 1310. Analytical balance: SARTORIOUS CPA224S. Micropipette: 100 to 1000 µL Eppendorf.

2.2.2. Chromatographic System

Injection port information

- Carrier gas : Nitrogen for chromatography R.
- Injection mood : Split
- Temperature : 250°C
- Flow Rate : 0.9 ml/min
- Split ratio : 100:1
- Injection Volume: 1 µL

Detector information

- Detection : Flame Ionization
- Temperature : 250°C
- H_2 Flow : 40.0 ml/min
- Air flow : 400 ml/min

Column temperature program

Time	Temperature (°C)
0-55 min	215

Standard and Sample Preparation

- Blank: Use n-Heptane as blank solution.
- Diluent: Use 1,1- dimethyl ethyl methyl ether as Diluent.
- Preparation of 12g/L sodium hydroxide solution: 12 mg of Sodium metal into 10 ml with methanol.

Reference solution (A) or Mixture of calibrating substances

Prepare 0.50 g of the mixture of calibrating substances with the composition described in below table. Dissolve in heptane and dilute with heptane to 50.0 ml.

Mixture of the following substances	Composition (%)
Methyl Myristate	5.0
Methyl Palmitate	10.0
Methyl Stearate	15
Methyl Arachidate	20
Methyl Oleate	20
Methyl Eicosenoate	10
Methyl Behenate	10
Methyl Lignocerate	10

NOTE—Commercially available mixtures of fatty acid methyl esters (FAME Mixture) may also be used.

2.3. Reference solution

Dissolve 50 mg of methyl 12-hydroxystearate CRS and 50 mg of methyl stearate CRS in 10.0 mL of 1, 1- dimethylethyl methyl ether.

2.4. Test solution

Introduce 75 mg of the substance to be examined into a 10 mL centrifuge tube with a screw cap. Dissolve in 2 mL of 1, 1-dimethylethyl methyl ether R1 by shaking and heat gently (50-60 °C). Add, when still warm, 1 mL of a 12 g/L solution of sodium R in anhydrous methanol R, prepared with the necessary precautions, and mix vigorously by hand for at least 5 min. Add 5 mL of milli-Q water and mix vigorously for about 30 s. Centrifuge for 15 min at 1500 g (Equivalent to 3500 rpm). Use the upper layer.

2.5. System suitability

Asymmetry: 0.7 to 1.5 for the peak due to methyl stearate in the chromatogram obtained with the test solution [1, 5].

3. Results and Discussion

GC analysis is very sensitive to detection, so GC grade chemicals and standards should be used for analysis [2, 7].

Some unknown peaks were observed in the chromatograms. However, no other peaks were detected at the retention times of Methyl 12-Hydroxy Stearate, Methyl Palmitate, Methyl Arachidate, Methyl stearate, Methyl Tetracosanoate (Methyl Lignocerate), Methyl behenate, Methyl Oleate, Methyl Myristate, Methyl Cis-11-eicosenoate (Methyl Eicosenoate), Methyl Stearate, Methyl 12- Hydroxy Stearate in the blank solution and diluent.

Therefore, no interference was found from the blank with the targeted peaks indicating that the method is specific for the respective solvents [4, 6]. From the precision study, it was observed that this method gives reproducible results [4, 6]. The %RSD found from the six replicate injections is less than 5.0.

3.1. Specificity

Each fatty acid composition Methyl Palmitate, Methyl Stearate, Methyl Arachidate, Methyl Eicosenoate, 12-Oxostearic acid, Methyl 12- Hydroxystearate was separated to confirm the interference between fatty acids in the sample solution when compare to FAME mixture and reference solution(b). The retention time for Methyl Palmitate, Methyl Stearate, Methyl Arachidate, Methyl Eicosenoate, 12-Oxostearic acid, Methyl 12- Hydroxystearate were found to be 4.51, 6.31, 9.46, 10.05, 19.06 and 25.88 min, respectively.

