

File No. 11014/07/2021-QA
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: 7th May, 2024

Order

Subject: Insertion of HPLC method for determination of gamma oryzanol content in rice bran oil and other vegetable oils - reg.

The HPLC method for determination of gamma oryzanol content in rice bran oil and other vegetable oils (FSSAI 02.053:2024) has been approved by Food Authority in its 41st meeting.

2. The Method is enclosed herewith and the same has also been inserted in the Manual of Methods of Analysis of Foods-Oils and Fats.
3. This method shall be used by the laboratories with immediate effect.
4. Since the process of updation of test methods is dynamic, any changes happening from time to time will be notified separately. Queries/concerns, if any, may be forwarded to email: sp-sampling@fssai.gov.in, dinesh.k@fssai.gov.in.

Encl: as above

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(Dr. Satyen Kumar Panda)
Advisor (QA)

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories
3. CEO, National Accreditation Board for Testing and Calibration Laboratories (NABL)

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</p>	HPLC method for determination of gamma oryzanol content in rice bran oil and other vegetable oils		
Method No.	FSSAI 02.053:2024	Revision No. & Date	0.0
Scope	<p>Method is useful to determine gamma oryzanol content in rice bran oil as well as other vegetable oils. Gamma oryzanol / Oryzanol is mixture of four compounds viz., cycloartenyl ferulate (CycloFer), 24-methylene cycloartanyl ferulate (24-MCFer), campesteryl ferulate (CampFer), and β-sitosteryl ferulate (β-SitFer).</p> <p>Note1: This method is also useful to determine tocopherols and sterols present in oils. However, details are not provided here.</p> <p>Note 2: For total oryzanol content, UV Spectrophotometer method may be used and in case of label claim for gamma oryzanol, HPLC method shall be used.</p>		
Caution	<ol style="list-style-type: none"> 1. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. 2. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns. 		
Principle	<p>Fats and Oils are diluted, filtered and analysed by RP-HPLC using PFP column and diode array detector@328nm for total oryzanol content.</p> <p>Notes:</p> <ol style="list-style-type: none"> 1. Method validation showed linearity of calibration curves ($\alpha=0.05$). 2. RSD of intra-day, inter-day and inter-laboratory precision were less than 4.88%. 3. The limit of detections (LODs) and limit of quantifications (LOQs) were low (0.009–2.166 $\mu\text{g/g}$) with recoveries around 96.0–102.9%. 		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. General standard glass ware. 2. HPLC connected to a diode array detector. 3. Pentafluorophenyl propyl (PFP) core-shell column (4.6×250 mm, 5 μm; It is alternative to the widely used C18 and C8 phases. 4. 0.45 μm syringe nylon filter. 5. HPLC syringe. <p>Notes:</p> <p>(i) Any HPLC system with a diode array detector and PFP column, which can provide proper resolution of the target compounds).</p> <p>(ii)The PFP core-shell column (4.6×250 mm, 5 μm) is a robust core- phase that reduces method development time with its dynamic and responsive chemical functionality. With five retention mechanisms and five separation modes, this column is an alternative to the widely used C18 and C8 phases.</p>		
Materials and Reagents	<ol style="list-style-type: none"> 1. Rice bran oil 2. Vegetable oils 3. Individual standards of γ-oryzanols (CycloFer, 24-MCFer, CampFer, and β-SitFer). (or) a Mixture of standard γ-oryzanols (CycloFer, 24-MCFer, CampFer, and β-SitFer at a known ratio). 		

	<ol style="list-style-type: none"> 4. Methanol – HPLC grade. 5. Water – HPLC grade. 6. Dichloromethane – HPLC Grade.
Preparation of Reagents	Degass all the HPLC solvents before use.
Sample Preparation	Dilute the oils (0.5 g) with dichloromethane and adjust the volume to 1.00 mL. Filter the resulting solution through a 0.45 µm syringe nylon filter and analyze.
Method of analysis	<p>HPLC analysis</p> <ol style="list-style-type: none"> 1. Mobile Phases - Component A: Methanol; Component B: Water 2. The gradient elution was 90% A (0–13 min), linearly changed to 95% A (13–14 min), linearly changed to 85% A (14–17 min), linearly changed to 95% A (17–22 min), and then held at 95% A (22–30 min). 3. The flow rate is 1.0 mL min⁻¹ 4. The column temperature is 30 °C. 5. Sample Volume – 5 µL/each injection. 6. Determine LOD and LOQ using standards. 7. Signal-to-noise (S/N) ratio was determined by comparing signals from sample with known low concentrations of compound with noise of blank samples. S/N ratios of 3 and 10 were for LOD and LOQ, respectively. 8. Prepare the standard curves using known concentrations (five or eight). Concentration vs peak area is standard curve for respective compound. 9. Inject the oil samples and analyse. Triplicate injections are preferred. 10. Note the peak areas. <p>Notes: (i)The elution order (retention times) derived from the PFP column is CycloFer (24.52 min), 24-MCFer (25.63 min), CampFer (26.62 min), and β-SitFer (27.50 min). These may vary depending on the column phase, mobile phase and flow rate.</p> <p>(ii) LODs for CycloFer, 24-MCFer, CampFer and β-SitFer are 0.215, 0.218, 0.216 and 0.714 µg/mL respectively. LOQs for CycloFer, 24-MCFer, CampFer and β-SitFer are 0.651, 0.647, 0.632 and 2.166 µg/mL respectively.</p>
Calculation with units of expression	<p>Calculate the quantities of each oryzanol components using respective peak areas and standard curves.</p> <p>Total oryzanol quantity is determined by adding /combining all the quantities of oryzanol components.</p> <p>Express the Total oryzanol quantity for 100 g of oil.</p>
Reference	1. Simultaneous determination of tocopherols, γ-oryzanol, phytosterols, squalene, cholecalciferol and phylloquinone in rice bran and vegetable oil samples by Piramon Pokkanta, Phumon Sookwong, Manatchanok Tanang, Saranya Setchaiyan, Pittayaporn Boontakham, Sugunya Mahatheeranont, Food Chemistry 271 (2019) 630–638.
Approved by	Scientific Panel on Methods of Sampling and Analysis