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Food Safety and Standards Authority of India

(A statutory Authority established under the Food Safety and Standards Act, 2006)

(Quality Assurance Division)

FDA Bhawan, Kotla Road, New Delhi – 110002

Dated, the 25th March, 2019

ORDER

Subject: Method for detection of adulteration in ghee (clarified milk fat) with vegetable oils - reg.

The Scientific Panel on Methods of Sampling and Analysis, Scientific Committee and Food Authority has approved the Method for detection of adulteration in ghee (clarified milk fat) with vegetable oils **(Annexure – I)**.

2. The food testing laboratories are hereby requested to use the aforesaid method, with immediate effect.

3. Kindly note that this method is applicable to only four vegetable oils viz Soybean Oil, Groundnut Oil, Coconut Oil and Sunflower Oil.

Encl: Method

(Bhaskar N.)
Advisor (QA)

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories

fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food	**Method for Determination of adulteration of Vegetable Oil in Ghee by Reversed Phase- High Performance Liquid Chromatography (RP-HPLC)**		
Method No.		**Revision No. & Date**	
Scope	Applicable to determination of vegetable fat (soybean oil, coconut oil, sunflower oil, groundnut oil) in ghee (clarified milk fat).		
Principle	The method is based on the detection of cholesterol and β- sitosterol as markers in the unsaponifiable matter (USM) of pure ghee and adulterated ghee samples. β-Sitosterol serves as an indicator for the adulteration in ghee by plants and specific dose manner (1-5g/100g of ghee). The RP-HPLC method resolves cholesterol, stigmasterol and β- sitosterol at Retention Time (RT values) of 22.8±0.2, 25.5±0.2, 29.05±0.2 minutes under prescribed conditions of the method.		
Apparatus	Beakers (100 ml, 1000 ml); Volumetric flasks (10ml,100ml, 200ml); Screw capped test tubes (50ml); Micro centrifuge stand (2); Adjustable micro pipettes (1 ml and 10 ml); Millipore filter paper (Nylon 0.22µm); Weighing balance (Minimum weighing capacity 1 mg); Shaking water bath; Vortex mixture; Centrifuge; Refrigerator; HPLC with PDA detector, pumps, auto sampler, column oven and degasser; Micro concentrator.		
Chemicals	β- sitosterol- Sigma; Sigmasterol- Sigma; 5% Methanolic KOH; n-Hexane (HPLC grade); Chloroform; Methanol; Cholesterol		
Preparation of stock solution of standards	**1) Stock solution A (Cholesterol):** Prepare 1 mg/ml of stock solution in chloroform. **2) Stock solution B (Stigmasterol):** Prepare 1 mg/ml of stock solution in chloroform. **3) Stock solution C (β- sitosterol):** Prepare 1 mg/ml of stock solution in chloroform. Prepare diluted standards up to 1µg/ml of individual and mixture of (1 to 3) in methanol.		
Preparation of Test Samples	1. Weigh 1 gm of fat in a screw capped test tube. 2. Add 25 ml of 5% Methanolic KOH. 3. Keep the tubes in water bath maintained at 90°C for 50 minutes with vigorous shaking at regular intervals. 4. Take out tubes from water bath and add 5 ml water and 15 ml hexane. 5. Vortex the mixture for one minute. 6. Centrifuge at 3000 RPM for 5 minutes. 7. Pipette out the upper hexane layer completely and dry (preferably in micro concentrator) to obtain unsaponified matter (USM).		

	8. Dissolve the dried USM in 300µl of Chloroform and add 500µl methanol, vortex and mix. 9. Filter the above extract through 0.22µm nylon filter paper. 10. Run the reference standards of cholesterol, sigmasterol and β- sitosterol and samples (from step 9) on HPLC.
HPLC Condition	Column : C-18, 4.6 × 250mm ID, 5µm Column Oven Temp : 30°C Flow rate : 1.5 ml/min PDA wavelength : 205 nm Injection volume : 20 µl Run time : 35 min Washing solution : Acetonitrile: Isopropanol (9:1) **Mobile Phase:** Acetonitrile: Isopropanol (9:1), filtered and degassed
Peak identification and Confirmation	In pure/genuine ghee sample no peak of β- sitosterol is expected to be found. The detection of β- sitosterol peak of ghee denotes the adulteration with vegetable fat. Identify the peaks in the sample by comparing the retention time with that of reference standards. The peak for β- sitosterol in adulterated sample confirms the presence of vegetable oil. Limit of Detection (LOD) would vary with the type of oil adulterated. Method is capable of detecting coconut oil at 5%, refined soybean oil at 1%, Groundnut oil at 2% and Sunflower oil at 1%. The additional oil can also be included for detection of adulteration, however detection would require extensive verification/validation. The detection and reporting of any result below than 1 % should be done cautiously.
Reference	Anupama Rani, Vivek Sharma, Sumit Arora, Darshan Lal Ghai (2015) : Int. J. of food Properties: Comparison of rapid reversed phase high performance liquid chromatography(RP-HPLC) method with rapid reversed phase thin layer chromatography (RP-TLC) method for detecting vegetable oil in ghee.
Approved by	Scientific Panel on Methods of Sampling and Analysis