All the solutions were prepared separately, and injected to the chromatographic system to confirm the identification of retention time [2, 4, 6, 7]. Chromatograms of identification solution are presented from Figure 1-6.

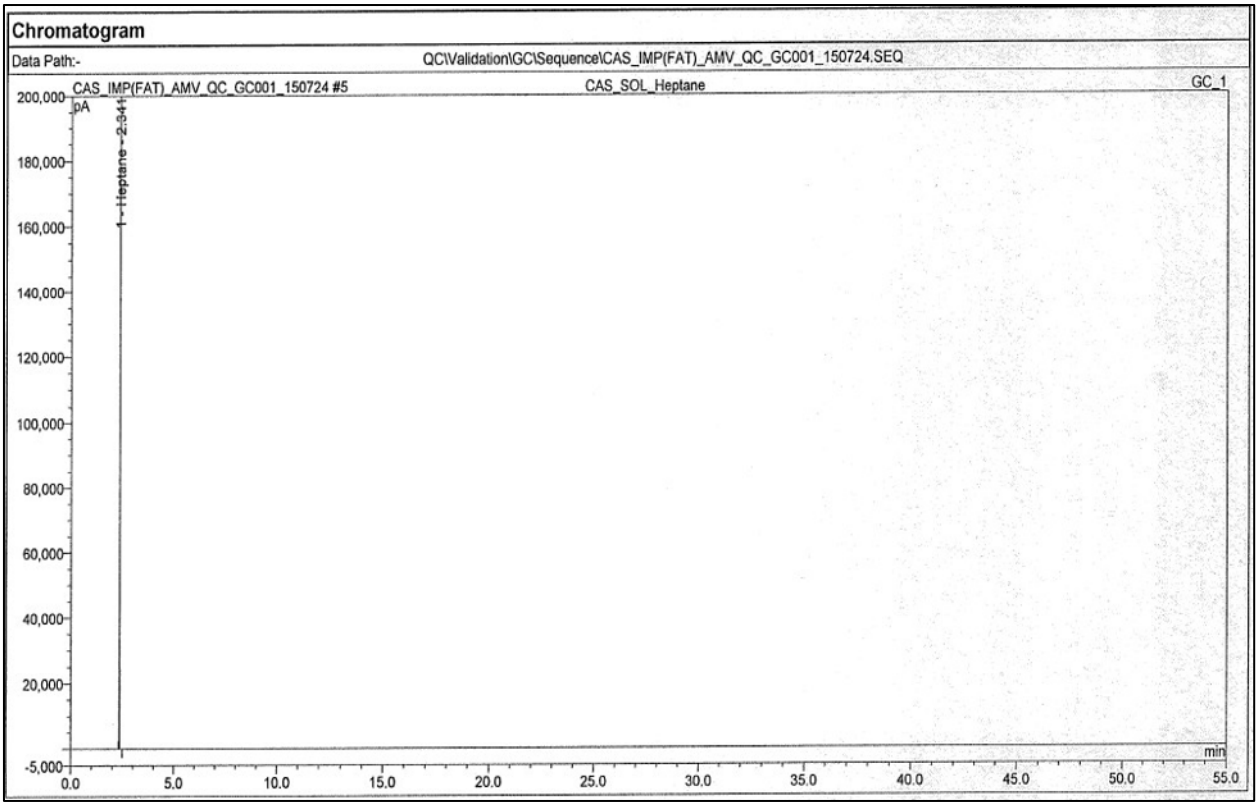

