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Application News

Gas Chromatograph Nexis™ GC-2030, AOC-20i+s Plus

A Simple Method for Determination of β-Sitosterol as a Marker of Vegetable Oil Adulteration in Ghee by GC

Mohit Sharma, Satyendra Singh, Rahul Dwivedi, Durvesh Sawant, Jitendra Kelkar and Pratap Rasam
Shimadzu India Pvt. Ltd.

User Benefits

- Shimadzu Nexis GC-2030 can be effectively used for determination of adulteration of Ghee with vegetable oil.
- The Nexis GC-2030 easily meets the acceptance criteria as per the proposed FSSAI method no.01.097:2022.
- This method is suitable for routine food industry analysis and academic research.

■ Introduction

The most common milk product in Indian cuisine is ghee or clarified butter. Due to its high cost, ghee is adulterated with vegetable oil, to gain commercial benefits. β-sitosterol, a common phytosterol, found in a variety of natural sources, including vegetable fat or oil from plants (Figure 1). It is used as a marker of vegetable oil adulteration in ghee[1].

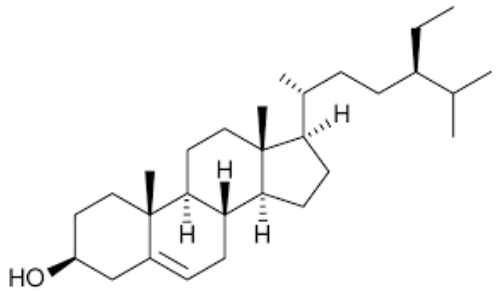

Figure 1 Structure of β-Sitosterol

The primary goal of this study is to assess the vegetable oil adulteration in ghee by analyzing β-Sitosterol as a marker using gas chromatography (GC). This will establish a foundation for the rapid detection of ghee adulteration. In this study, a simple, sensitive, and inexpensive method is developed for extraction of β-Sitosterol from ghee which is then subjected to gas chromatography with flame ionization detection for quantitation. The effects of several important parameters influencing the extraction efficiencies of β-Sitosterol including water-immiscible organic solvent (type and volume), volume of water, sonication time, surfactant (type and concentration), and organic modifier (type and volume) were investigated.

This method is ideal for routine analysis in the food industry during the manufacturing, processing, and commercial testing of milk fat samples, or for academic research purposes.

Figure 2 Nexis™ GC-2030 system

■ Sample Preparation and Analysis Conditions

Standard and sample preparations were conducted following the guidelines outlined in the proposed FSSAI method No. 01.097:2022[2]. The analysis was performed using a Shimadzu Nexis GC-2030 equipped with a Flame Ionization Detector (FID), as shown in Figure 2. The specific configuration and analytical conditions used during the study are provided in Table 1

Table 1 Analysis Conditions of the GC-2030

System	: GC-2030
Column	: SH-I-Rxi-5Sil MS Cap. Column, (30m x 0.25 mm ID., df=0.25µm)*1
Injection Mode	: Split
Flow Control Mode	: Constant Flow
Injector Port Temp.	: 320 ˚C
Carrier Gas	: Nitrogen
Column Flow	: 0.7 mL/min
Split Ratio	: 15:1
Injection Volume	: 0.5 µL
Oven Temp.	: 140 ˚C (2.0min), 15 ˚C/min to 320 ˚C (16min)
Equilibration Time	: 1.0 min
Detector	: Flame Ionization Detector
Detector Temp.	: 320 ˚C
Detector Gas	: Air 200mL/min, H 32mL/min, N 24mL/min

*1 P/N: 221-75954-30

Standard and Sample Preparations:

β-Sitosterol Stock Preparation: To Prepare a stock solution of 1000 mg/kg, accurately weigh 10 mg of β-Sitosterol and place it in a 10 mL volumetric flask. Add 5 mL of chloroform, sonicate to dissolve, and then dilute with chloroform to the final volume.

Preparation of Calibration Standard: A set of seven calibration standards were prepared from the 1000 mg/kg stock solution of β-Sitosterol, using hexane as a diluent. The final concentrations of the standards were 1, 2, 5, 10, 20, 50, and 100 mg/kg.

Preparation of Sample: Sample preparation was carried out by accurately weighing 1 g of ghee into a round-bottom flask, followed by the addition of 25 mL of 5 % methanolic KOH. The mixture was then refluxed at 95˚C for 45 minutes to ensure complete reaction. After refluxing, the solution was allowed to cool, and the mixture was extracted three times with 15 mL portions of n-hexane. The combined n-hexane extracts were subsequently passed through anhydrous sodium sulphate to remove any residual moisture. The dried extract was then washed with water, and the n-hexane layer was evaporated under a gentle stream of nitrogen. The resulting residue was dissolved in 0.5 mL of n-hexane, filtered through a 0.45 µm filter, and finally injected into the gas chromatograph for analysis.

■ Calibration and Linearity

For quantitative analysis multipoint calibration curve was obtained using analytical conditions described in Table 1. Good linearity with a regression coefficient R2 = 0.99 was obtained (Figure 3).

