

 <p>FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA <small>Insuring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India</small></p>	HPLC method for determination of gamma oryzanol content in oils (rice bran oil and other vegetable oils)		
Method No.		Revision No. & Date	
Scope	Method is useful to determine gamma oryzanol content in rice bran oil as well as other vegetable oils. gamma 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). Note: This method is also useful to determine tocoferols and sterols present in oils. However, details are not provided here.		
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	Fats and Oils are diluted, filtered and analysed by RP-HPLC using PFP column and diode array detector@328nm for total oryzanol content.		
Apparatus/Instruments	<ol style="list-style-type: none"> 1. General standard glass ware. 2. Agilent HPLC 1100 connected to a diode array detector (Model G1315 A, Agilent Technologies). 3. Kinetex PFP column (4.6×250 mm, 5 μm, Phenomenex, Inc.). 4. 0.45 μm syringe nylon filter. 5. HPLC syringe. Notes: (i) Any equivalent HPLC system as well as any equivalent column may be used, which can provide proper resolution of the target compounds. (ii) Kinetex F5 is a robust PFP core- shell phase that reduces method development time with its dynamic and responsive chemical functionality. With five retention mechanisms and five separation modes, Kinetex F5 is an alternative to the widely used C18 and C8 phases.		
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). 4. Methanol – HPLC grade. 		

	<p>5. Water – HPLC grade.</p> <p>6. Dichloromethane – HPLC Grade.</p>
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 gamma oryzanol quantity for 100 g of oil.</p>
Reference	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

The following 'note' need to be added in all manuals:

Note: The test methods given in the manual are standardised/ validated/ taken from national or international methods or recognised specifications, however it would be the responsibility of the respective testing laboratory to verify the performance of these methods onsite and ensure that it gives proper results before putting these methods in to use".

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MoM – General
MoM - Pesticides
MoM – Sampling
MoM – Product Category
MoM – Contaminants

(to be written depending upon
concerned manual)