Figure 1 Chromatogram of Blank (Heptane) in Composition of fatty acid method

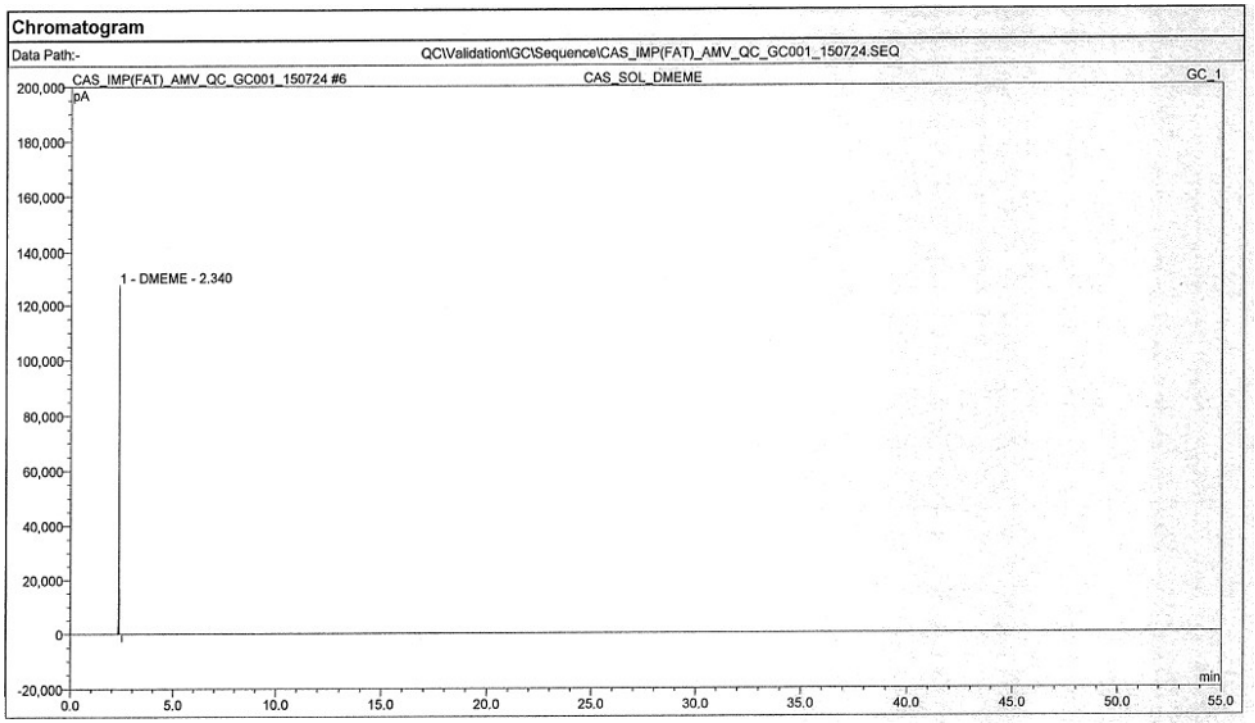

Figure 2 Chromatogram of Blank (DMEME) in Composition of fatty acid method

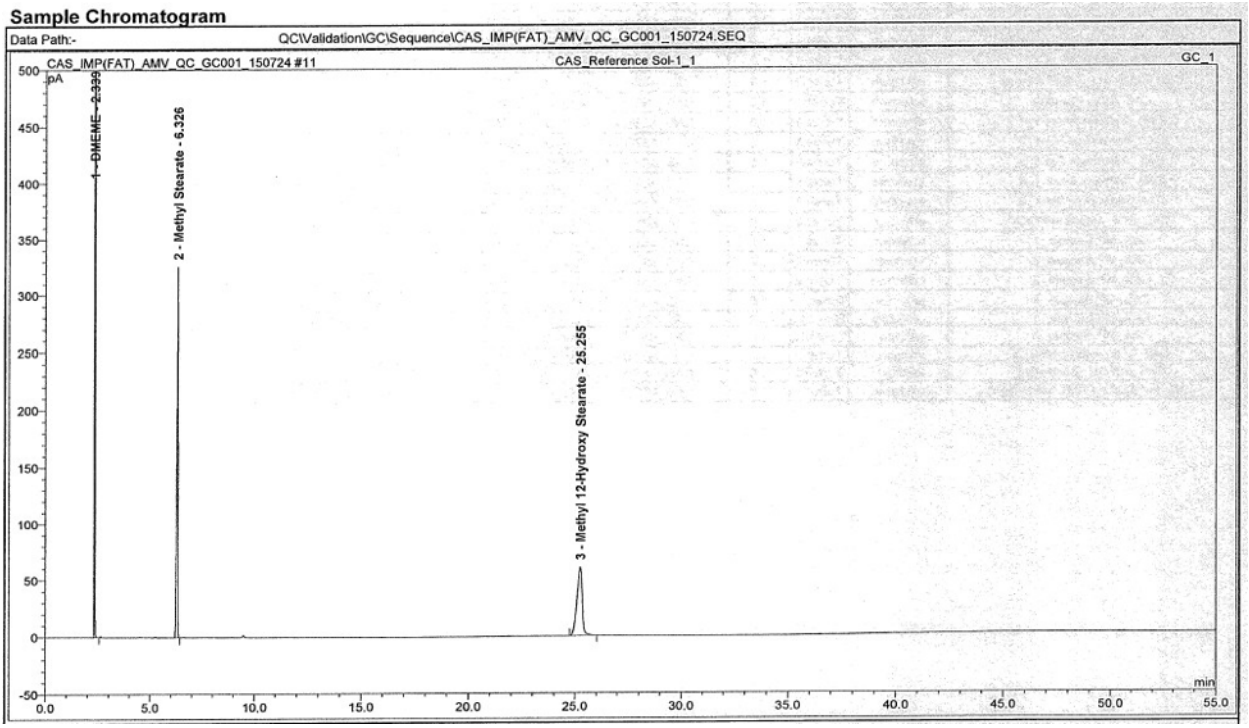

Figure 3 Chromatogram of Reference Solution Composition of fatty acid method