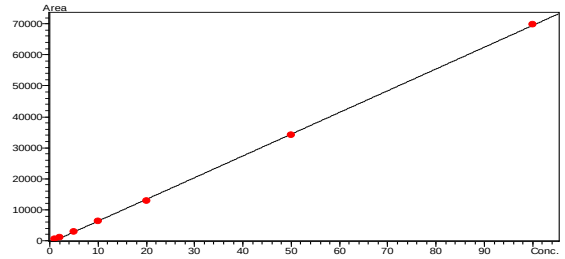

Figure 3 Calibration curve for β-Sitosterol

Table 2 Repeatability of the peak area of β-sitosterol at various concentrations (n=3)

Conc. (mg/kg)	Peak Area of β-Sitosterol (n=3)			
	Replicate-1	Replicate-2	Replicate-3	Average
1	504	561	466	510
2	1002	1107	1022	1044
5	2777	2910	2682	2790
10	6236	6083	6351	6223
20	12542	12922	12720	12728
50	34145	34002	34089	34079
100	70164	69458	69697	69773

Table 2 provides detailed repeatability data for the peak area of β-Sitosterol at various concentrations, while Table 3 provides detailed repeatability of the method at a 10 mg/kg concentration by injecting five replicate standard samples. For these replicates, the % RSD for both retention time and peak area were found to be less than 0.1 % and 5 %, respectively.

Table 3 Repeatability of retention time and peak area

Compound name	Conc. (mg/kg)	RT (%RSD)	Area (%RSD)
β-Sitosterol	10	0.05	1.72

Recovery Study: Matrix Blank samples were tested to determine the suitability of sample preparation procedure. The ghee samples were spiked at concentration of 20 mg/kg and 50 mg/kg of β-Sitosterol and recoveries values were found in the range of 70-120 % (Table 4).

Table 4 Recovery study for β-Sitosterol in ghee samples

Compound Name	Spiked Conc. (mg/kg)	Recovery(%)
β-Sitosterol	20	91
	50	98

Vegetable oil adulteration study: To evaluate the applicability of the proposed method, ghee samples were intentionally adulterated with vegetable oil at three concentration levels i.e., 2 %, 5 %, and 10 %. Each adulterated sample was processed and analysed using the developed procedure. Blank sample doest not show any peak at retention time of β-Sitosterol (Figure 4). The method successfully detected vegetable oil adulteration even at the lowest level of 2 %, demonstrating good sensitivity (Figure 5). The observed concentrations (mg/kg) corresponding to each adulteration level are summarized in Table 5, which clearly shows the increasing analyte response with higher percentages of vegetable oil adulteration.

Table 5 Concentration of β-Sitosterol at various adulteration levels

Compound Name	Adulteration Level (%)	Observed Conc. (mg/kg)
β-Sitosterol	2 %	4
	5 %	11
	10 %	22

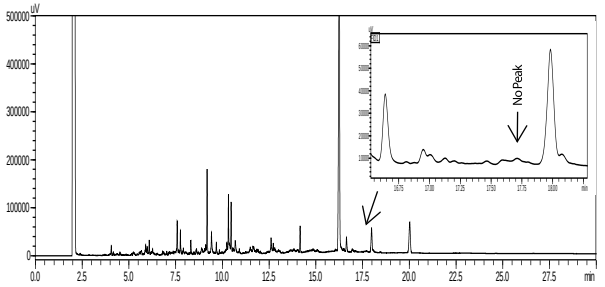

Figure 4 Chromatogram of unadulterated pure cow ghee

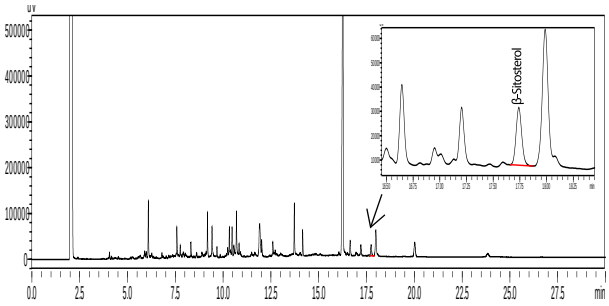

Figure 5 Chromatogram of adulterated pure cow ghee

■ Conclusion

A gas chromatography–based method using GC-2030 was developed for the estimation of β-Sitosterol in ghee. The method exhibits a detection limit of 2 %, enabling the identification of vegetable oil adulteration in ghee at levels as low as 2 %. At this adulteration level, the signal of β-Sitosterol achieved a signal-to-noise (S/N) greater than 3, confirming the reliability of detection at the established limit. This method provides a reliable and effective approach for the quantification of β-Sitosterol, which serves as a marker for vegetable oil adulteration in ghee.

■ References

1. Kala, A. et al. (2016). Determination of triacyl glycerol and sterol components of fat to authenticate milk fat based sweets. Journal of Food Science and Technology. 53. 10.1007/s13197-016-2182-3.
2. Method for detection of adulteration in milk fat (clarified milk fat) with vegetable oils. File No. 1-90/FSSAI/SP (MS&A)/2009 dated 25th March, 2018. Food Safety Standards Authority of India, New Delhi.

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