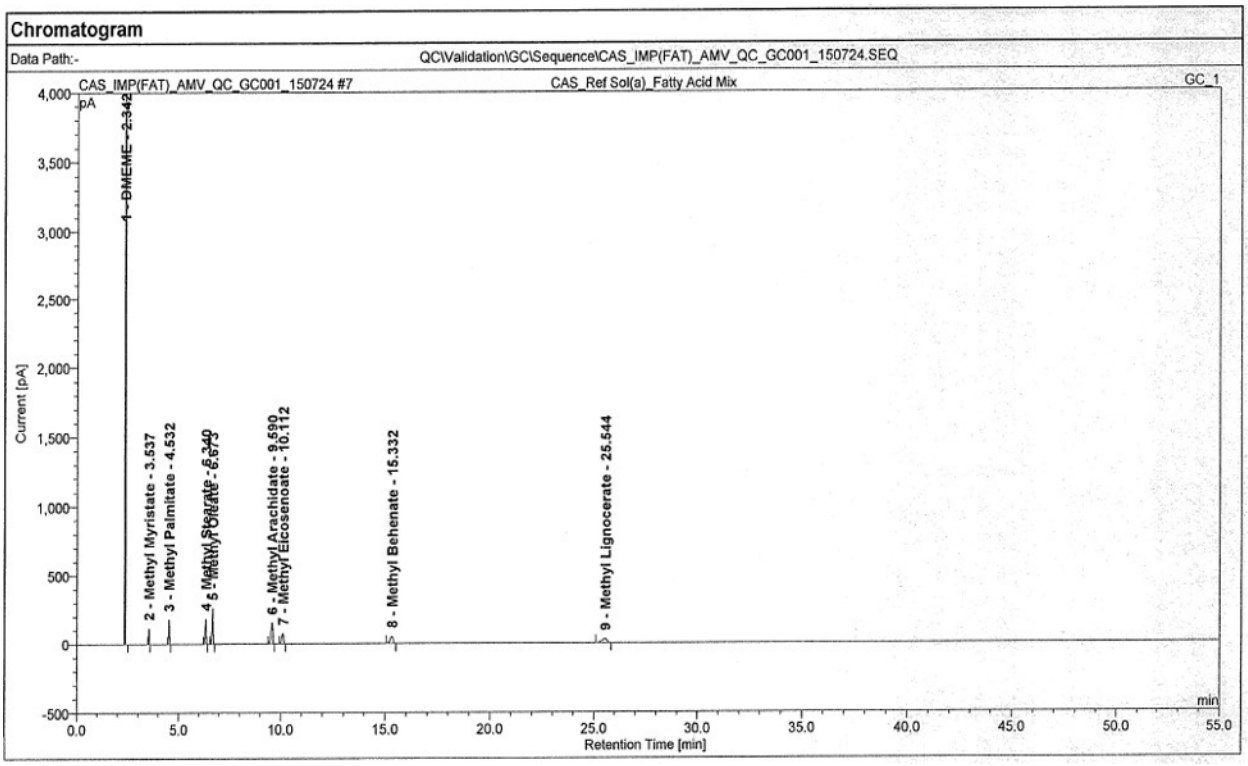

Figure 4 Chromatogram of Fatty acid mixture in Composition of fatty acid method

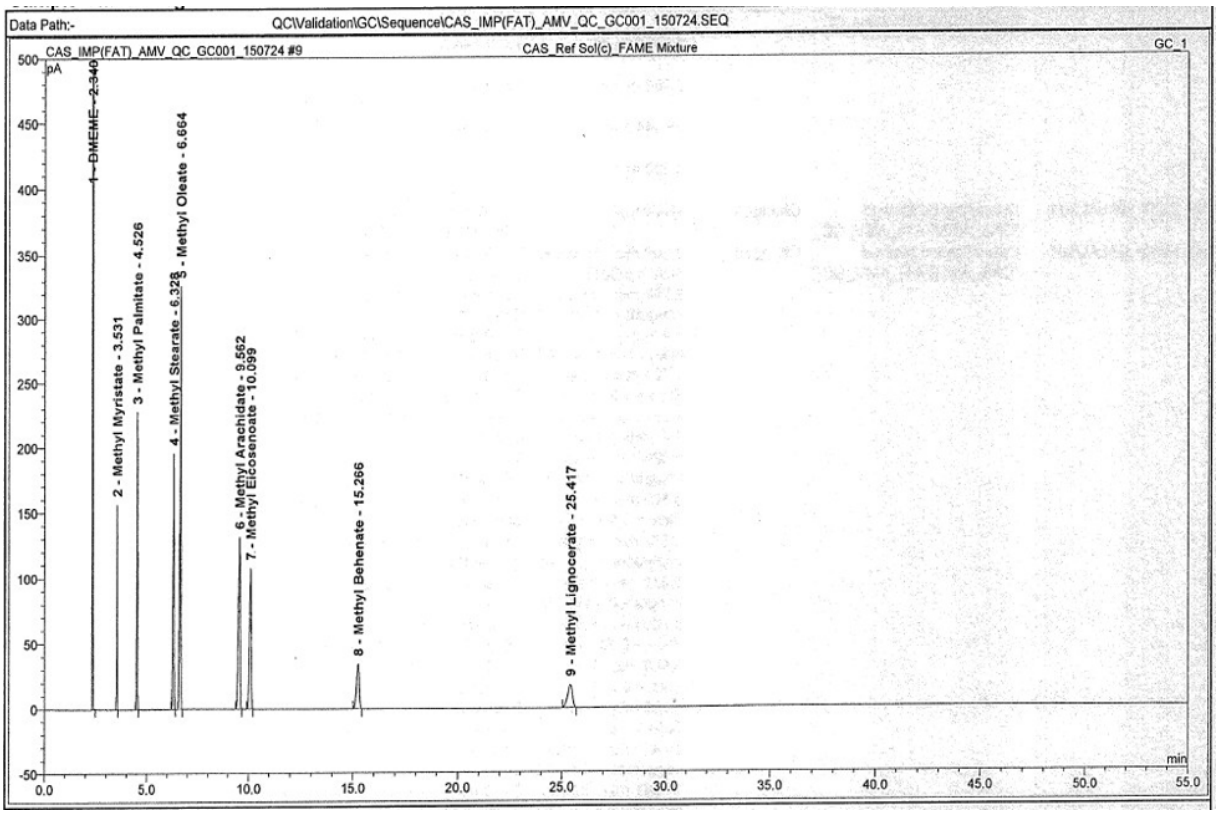

Figure 5 Chromatogram of FAME mixture in Composition of fatty acid method

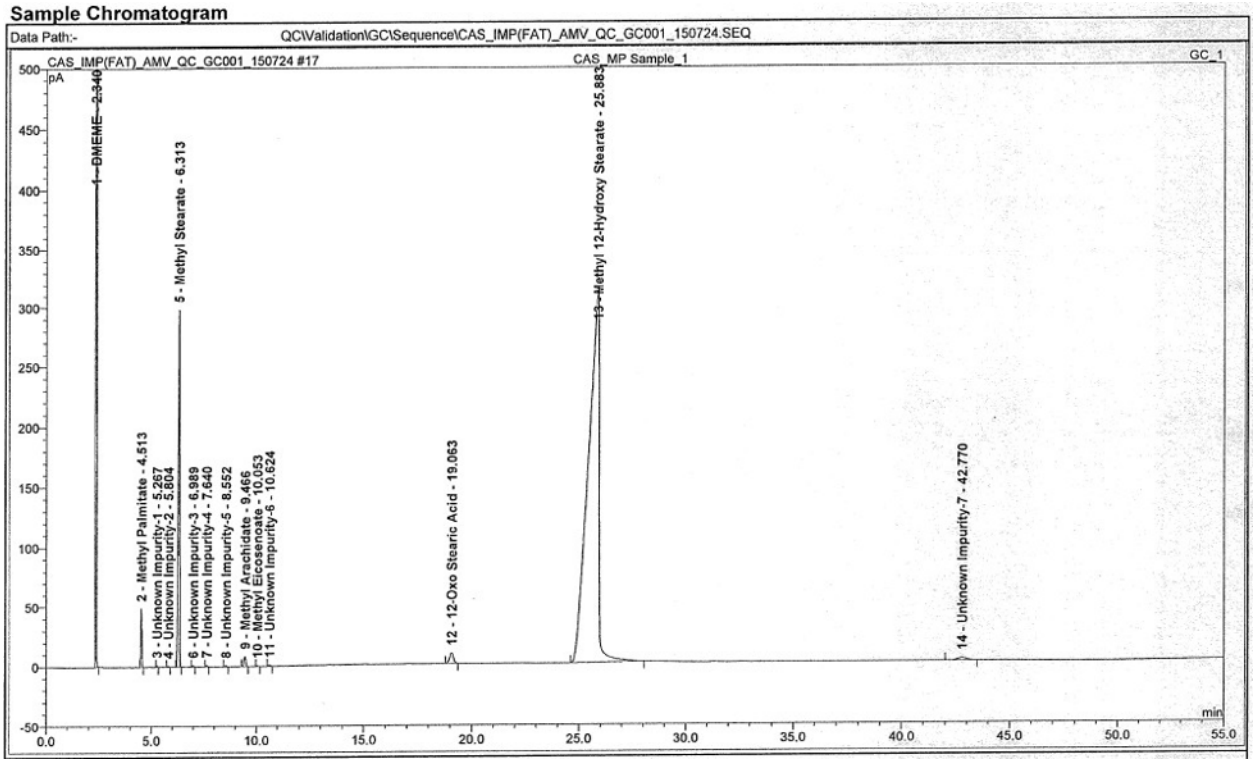

Figure 6 Chromatogram of Method Precision Sample in Composition of fatty acid method

3.2. System Suitability

As a part of this study, system precision was performed using repeated injections of standard solutions [4,6]. For the system precision, FAME Mixture was injected to see resolution between each component [1,5]. Standard solution was injected for five times and observes the chromatogram.

Table 1 represents the Resolution results.

Table 2 represents the % RSD of areas of was found below 15.0.

Table 1 System Suitability Resolution

Resolution								
Solutions	**Methyl Myristate**	**Methyl Palmitate**	**Methyl Stearate**	**Methyl Oleate**	**Methyl Arachidate**	**Methyl Eicosenoate**	**Methyl Behenate**	**Methyl Lignocerate**
FAME Mixture	17.27	21.70	3.34	22.52	3.47	25.93	30.31	NA
Fatty Acid Mixture	18.08	22.42	3.42	23.13	3.40	26.02	29.25	NA

Table 2 % RSD of Area of Reference Solution

Reference Solution	Area of Methyl Stearate	Area of Methyl 12- Hydroxy Stearate
% RSD	6.9%	6.9%

3.3. Precision Study

As a part of this study, method precision was performed. For the method precision, six individual sample solution was injected and observe the chromatogram.

Table 3 Represents the % RSD of areas of was found below 5.0%

Solutions	Methyl Myristate	Methyl Palmitate	Methyl Stearate	Methyl Oleate	Methyl Arachidate	Methyl Eicosenoate	Methyl Behenate	12- hydroxy stearate	12-oxostearate	Methyl Lignocerate
% RSD of six sample	ND	0.3 %	0.1 %	ND	0.6 %	4.2 %	ND	1.0%	0.0 %	ND

4. Conclusion

To develop a simple GC method for the identification and quantification of composition of fatty acids, this study was conducted. According to pharmacopeia and ICH guidelines, recommended GC column was not giving system precision and resolution. So, this compendia method was developed and verified with new column.

Pharmaceutical manufacturing companies, quality control scientists, and researchers will benefit from this method. Additionally, it can serve as study material for students learning about method development through liquid injector gas chromatography. This method can be employed to quantify composition of fatty acid in hydrogenated castor oil - Myristate, palmitate, stearate, oleate, arachidate, eicosenoate, behenate, 12-oxostearate, 12- hydroxy stearate, lignocerate. The developed method is precise, specific and should be validated according to ICH guidelines before being used to release the commercial products.

List of Abbreviations

- RT: Retention Time
- HCO: Hydrogenated Castor Oil
- FAMEs: Fatty Acid Methyl Esters
- IPA: Isopropyl alcohol
- THF: Tetrahydrofuran
- DMEME: 1,1- dimethyl ethyl methyl ether
- GDP: Good Documentation Practices
- GC: Gas Chromatography
- FID: Flame Ionization Detectors
- ICH: International Council for Harmonization
- µm: Micrometer
- mL: Milliliter
- µL: Microliter
- g/L: Gram/ Liter
- RSD: Relative Standard Deviation
- ppm: Parts Per Million
- Conc.: Concentration
- ND: Not Detected

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

Authors' Contribution

This research was designed and performed by Sukanta Nath Dalal. The co-author reviewed the content, data presentation, and overall layout of the study.

References

[1] European Directorate for the Quality of Medicines & HealthCare (EDQM). *European Pharmacopoeia*, 11th Edition. Strasbourg: Council of Europe; 2023.

[2] Christie WW. *Gas Chromatography and Lipids: A Practical Guide*. Ayr, Scotland: The Oily Press; 1989.

[3] Knothe G, Van Gerpen J, Krahl J. *The Biodiesel Handbook*. 2nd ed. Urbana, IL: AOCS Press; 2010.

[4] International Council for Harmonization (ICH). *ICH Q2(R2): Validation of Analytical Procedures*. Geneva, Switzerland; 2023

[5] European Pharmacopoeia, latest edition, *Hydrogenated Castor Oil Monograph and Fatty Acid Composition – Gas Chromatography (General Chapters), European Directorate for the Quality of Medicines & HealthCare (EDQM), Strasbourg.*

[6] International Council for Harmonization (ICH), ICH Q2(R2): Validation of Analytical Procedures: Geneva, Switzerland; 2023.

[7] AOAC International, Official Methods of Analysis, Fatty Acid Methyl Ester (FAME) Analysis by GC.